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GERMINATION AND EARLY GROWTH RESPONSE OF *GLYCINE MAX* VARIETIES IN TEXTILE AND PAPER INDUSTRY EFFLUENTS

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Abstract

Present investigation was carried out to assay the effects of industrial effluents on different varieties of soybean. For that purpose Textile effluent and Paper & Board effluent were chosen. Concentrations used for both the effluents were 0, 10, 20, 40 & 60%. On the other hand 5 varieties of *Glycine max* viz., PSC-82, NARC-2, NARC-5, NARC-7 & William-82 were used. Physico-chemical characteristics of these effluents revealed that both of them contained high amounts of sulphates, nitrates, calcium, various heavy metals etc., while DO was very low, which confirms their highly polluted conditions. Maximum improvement in seedling length was found in 60% of textile effluent (NARC-2, NARC-7 & Williams-82), and in paper & board effluent (NARC-2, NARC-5 & NARC-7). For others varieties, lower concentrations enhanced the growth. Number of leaves was unaffected and remained same in all concentrations of the two effluents. Overall seedling lengths of PSC-62, NARC-2 and NARC-5 were comparatively longer in paper and board effluent as compared to textile effluent but it was other way round for the varieties NARC-7 and Williams-82, while there was no set pattern for the weight parameters.

Introduction

Rapid industrialization leads to the production of heavy loads of wastes. These wastes (effluents) are released in the environment after treatment (developed countries) or mostly without treatment (developing countries such as India and Pakistan etc). These effluents are either released into some water body or directly on to the lands, which are mostly agricultural. Some times these effluents are purposely used for irrigation due to scarcity of water, especially for raising vegetables and fodder etc (Ghafoor *et al.*, 1994).

In Pakistan, very few industries are equipped with satisfactory operating treatment facility set up, so there is common trend that industries dispose off untreated effluents *via* open and covered routes into the water ways which degrade water quality (Farid, 2003). Hence these industrial effluents are the most potential source of water and soil pollution. These effluents contain heavy metals as well as nutrients, which affect plant and soil in variety of ways (Dhevagi & Oblasam, 2002). Heavy metals are accumulated in the living cells causing a reduction of cell activities, inhibition of growth and various deficiencies/ diseases in plants (Shafiq & Iqbal, 2005; Kabir *et al.*, 2008; Farooqi *et al.*, 2009).

Most of the studies conducted so far are to investigate the effects of different industrial effluents on vegetable crops and cereals. Information regarding the effect of effluents on oil producing crops is still scanty. Keeping in view the importance of pollution and lack of knowledge regarding the effects of industrial effluents on oil producing plants, present study was carried out. As agricultural crops vary widely in their tolerance to toxic metals, one objective of the study was to evaluate different cultivars of *Glycine max* under different industrial effluents and to identify the tolerant variety so that its cultivation be recommended in a particular environment. Still another aspect of the study was to evaluate the potential of industrial effluents, which may reduce the water scarcity issue.

Materials and Methods

Effluents of Textile Industry and Paper & Board Industry were collected and subjected to various physico-chemical tests using the standard methods (Eaton *et al.*, 1998). Heavy metal analysis was also done using Atomic Absorption spectrophotometer.

For the germination experiments, certified seeds of different varieties of *Glycine max* were obtained from NARC, Islamabad. Five varieties PSC-62, NARC-2, NARC-5, NARC-7 and William-82 were used in the present study. Experimental setup was the same as described by Nawaz *et al.* (2006). Healthy and equally sized seeds of all five varieties were sterilized with 0.1% $HgCl_2$. After repeated washings with sterilized distilled water, seeds were soaked in the same water for 4hrs. Then ten sterilized seeds were arranged in sterilized Petri dishes, lined with double layer of filter paper. Plates were labelted as per type, concentration of the effluent and variety of soybean. These were then supplied with respective effluent concentration and incubated for three days at 26±2^{o}C for germination. Daily observations were made for the germinated seeds, and then were shifted to light (10kLux) for next 7 days. Before shifting, 15 ml of nutrient solution (Hewitts, 1963) was provided with the same concentration of effluent. After seven days, seedlings were harvested; root, shoot & seedling lengths and fresh weights were recorded. For dry weights seeds were incubated at 60^{o}C for 24 hrs. Data was subjected to statistical analysis to see the significance.

Results and Discussion

Textile as well as paper industry effluents contain organic and inorganic chemicals, balance of which may affect plant growth adversely. Physico-chemical analysis of both the effluents indicated very high conductivity, which is a clear reflection of the presence of large number of metals and salts. Paper mill effluent was highly turbid containing huge amount of total solids. Amounts of calcium, nitrates and sulphates were very high, which are among the plant nutrients and help in their growth. DO was very low in both effluents, which confirms their highly polluted and deteriorating condition (Table 1). Heavy metal contents of effluents were higher than US-EPA guidelines for irrigation water. Heavy metals are toxic to living organisms if present in higher concentrations (Alloway, 1990). An excess of metal ions or soluble metal chelates may induce a series of biochemical and physiological alterations in plants (Lepp, 1981).

For most of the Soybean cultivars (PSC-62, NARC-5 & Williams-82), the percentage germination was significantly improved in the presence of different concentrations of the two effluents, though there was no particular trend (Table 2). Few seeds were able to grow at 80 & 100% concentrations but they could not survive (data not shown) so these concentrations were not used further. Enhanced seed germination of *Vigna mungo* is also documented at 25% concentration of textile effluent (Wins & Murugan 2010). Mohammad & Khan (1985) reported adverse effects of textile effluent (75 & 100% concentrations) on the germination of *Phaseolus aureus* and *Abelmoscus esculentus* seeds, while there was no effect upto 50% concentration of the same effluent on this parameter. Dhanam (2009) also documented increased percentage germination of paddy in low concentrations of dairy effluent, though the higher concentrations were injurious. Many researchers related decrease germination at high concentration of different effluents with high osmotic pressure of these effluents (Ramana *et al.*, 2002; Nagada *et al.*, 2006).

Table 1. Physico-chemical analysis of the two effluents.

Parameter	Textile effluent	Paper & board effluent
Color	Dark Brown	Gray
Temperature ºC	33.2	30.9
pH	7.21	6.23
Turbidity (NTU)	14.3	97
Conductivity (µS/cm)	13350	14060
DO (mg/L)	0.921	0.37
Total hardness (mg/L)	495	525
Calcium (mg/L)	262.1	266.53
TSS (mg/L)	32	69
TDS (mg/L)	10012.5	10545
Alkalinity (mg/L)	140	110
Chlorides (mg/L)	152.43	76.21
Nitrates (mg/L)	20.35	16.94
Sulphates (mg/L)	116	220
Copper (µg/L)	954.2	1527.1
Zinc (µg/L)	183.9	303.6
Lead (µg/L)	726.4	649.1
Chromium (µg/L)	1235.2	876.4
Cadmium (µg/L)	872.5	1195.9
Nickel (µg/L)	10.2	37.0

In normal conditions (0% effluent), variety NARC-2 proved to be the best variety followed by NARC-7 and NARC-5. When different concentrations of both effluents were added, almost all varieties showed improved seedling length at 10% and 20% effluent concentration of both the effluents, as compared to the control. Higher concentrations (40 & 60%) also improved the seedling lengths with few exceptions (Table 3). Maximum improvement in seedling length of varieties NARC-2, NARC-7 & Williams-82 was found in 60% of textile effluent, whereas for varieties NARC-2, NARC-5 & NARC-7, 60 % concentration of paper and board effluent was best. Many researchers documented improved seedling lengths of various crops/plants under different concentrations of textile, paper, marble, dairy and brewery effluents etc. (Orhue *et al.*, 2005; Nawaz *et al.*, 2006; Akbar *et al.*, 2007; Dhanam, 2009).

Reasons for increased seedling growth towards higher concentrations might be that the effluents contain appreciable amount of nitrates and sulphates (Table 1), which stimulate the protein production and other organic molecules such as chlorophyll, required for the growth of plants. In diluted concentrations those nutrients might not have been available in sufficient amounts to enhance the seedling growth. Another reason for such behavior might be due to the fact that heavy metals trigger the release of protective organic acids and chemical compounds specifically from the root-tips into the adjacent environment. These chemicals form complex with the toxic pollutants when released, prevent their entry into the roots and allow the essential nutrients to enter the body. Orhue *et al.* (2005) also reported enhanced growth of maize plant as well as its chlorophyll content with brewery effluent treatments.

Root lengths for all varieties increased under various concentrations of effluents as compared to control (0% effluent). The increase was some times more than two folds

(Table 4). Behavior of seedlings' roots were variable for the two effluents, for example NARC-2, NARC-7 and Williams-82 gave maximum root growth in textile effluent at 60% concentration, but the maximum increase for the same varieties were found in 40%, 20% and 10% of paper and board industry effluent, respectively. While the improvement of shoot lengths were very less as compared to roots (Table 5). Maximum increase was mostly observed at lower concentrations (10 & 20%) of textile industry effluent, while they were variable in paper and board effluent. Seedling growth in the textile mill effluent indicated that mostly the cultivars of Soybean were insensitive to higher concentrations of effluents. All cultivars showed better seedling growth at higher concentrations than in more diluted treatments. Vijayakumari (2003) also observed a reduction in various growth parameters of Soybean in various concentrations of textile dying effluent.

Seedlings grown in lower concentrations had light green and soft cotyledons and fleshy shoots. In higher concentrations, seedlings had dark green cotyledons with deep slits. Shoots and cotyledons were harder having dark brown patches. The reason for the presence of dark patches might be the 'toxic metals induce interference' with the uptake of ions that lead to deficiency of micronutrients in plants. Kochhar & Krishnamoorthy (1981) reported that excessive nitrogen inhibits the development of mechanical tissues like sclerenchyma, as a result the leaves become dark green. Morphologically the root tips of Soybean cultivars showed necrosis, which lead to root growth inhibition. This might be due to excess of minerals which cause burning and toxicity of roots. It may also be in conformity with the study of Becker (2000) that in higher plants, roots are the first organ to contact the toxic metal concentrations and root tip is a key site of injury, leading to inhibit root growth, a stunted root system and reduced yield from decreased uptake of water and nutrients. Number of leaves was unaffected and remained same in all concentrations of the two effluents. Same observation was made by Orhue *et al.* (2005).

Seedlings of PCS-62 showed a matching behavior in both the effluents for the length and weight parameters. Maximum growth was observed at 20% concentration of both the effluents and the same was observed for fresh weight & dry matter accumulation. This behavior was not observed in any other variety (Tables 6 & 7). NARC-2 seedling length increased with increase in effluents' concentrations. Maximum increase was observed in 60% concentration of both effluents, whereas there was a systematic improvement in fresh weight and a regular decrease in dry matter accumulation with the increasing concentration of both effluents for 0-60% concentrations. For NARC-5 seedlings, fresh weight decreased but dry matter accumulation increased with the increase in textile effluent concentrations. On the other hand its length parameters though improved but the level of significance was less as compared to other varieties. Same trend for the weight parameter was observed when the seeds were grown in paper and board effluent but here the growth was much better as compared to the textile effluent. Maximum improvement in NARC-7 was observed in 10 & 60% concentration of textile effluent and 60% concentration of paper and board effluent. It could also be correlated with higher weights in the same concentrations. But surprisingly dry matter accumulation was more at 0% textile effluent and 10% paper and board effluent. Seedlings of William-82 showed a gradual increase in textile effluent (maximum at 60%) but decreased in paper and board effluent except at 10% concentration and same trend was seen for fresh weights, whereas quite a mixed pattern was observed for dry matter accumulation (Table 6 & 7). Overall seedling lengths of PSC-62, NARC-2 and NARC-5 were comparatively longer in paper and board effluent as compared to textile effluent but it was other way round for the varieties NARC-7 and Williams-82, while there was no set pattern for the weight parameters.

Table 2. Effect of industrial effluents on the percentage germination of *G. max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G. max* var↓**									
PSC-62	50±5.2	50±7.0	60±0	65±7.0	65±3.5	55±0	55±3.5	75±3.5	75±3.5
NARC-2	95±3.5	85±3.5	90±7.0	95±3.5	95±3.5	100±0	100±0	100±0	90±7.0
NARC-5	60±3.5	70±7.0	95±3.5	75±3.5	55±3.5	85±3.5	80±0	80±0	85±3.5
NARC-7	95±3.5	90±0	95±3.5	85±3.5	95±3.5	95±0	90±0	80±3.5	80±3.5
Williams-82	65±3.5	90±0	75±3.5	95±3.5	90±0	75±0	75±0	90±0	95±3.5

Table 3. Effect of industrial effluents on the seedling lengths (cm) of *G. max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G. max* var↓**									
PSC-62	11.0 ± 0.4	11.6±1.5	16.42±0	11.17±0.22	11.96±1.4	11.97±0.5	18.48±0	15.15±1.23	14.05±0.89
NARC-2	13.87±0.37	14.91±0.62	15.70±0.08	15.47±1.2	17.38±0	14.26±0.4	15.77±0.65	17.74±1.1	17.98±0.51
NARC-5	13.0±0.76	13.13±0.43	13.84±0.66	10.62±0.5	10.13±0	14.33±0.85	12.07±0.2	13.66±0.7	19.3±1.7
NARC-7	13.0±0.34	15.0±1.4	12.0±0.8	12.44±0.15	16.1±0.72	12.51±0.87	13.71±0	12.64±0	14.23±1.1
Williams-82	12.4±0.69	13.3±0.87	13.78±1.4	14.07±0.35	17.95±1.4	15.13±0.45	10.77±1.2	10.77±1.2	14.31±0.9

Table 4. Effect of industrial effluents on the root lengths (cm) of *G. max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G. max* var↓**									
PSC-62	3.8±0.19	5.25±0.70	7.26±0	4.93±0.01	4.58±0.7	4.95±0.03	10.1±0	7.29±0.8	6.11±0.28
NARC-2	6.16±0.29	7.52±0.10	7.99±0.02	7.98±0.70	10.0±0	6.77±0.22	8.27±0.48	9.35±0.6	8.89±0.39
NARC-5	4.38±0.47	5.13±0.33	4.86±0.32	2.52±0.18	2.48±0	5.94±0.7	3.97±0.18	5.44±0.6	10.75±1.1
NARC-7	5.78±0.08	7.53±1.0	4.41±0.79	5.11±0.06	8.87±0.69	5.36±0.7	6.79±0.09	4.82±0	6.08±0.73
Williams-82	4.01±2.25	4.54±0.48	6.24±0.69	6.640.29	10.2±0.93	6.36±0.41	5.03±0.33	4.75±0.50	6.14±0.6

Table 5. Effect of industrial effluents on the shoot lengths (cm) of *G.max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G.max* var↓**									
PSC-62	7.13±0.21	6.35±0.84	9.16±0	6.24±0.21	7.38±0.7	7.02±0.47	8.38±0	7.86±0.43	7.94±0.61
NARC-2	7.71±0.08	7.39±0.52	7.48±0.6	7.74±0.06	7.38±0	7.49±0.18	7.5±0.17	8.39±0.52	9.09±0.12
NARC-5	8.7±0.29	8.0±0.1	8.98±0.34	8.1±0.35	7.65±0	8.39±0.15	8.1±0.02	8.22±0.1	8.55±0.6
NARC-7	7.24±0.26	7.5±0.44	7.61±0.09	7.33±0.09	7.23±0.03	7.15±0.17	6.92±0.6	7.82±0	8.15±0.37
Williams-82	8.39±0.44	8.76±0.39	7.54±0.80	7.43±0.06	7.75±0.53	8.77±0.04	7±0.73	6.02±0.79	8.17±0.3

Table 6. Effect of industrial effluents on the fresh weight (mg) of *G.max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G.max* var↓**									
PSC-62	629±18.6	679±67.3	643±0	727±44.3	864±5.31	792±51.4	687±0	670±47.8	631±44
NARC-2	533±32.5	515±33.3	634±31.9	698±12.3	701±0	547±22.3	570±22.9	672±46.4	681±48.5
NARC-5	872±94.2	796.5±32.2	750±24.4	778±29.4	631±0	732±59.2	689±43.2	631±32.6	827±46.4
NARC-7	555±14.1	712±49.0	662±16.6	626±29.7	726±51.7	623±3.9	647±31.9	678±0	718±46.8
Williams-82	632±12.2	688±19.1	699±34	719±29	665±27.3	750±76	671±22.6	669±0	682±6.38

Table 7. Effect of industrial effluents on the dry matter accumulation (mg/g) of *G.max* varieties.

Effluents from →		Textile effluent				Paper & board effluent			
Conc.→	**0%**	**10%**	**20%**	**40%**	**60%**	**10%**	**20%**	**40%**	**60%**
***G. max* var↓**									
PSC-62	144.6	115.7	164.8	135.4	115.0	109.7	243.0	193.8	105.3
NARC-2	230.9	179.6	166.9	165.6	152.6	175.3	178.0	175.4	164.3
NARC-5	129.6	156.9	158.5	141.2	177.4	149.4	159.6	190.9	103.9
NARC-7	169.3	145.9	147.9	142.1	137.0	191.6	182.8	132.7	167.1
Williams-82	161.3	156.9	173.8	157.7	165.4	141.5	97.6	191.3	171.5

Present study revealed that industrial effluents may be used after appropriate dilutions, which may enhance various parameters of *G. max* growth. Response of various varieties was dilution/effluent type dependent. Use of these effluents in agriculture may be a step towards water shortage solution. However further work is required to understand the long-term effects of these effluents on various biochemical parameters of Soybean.

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