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EFFECT OF TEXTILE INDUSTRIAL WASTE WATER ON GROWTH, PROTEINS, LIPID PEROXIDATION AND PROLINE IN MUNG BEAN SEEDLINGS

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ABSTRACT

Industrial waste water and sewage water is extensively used to irrigate various crops in Karachi. The research was conducted to evaluate the effect of textile industrial waste water, on morphological and biochemical activity of mung bean seedlings as it is composed of various heavy metals. Mung bean genotype 'Ramzan' was used as a test material. Seeds were imbibed in deionized water for 12 h, germinated in Petri dishes for 24 h in deionized water. Treatment of various concentrations of textile industrial waste water (10, 50 and 100%) along with control (0%), was given till 72 h. A decrease in seedling length was observed in treated samples as compared to control with more decrease as the concentration of waste water was raised. The amount of soluble proteins was non-significantly increased in low concentrations (10 and 50%) as compared to control. However at high concentration (100%), there was significant reduction in mean protein content. Various abiotic stresses like metal stress is able to cause lipid peroxidation which can be detected by Malandialdehyde (MDA) content. We found that there was more accumulation of MDA as well of proline in treated samples in concentration dependent manner.

Key words: mung bean, industrial waste water, proline, MDA, metal stress, lipid peroxidation

INTRODUCTION

Mung bean (*Vigna radiata* L.) has been grown and consumed in Africa, South America, Australia and Asia including Pakistan where it is considered as a cheapest source of protein. Human activities such as industrial production, mining, agriculture and transportation release high amounts of heavy metals into surface, ground water, soils and ultimately become harmful for plant as well as animals and human life. In Karachi, untreated sewage water and the industrial effluents are discharged directly into water channels or canals and the polluted water is used for growing crops particularly vegetables and fodder (Khan *et al*.,2003). Ingestion of vegetables irrigated with waste water and grown in soils contaminated with heavy metals poses a possible risk to human health and wildlife (Otero *et al*., 2000; Mussarat *et al*., 2007). Textile industrial waste water contains heavy metal dyes, high amount of sulphuric acid, sodium hydro sulphide, sodium hydro oxide, silicon contents, calcium hypochloride and high amount of Cd, Fe, Zn, Pb and Ni contents. Certain heavy metals like, Cd, Pb and Ni are not essential for plant growth, but they are taken up and accumulated by plants to toxic levels (Qadir *et al*., 1999; Bhatti and Perveen, 2005; Mussarat *et al*., 2007), when irrigated with contaminated water.

Metabolic activities are disturbed when plants are under metal stress. Reactive oxygen species (ROS) are synthesized constantly during growth as normal product of plant metabolism, however various environmental stresses exceed the synthesis of ROS (Sharma *et al*., 2012), leads to peroxidation of membrane lipids. Product of lipid peroxidation is MDA, which can cross link to protein and enzymes and make them inactive. Proline is an essential amino acid and is accumulated during various abiotic stresses in plants, considered as a tolerance mechanism, as it is suggested to act as an osmolyte and component of cell wall (Matysik *et al.*, 2002), and scavenging of free radicals, compatible solute as well as a source of nitrogen during recovery from stress.

MATERIALS AND METHODS

Seeds of mung bean genotype '*Ramzan'* were obtained from Nuclear Institute for Food and Agriculture (NIFA) Peshawer, Pakistan. Mung bean seeds were imbibed for 12 h in deionized water, sterilized with 1% sodium hypochlorite solution for 5 min and rinsed several times with deionized water. Seeds were sown in Petri dishes at room temperature (30°C ± 2°C), treated with 3 mL of various concentrations (10%, 50%, 100%) of textile waste water per Petri dish, along with control (0%). Seedling length at 48 h after treatment was recorded. Seedlings of 96 h after treatment were harvested and stored at 4°C for the estimation of proteins, malandialdehyde (MDA) content and proline.

Extraction of protein and MDA

Mung bean seedlings (0.2 gm) were homogenized in 1 mL of 5% w/v Trichloroacetic acid (TCA), centrifuged at 10,000 rpm for 30 min and supernatant was saved for the estimation of protein and MDA content. Protein was estimated by the method of Lowry *et al*., (1951). Absorbance was taken at 750 nm against the reagent blank.

Estimation of lipid peroxidation

Lipid peroxidation was estimated by measuring MDA content using the method of Heath and Pecker (1968) with minor modifications. Reaction mixture contain 500 µL supernatant and 2 mL of 0.5% Thiobarbituric acid (TBA) in 20% TCA, incubated in water bath at 95°C for 25 min. The reaction was stopped by placing tubes quickly in ice chilled water following centrifugation at 2000 rpm for 10 min. Supernatant was assayed for MDA content by taking absorbance at 532 and nonspecific absorbance at 600 nm. Lipid peroxidation was calculated by subtracting the absorption value at 600 nm from 532 nm. MDA concentration was calculated using an extinction co-efficient (155 $mM^{-1}cm^{-1}$).

Estimation of proline

Proline was estimated by the method of Bates *et al.* (1973). Seedlings were homogenized in 3% sulphosalysalic acid and filtered through whatman No 1 filter paper. Reaction mixture composed of 2 mL supernatant, 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent (1.25 gm ninhydrin, 30 mL glacial acetic acid and 20 mL of 6N orthophospheric acid). Reaction mixture was placed in a boiling water bath at 100°C for 1h, immediately cooled in ice chilled water. Mixtures were extracted by adding 4 mL of toluene. After through mixing the brick red coloured toluene was separated and its absorbance was taken at 520 nm against toluene blank using Shumadzu UV spectrophotometer (Model UV 1601). Proline concentration was measured by using proline standard curve.

Statistical analysis

Experiment was performed in complete randomized design (CRD) with three replicates (n=3). Analysis of variance was performed by a computer program SPSS version 11. For mean comparison, DMR was performed at $P<0.05$ level of significance using Steel and Torrie (1980).

RESULTS

Seeds were treated with different concentrations of textile industrial waste water in order to observe the adverse effect of waste water on seedling length. Table 1 showed mean square values for seedling length, Protein, MDA and Proline, harvested after various concentrations of industrial waste water, possessed significant difference.

Mean seedling length was shown in Table 2. Results showed that seedling length was non significantly reduced ($P<0.05$) at 10 and 50% concentration of waste water but reduced significantly at undiluted (100%) waste water. It was inhibited by 6.35, 15.6, 40.7% respectively (Table 3).

Table 2 showed that the mean protein content was 94.66 µg protein/gm fwt in untreated sample (control), which increased non significantly to 99 µg protein/gm fwt (4.57% promotion, Table 3) at low concentration (10%) of waste water, then decreased to 97 µg protein/gm fwt at 50% concentration which was still higher than in control. It was later noted that there was significant reduction (80 µg protein/gm fwt) in mean protein content at 100% concentration. It showed that low concentration of textile industrial waste water induce protein synthesis but caused negative effect on proteins when the waste water was undiluted (100%) ,which showed 14.7% inhibition (Table 3).

Our results revealed that mean MDA content was 156.1 µmol/mg fwt in contol, which increased non significantly ($P<0.05$) at 10% and 50% concentration and significantly at 100% concentration of waste water (Table 2). It is suggested that the reduction in seedling length in treated samples could be due to more lipid peroxidation as indicated by more accumulation of MDA, represented by 0.89, 1.66 and 3.63% promotion respectively (Table 3).

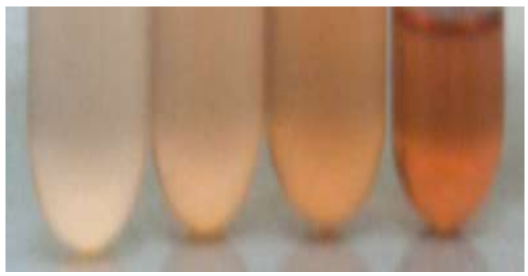
Fig 1 exhibited that there was more accumulation of proline as the concentration of waste water was increasing, which was estimated by the formation of brick red coloured praline ninhydrin complex in acidic medium. Table 2 represented mean proline in seedlings treated with 0% (control), 10, 50 and 100% concentrations of waste water. It showed significantly highest accumulation (20.33 µmol proline/gm Fwt), with 56.41% promotion (Table 3) at 100% waste water and lowest (13.00 µmol proline/gm Fwt) at 0% concentration (control).

DISCUSSION

Due to high cost of fertilizers, poor farmers prefer to use sewage water or industrial waste water for the irrigation of their crops however the use of industrial waste water is harmful for plants as well as humans. It can bring various morphological and biochemical disturbances in any crop, and also contaminate soil with heavy metals as it contains high concentration of different heavy metals (Naaz and Panday, 2010). These heavy metals are required by plants in trace amounts but the continuous use of waste water is responsible to accumulate these heavy metals in plants at high levels, which in turn become toxic and harmful for human as well as animals. Present research is an attempt to understand the toxic effect of textile industrial waste water using bioassay system of mung bean. Textile industrial waste water exposure led to the non significant retardation in mean seedling length in the concentration dependent manner which became significant only at 100% concentration. Hussain *et al.,* (2010), found the retardation of sunflower seedling length, treated with five concentration of tannery effluent. Khan *et al.*, (2011), also reported that the tomato biomass was significantly affected by different treatments of waste water. Similarly Sharma *et al.*, (2010) observed the decrease in radical and hypocotyl length of *Cicer arietinum L* at 75 and 100 mM solution of Zn.

Table 1. Mean sum of square for seedling length, protein, MDA and proline in mung bean after textile industrial waste water treatments.

Sources of Variations	df	MS			
		Seedling length (cm)	Protein (µg/gm Fwt)	MDA (µmol/mg rotein)	Proline (µmol/gm Fwt)
Between Treatment	3	11.625*	206.178*	17.470*	30.528*
Within Treatment	8				



0% 10% 50% 100%

Fig.1. Estimation of proline in mung bean seedlings treated with different concentration of textile industrial waste water, shown by the production of brick red coloured ninhydrin complex.

Table 2. Mean comparison of seedling length, protein, MDA and proline in mung bean seedlings after treatment with textile industrial waste water.

Conc. of Waste water	Seedling length(cm)	Protein (µg/gm Fwt)	MDA (µmol/mg protein)	Proline (µmol/gm Fwt)
cont	7.766±1.718a	94.666±6.565a	156.100±1.5561b	13.000±1.527b
10%	7.273±1.681a	99.000±1.00a	157.500±1.300ab	14.333±1.201ab
50%	6.551±1.492a	97.000±1.00a	158.700±1.320ab	16.000±1.527ab
100%	4.598±0.900b	80.666±0.666b	161.766±1.442a	20.333±3.282a

Table 3. Percent promotion/ inhibition in mean seedling length, proteins, MDA and proline in mung bean treated with various concentration of textile industrial waste water

Conc. of waste water (%)	% promotion / inhibition			
	Seedling Length	Proteins	MDA	Proline
10	-6.35	4.57	0.89	10.25
50	-15.6	2.46	1.66	23.07
100	-40.7	-14.7	3.63	56.41

There was an increase in soluble proteins at 10 and 50% concentration of waste water which could be due to the triggering of stress proteins including some heat shock proteins or antioxidant enzymes Wollgieh and Newmann (1995) and Namjooyan *et al.*, (2012) reported induction of heat shock proteins in tomato cultivar and safflower callus under metal stress, which may have a role in the adaptive response. However our results showed that soluble proteins decreased when plants were exposed to high concentration (100%) of textile industrial waste water.

Doganlar *et al.,* (2012), found that the Mn and Ni first induced soluble proteins then reduced in *Lemna gibba* by increasing concentration. *Lemna minor* showed decrease in protein content when treated with industrial waste water (Singh and Singh 2006)

Reactive oxygen species (ROS) are generated as a result of various abiotic stress like heavy metals, and caused damage to lipid membranes which can be observed by the synthesis of MDA. Increase in the synthesis of ROS will enhance the degree of lipid peroxidation. Our results revealed that the exposure of textile industrial waste water lead to increase lipid peroxidation as indicated by MDA content. These results are in agreement with the findings of Hatata and Abdel-Aal (2008), who reported that Cd concentration and time of exposure showed positive effect of MDA on sunflower. Singh and Agarwal (2010) also found the increase in MDA content when *Beta vulgaris* was treated with municipal waste water

We observed accumulation of proline under stress in concentration dependent manner Accumulation of proline has been reported during number of stresses, Mohammed (2007) reported the accumulation of proline in salt stressed mung bean, and Nayek *et al.,* (2010) found that heavy metal stress by irrigating vegitables with industrial waste water possessed higher amount of proline. It is suggested that proline protects proteins during various abiotic stresses. Under the stress condition many plant species accumulate proline as an adaptive response to adverse conditions (Verbruggen and Hermans, 2008) and the increase in proline content following stress injury is beneficial for the plant cell (Hare and Cress, 1997).

It is concluded that mung bean genotype 'Ramzan' seemed tolerant against industrial waste water, as the reduction in seedling length was not considerable and there was increase in protein at low (10 and 50%) concentrations. However the length was retarded significantly only at high (100%) concentration with reduced protein synthesis. Similarly degree of lipid peroxidation and proline accumulation was also non significant at low concentrations of waste water, which became significant only at 100% concentration.

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