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Morpho-Biochemical Evaluation of Mung Bean under Textile Industrial Wastewater Stress and Alleviation of Stress by Exogenous Application of Calcium

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Abstract

The study was conducted to evaluate the effect of textile industrial waste water on morpho-biochemical properties of four genotypes of mung bean. The aim of this study was, to analyses the adverse effect of textile waste water irrigation on crops. Seeds were obtained from National Agricultural Research Center (NARC), Pakistan. Genotype Ramzan and NM 51 were released varieties where as NM-5-63-19 and NFM-12-7 were advanced lines. Seeds were grown in textile industrial wastewater with and without 10 mM $CaCl_2$. Results showed calcium acted as stress inhibitor on seedlings length and some biochemical parameters. Textile wastewater treatment without calcium adversely affect germination, seedling length, fresh weight, with accumulation of MDA, Proline and Tannin. However when $CaCl_2$ was given along with textile wastewater, MDA and Proline accumulation was decreased. Genotypic variations with respect to antioxidant enzymes were also observed for textile industrial wastewater treatment with and without $CaCl_2$.

Genotype Ramzan showed increased ascorbate activity while others exhibited decreased ascorbate activity in presence of calcium, so these results showed some varietals variation. There was promotion in antioxidant enzymes under textile waste stress. It is concluded that by the application of calcium one can reduce the effect of textile industrial wastewater, as it is frequently used to irrigate various crops in developing countries.

Keywords: Mung bean, Textile industrial wastewater, Malondialdehyde, Antioxidant enzymes, Ascorbate peroxidase, Catalase, Guaiacol peroxidase, Tannin, Proline, Calcium.

1. Introduction

Mung bean (*Vigna radiate L*) has been grown and consumed in Africa, Australia and Asia including Pakistan where considered as a cheapest source of proteins (Weinberger, 2003). Water pollution by toxic chemicals present in industrial waste effluents is a world wide problem now a days. Industrial wastewater is commonly used for irrigating agricultural fields in developing countries (Sharma *et al*., 2007), which increases the amount of macro and micronutrients including heavy metals and these under high concentrations are harmful for plants (Kocak *et al.*, 2005). Plant exposure to micropollutants may occur naturally or results directly from contaminated industrial discharges or from the discharge of sewage effluents and sewage sludges. The chemical reagents used in textile industries are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products. The presence of dyes and heavy metals in effluent could be toxic.

Unfavourable environmental conditions such as temperature extremes, heavy metals, drought, water availability, or salt stress can alter physiological and metabolic activities of plants by producing reactive oxygen species (ROS) (Azevedo *et al.,* 2006), which causes lipid peroxidation and plant cell damage (Gajewska *et al*., 2006). High level of ROS can cause oxidative damage to the biomolecules leading to cell membrane peroxidation, loss of ions, protein hydrolysis and DNA strand break (Shu *et al.,* 2011). Plant defense to environmental stresses like metal toxicity is the development of antioxidative enzyme system in various cell organelles (Cakmak and Horst, 1991). Enzymatic antioxidants include Superoxide dismutase (SOD), Caltalase (CAT), Ascorbate peroxidase (APX), Guaiacol peroxidase (GPX), Glutathione reductase (GR).

Calcium is an essential plant macro-nutrient which is taken up by roots and delivered to shoot via xylem to regulate many physiological and metabolic processes (Tuteja and Mahajan, 2007). Various abiotic stresses like salt, heat, metal, and water can induce changes in calcium (Takano *et al.*, 1997). It is suggested that calcium play important role in providing stress tolerance in plants (Tuleja and Sopory, 2008).

2. Materials and Methods

2.1. Test System

Seeds of mung bean genotypes ‘Ramzan, NM-5-63-19, NM-51 and NFM-12-7 were obtained from National Agricultural Research Center (NARC), Pakistan and used as test system.

2.2. Test Material

Undiluted textile industrial wastewater was collected from a well known Textile industry of Karachi, Pakistan and used as test material.

2.3. Growth Condition and Treatments

Mung bean seeds were imbibed for 19h 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 for 24h at room temperature, treated with 3 mL of textile wastewater alone (W.W) or along with 10 mM $CaCl_2$ (W.Ca) and deioniozed water will serve as control (Cont).

2.4. Morphological Parameters

Seedling length, fresh weight, dry weight and moisture content at 48h were recorded. Moisture content (M.C) was measured by;

M.C % = fresh weight – dry weight/ fresh weight × 100

2.5. Biochemical Parameters

Seedlings were also harvested at 48h and saved at 4°C for biochemical analysis that include total proteins (Lowry et al., 1951) malandialdehyde content (Heath and Packer, 1968), antioxidant enzymes, Proline and Tanins.

Extraction of antioxidant enzymes was done by taking 0.2 g seedlings and homogenized in 4 mL of 50 mM phosphate buffer (pH 7.0) containing 1% w/v PVP (polyvinyl pyrroliodine) and 0.2 mM ascorbic acid. The homogenate was centrifuged at 10,000 rpm for 30 min, supernatant was used for antioxidant enzyme activity assays (Jiang and Bingru, 2001).

Ascorbate Peroxidase (APX) activity was determined according to Nakano and Asada (1981) by measuring a decrease in the absorbance of the oxidized ascorbate at 290 nm against the reagent blank using extinction co-efficient of 28.0$mM^{-1}cm^{-1}$.One unit of APX was defined as the amount of of enzyme required to consume 1 µmol ascorbate/min/mg protein. Guaiacol Peroxidase (GPX) activity assay was based on the method of Evers *et al.,* (1994). The oxidation of Guaiacol into Tetraguaiacol was estimated by measuring the absorbance at 470nm against the reagent blank using extinction co-efficient of 26.6 mM^{-1} cm^{-1}. Calatase (CAT) activity was estimated on the method of Aebi (1984). The oxidation of Guaiacol into Tetraguaiacol was estimated by measuring the absorbance at 470nm against the reagent blank using extinction co-efficient of 26.6 mM^{-1} cm^{-1}. Proline was estimated by spectrophotometric analysis at 520 nm against toluene as blank of the ninhydrin reaction, according to

Bates (1973) method. Tannin content was estimated by Folin's Denis method with few modification. 0.2 g of seedlings homogenized in 4 mL of autoclave deionized water, homogenate filtered through Whatman No.1 filter paper and collected filterate. 100 µL of extract was taken in the reaction mixture, add 500 µL of folin's denis reagent, 1 mL of 35% freshly prepared sodium carbonate solution and raised with 7.5 mL of deionized water, reaction mixture incubated at room temperature for 30 minutes, deep blue colour developed, its absorbance was read at 750 nm in spectrophotometer against blank sample and proline.

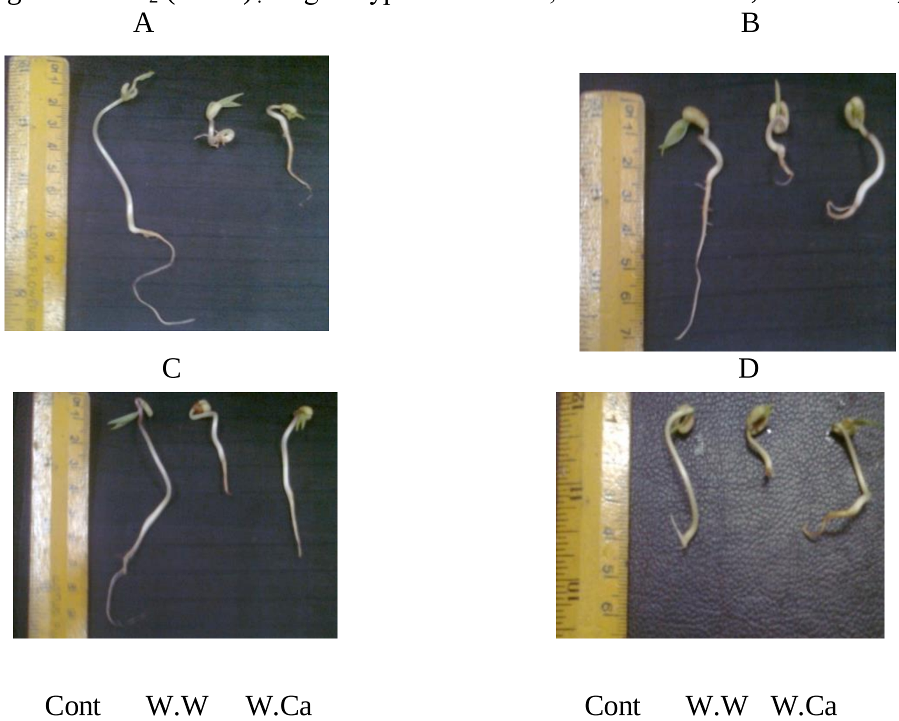
2.6. Statistical Analysis

Experiment was conducted in RCBD as factorial with three replications. Analysis of variance was performed by using computer software SPSS 11 for all morphological and biochemical parameters. DMRT was performed by using the method of Steel and Torrie (1980).

3. Results

3.1. Morphlogical Parameters

Fig-1. Seedlings length (cm), treated with deionized water (cont), Wastewater (W.W) and Wastewater along with $CaCl_2$ (W.Ca) . A=genotype "Ramzan" ; B=NM-5-63-19; C= NM-51; D= NFM-12-7



Analysis of variance for seedling length of four mung bean genotypes was presented in Table 1. There was significant difference for genotypes, treatments as well as interaction represented that genotypes and treatments were dependent upon each other far as seedling length is concerned. Table 3 and Figure 1 represents comparisons for seedling length at all three treatments of four genotypes of mung bean, demonstrated that wastewater without $CaCl_2$ (W.W) significantly reduced seedlings length for all four genotype, however the application of $CaCl_2$ during treatment of wastewater (W.Ca), seedling length was improved significantly as compared to (W.W) but not more than in control for all genotypes. Percent promotion/inhibition of seedlings length graphically represented in Figure 2. These results showed that calcium reduces the stress of wastewater.

Analysis of variance exhibit significant difference for genotypes but non significant differences for treatment and interaction, indicating that genotypes and treatments were independent upon each other as far as fresh weight was concerned (Table 1). There was reduction in fresh weight during wastewater stress which increased non significantly in the presence of calcium for all genotypes, except for genotype Ramzan where there was non significant reduction (19.6% inhibition) in fresh

Table-1. Mean sum of square for Morphological parameters of four mung bean genotypes, harvested 48 hr after textile industrial waste water, with and without Calcium

Sources of Variations	MS			
	Seedling length Content	Fresh Weight	Dry Weight	Moisture
Genotypes(G)	26.431*	6.649 $^{N.S}$	2.584*	9.980 $^{N.S}$
Treatments (T)	63.641*	4.410$^{N.S}$	3.191$^{N.S}$	9.771 $^{N.S}$
GXT	2.707* 27.477$^{N.S}$	5.811$^{N.S}$	1.866$^{N.S}$	
Error	0.634	8.51	1.942	18.70

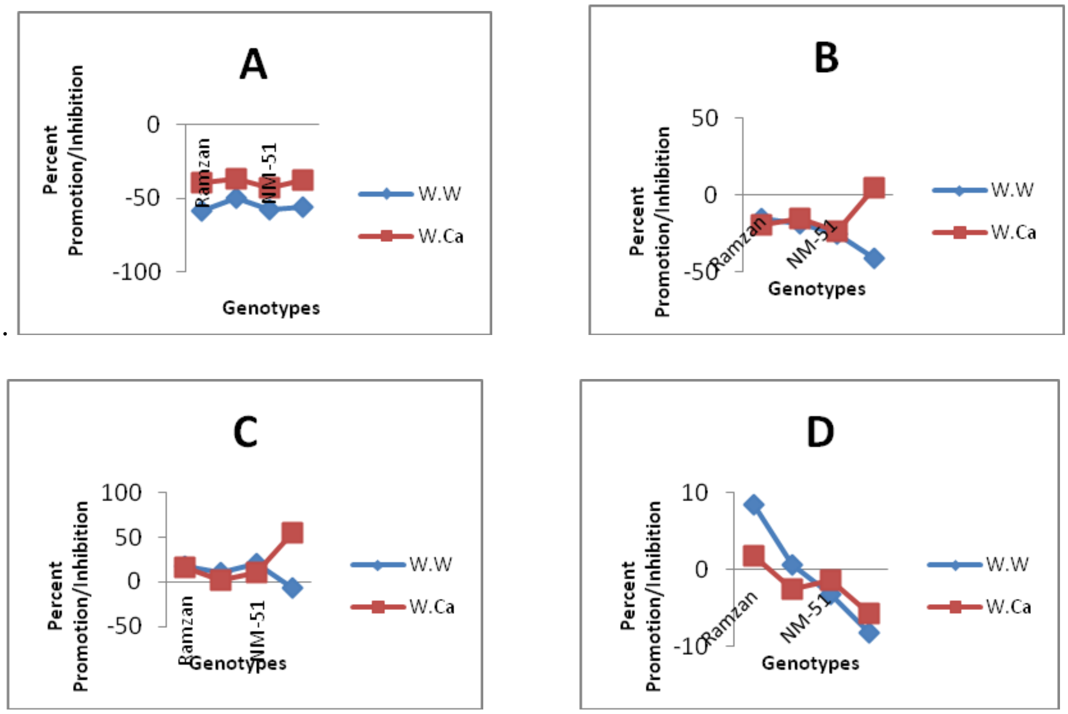
weight for W.Ca as compared to W.W (15.6% inhibition) explained in figure 2. Whereas for genotype NFM-12-7 there was 4.7% promotion in fresh weight when treated with W.Ca. These results showed that Calcium may act as good stress inhibitor for genotype NFM-12-7, but there was non significant effect on genotypes NM-5-63-19 and NM-51, but calcium showed non significant negative effect on fresh weight of genotype Ramzan. The pattern of percentage inhibition observed was same almost in all genotypes of mung beans with the exception of genotype NFM-12-7, which showed 4.7% promotion in the presence of calcium.

Analysis of dry weight of four genotypes of mung bean, treated with textile industrial wastewater alone and with calcium explained that all four genotypes harvested at 48h were significantly different from each other, While the treatments and interactions imposed non significant difference on dry weight (Table 1). It was observed that dry weight of all the genotype increased in waterwater, with the exception of genotype NFM-12-7 dry weight that showed 7.14% inhibition and recorded highest promotion (54.76%) in wastewater with calcium (Figure 2).

Analysis of variance revealed non significant differences for genotypes, treatments as well as interaction between genotypes and treatments as far as moisture content is concerned (Table 1). Non significant interaction indicated that genotypes and treatments were independent on each other as far as moisture content was concerned. There was 8.4% promotion in moisture in genotype Ramzan at wastewater treatment content in different treatments, there was 8.4% promotion in proteins c which was reduced to 1.69% at treatment with calcium. Genotype NM-5-63-19 showed promotion of 0.53% in moisture content when treated with wastewater treatment, however reduced to 2.6% when calcium was added in the wastewater treatment (Figure 2). Remaining two genotypes exhibited inhibitory effects on mean moisture content, however less inhibition was seen when calcium was added in the treatment

Total proteins content was measured in seedlings of all four mung bean genotypes, harvested after wastewater treatment with and without calcium. Table 2 demonstrated mean squares values of total proteins content, where differences were significant for genotypes, treatments as well as interaction. Mean comparisons for total proteins between treatments was performed by Duncan's multiple range test (Table 3). Varietal variations were seen for mean protein content for different treatments. It was detected that total protein content was significantly increased to (52.66 µg g^{-1} fwt) in wastewater treated sample of genotype 'Ramzan' which reduced significantly to 30.0 µg g^{-1} fwt in the presence of calcium. But genotype NM-5-63-19 showed significant induction (33.33µg g^{-1} fwt) in protein content under wastewater stress which increased further (46.66 µg g^{-1} fwt) when calcium was added in the wastewater sample. NM-5-63-19 showed more percent promotion (125.84%) in mean protein content in the calcium treated sample while NM-51 showed 23.08% inhibition and NFM-12-7 exhibited 51.7% inhibition (Table 4)

Figure-2. Percent promotion/inhibition on mean seedling length (A), mean fresh weight (B), mean dry weight (C), and mean moisture content (D) of four mung bean genotypes treated with wastewater (W.W) and wastewater with calcium (W.Ca)



3.2. Biochemical Parameters

Table-2. Mean sum of square for Biochemical parameters of four mung bean genotypes, harvested 48 hr after textile industrial waste water, with and without Calcium

Sources of Variations	MS						
	Total Protein	MDA	APX	GPX	CAT	Proline	Tannin
Genotypes (G)	590.85*	538713.66*	2834.326*	1.377.985*	95.731*	7.435*	2.824$^{N.S}$
Treatments (T)	451.194*	1086715.15*	1700.575*	1972.161*	147.013*	39.361*	21.5*
G x T	378.380*	200167.77*	633.312*	398.167*	18.459*	0.213$^{N.S}$	1.573$^{N.S}$
Error	6.604	3462.38	4.364	2.361	0.859	0.604	1.152

Lipid peroxidation as MDA content was measured in seedlings of all four genotypes of mung bean, harvested after textile wastewater treatment with and without calcium. Mean squares values of MDA content in all four genotypes possessed significant difference for genotypes, treatments as well as interaction (Table 2). It was seen that mean MDA content (µmol MDA mg^{-1} protein) was lowest in control samples in all genotypes, and a significant increase was observed in wastewater treated treated sample, however by the application of calcium in the wastewater sample significantly reduced lipid peroxidation in all genotypes except in NM-51 (Table 3). There was 59.08% promotion in MDA content in wastewater sample and 14.94% promotion was recorded in calcium treated sample of genotype Ramzan, and in genotype NM-51 60.80% promotion recorded in wastewater and 81.87% promotion in calcium treatment. But the results observed

Table-3. Mean comparisons of seedling length and biochemical parameters of seedlings of four mung bean genotypes, treated with wastewater alone and along with Calcium

Genotypes	Treatment	Seedling length	Total Proteins	MDA content	APX	GPX	CAT	Proline	Tanin
Ramzan	Cont	9.50±0.93^a	20.66±0.66^c	73.13±13.45^c	16.90±0.04^c	12.47±1.07^c	5.60±0.32^b	7.33±0.33^c	0.33±0.10^c
	W.W	3.92±0.37^b	52.66±1.45^a	116.34±39.34^a	35.16±2.11^b	27.73±0.77^a	16.66±0.88^a	11.00±0.00^a	0.43±0.25^b
	W.Ca	5.72±0.67^b	30.00±1.73^b	84.08±21.25^b	44.80±1.82^a	14.34±1.28^b	4.28±0.21^b	9.00±0.57^b	0.58±0.01^a
NM-5-63-19	Cont	7.45±0.46^a	20.66±0.66^c	106.26±17.52^c	40.38±0.60^b	1.14±0.15^c	1.74±0.26^b	5.66±0.33^c	0.31±0.00^b
	W.W	3.71±0.46^b	33.33±2.40^b	211.82±64.28^a	79.15±0.32^a	8.153±0.19^a	4.08±0.46^a	9.66±0.33^a	0.366±0.03^b
	W. Ca	4.71±0.60^b	46.66±0.66^a	116.02±24.02^b	24.11±0.82^c	5.79±0.55^b	0.79±1.88^c	7.33±0.66^b	0.57±0.01^a
NM-51	Cont	10.82±0.12^a	17.33±1.45^b	85.5±20.23^c	63.14±1.05^b	6.68±0.88^c	4.31±0.21^b	6.00±0.57^c	0.27±0.03^c
	W.W	4.59±0.56^c	25.00±1.52^a	137.52±48.17^b	85.01±1.72^a	60.20±1.38^a	10.76±0.46^a	9.66±0.33^a	0.40±0.01^b
	W.Ca	6.16±0.28^b	13.33±0.88^c	155.59±18.74^a	65.48±1.44^b	38.21±1.62^b	0.38±1.17^c	7.00±0.57^b	0.55±0.00^a
NFM-12-7	Cont	4.42±0.16^a	29.66±0.88^a	81.72±35.73^b	36.00±0.19^b	15.430±0.84^b	0.60±0.19^b	5.66±0.33^c	0.28±1.10^c
	W. W	1.92±0.15^c	26.00±22.30^b	121.20±29.70^a	42.05±0.65^a	40.33±0.89^a	4.23±0.21^a	8.66±0.33^a	0.43±0.01^b
	W.Ca	3.23±0.38^b	14.33±1.45^c	97.39±16.644^b	27.22±1.07^c	11.14±0.37^c	0.60±0.19^b	6.66±0.33^b	0.55±0.00^a

Similar superscript letters for three treatments of each genotype, are representing homogenous means at P<0.05

in genotype NM-5-63-19 showed 0.17% inhibition in presence of calcium, and 99.34% promotion in wastewater treated sample. The decrease in concentration of ascorbate oxidized was recorded at 290 nm at 2 minutes from initial rate of reaction using extinction coefficient of ascorbate (2.8 mM^{-1} Cm^{-1}). Table 2 explained mean sum of square for ascorbate peroxidation for all four genotypes treated with textile industrial wastewater with and without calcium, found significant differences for genotypes, treatments and interaction. Duncan's multiple range test was performed and showed that APX was increased in wastewater sample which reduced in the presence of calcium in all genotypes except 'Ramzan'(Table 3). There was 108.04% promotion of APX activity in wastewater treated sample of genotype Ramzan which showed further promotion of 165.08% (Table 4). In NM-5-63-19 there was 96.01% promotion for wastewater sample but 40.29% inhibition for calcium added sample. In case of NM-51, amount of APX was promoted to 34.63% for wastewater sample while showed 3.65% promotion in sample treated with wastewater and calcium. NFM-12-7 demonstrated 16.80% promotion in wastewater sample whereas 24.3% inhibition in presence of calcium.

GPX activity was recorded at 470 nm using extinction coefficient of 26.6mM^{-1} Cm^{-1}. Analysis of variance represented significant differences for genotype, treatments and interaction (Table 2). Mean comparisons were made by Duncan's multiple range test (Table 3). Wastewater showed significant increased in GPX activity while its activity was decreased when calcium was added in wastewater sample of all genotypes. Table 4 demonstrated that in genotype Ramzan there was 122.32% promotion in wastewater sample and 14.99% for calcium treated sample (Table 4). In NM-5-63-19 there was 613.29% promotion for wastewater and 406.56% promotion for wastewater along with calcium. In case of NFM-12-7 wastewater exhibited 161.37% promotion in GPX activity while 27.80% inhibition presence of calcium in wastewater sample.

Catalase (CAT) activity was recorded at 240 nm at 2 min using extinction coefficient of 40.0mM^{-1} Cm^{-1}. ANOVA for catalase (Table 2) represented significant differences for genotype, treatments and interaction. Mean comparison was made by Duncan's multiple range test for catalase activity (Table 3). It showed that wastewater significantly enhanced activity of catalase while presence of calcium significantly inhibited CAT activity for all genotypes. In Ramzan, catalase activity was

16.66±0.881 µg H_2O_2/mg protein/2min for wastewater and 4.287±0.211 µg H_2O_2/mg protein/2min for wastewater with calcium. In case of genotype NM-5-63-19 the activity of catalase was estimated as 4.083±0.463 µg H_2O_2/mg protein/2min in wastewater sample. For genotype NM-51, activity of CAT was 10.76±0.46 µg H_2O_2/mg protein/2min for wastewater, which reduced significantly to 0.383±1.17 µg H_2O_2/mg protein/2min for wastewater with calcium. In NFM-12-7 showed no change in catalase activity in wastewater sample and control (0.606±0.192) was seen. For genotype Ramzan, wastewater caused 197.18% promotion in catalase activity and 23.52% inhibition of CAT activity in the presence of calcium (Table 4). Similarly wastewater exhibited 133.98% promotion in NM-5-63-19 and 54.55% inhibition for wastewater with calcium. In case of NM-51, catalase activity was promoted with 149.65% by wastewater treatment but inhibited to 91.11% when calcium was added in wastewater. NFM-12-7 demonstrated that wastewater caused 599.0% promotion in CAT activity.

Proline as proline ninhydrin complex was measured in seedlings of all four genotypes of mung bean, harvested after textile wastewater treatment alone and along with Calcium. Table 2 represented mean squares values of proline content. It showed that the differences were significant for genotypes and treatments, while interaction was non significantly different. Mean comparisons for proline content was explained by Table 3, showed that proline content was lowest in control samples in all genotypes, and a significant increase was observed in wastewater samples, where as calcium in wastewater reduced proline content as compared to wastewater sample but still more than in control. Table 4 demonstrated that there was 50.60% promotion in proline content in wastewater sample and 16.75% promotion in genotype Ramzan. Similarly genotype NM-51 showed 61.00% promotion in wastewater sample and 16.66% promotion in treatment of wastewater along with calcium.

Table 2 showed ANOVA for Tannin, measured from seedlings of all four genotypes of mung bean, harvested after wastewater treatment alone and along with calcium. Differences were significant for treatments, while genotypes and interaction were non significant difference. Non significant interaction indicated that genotypes and treatments were independent on each other. Mean comparisons between treatments for tannins was performed by Duncan's multiple range test (Table 3). It was seen that tannin content was lowest in control samples in all genotypes, and significant increase was observed in treated samples. Hierarchy for mean tannin for all genotypes was W.W>W.Ca>Cont. The percentage promotion/inhibition of Tannin showed 32.42% promotion in tannin content in wastewater treated sample and 76.66% promotion in wastewater with calcium for genotype Ramzan. Similarly in genotype NM-51, wastewater caused 45.45% promotion and wastewater with calcium exhibited 101.45% promotion. Genotype NM-5-63-19 showed 82.69% promotion in presence of calcium and 17.30% promotion in wastewater sample. NFM-12-7 showed 54.41% promotion by the treatment of wastewater and 97.17% promotion in case of calcium treatment.

Table-4. Percent promotion /inhibition of biochemical p arameters of four mung bean genotypes harvested 48 hr after textile industrial waste water, with and without Calcium

Genotypes	Total Proteins	MDA	APX	GPX	CAT	Proline	Tannin
Ramzan							
W.W	154.88	59.06	108.04	122.32	197.18	50.60	32.42
W. Ca	59.72	14.97	165.08	14.99	-23.52	16.75	76.66
NM-5-63-19							
W.W	61.32	99.37	96.01	613.29	133.98	70.67	17.30
W. Ca	125.84	-0.17	-40.29	406.56	-54.55	29.50	82.69
NM-51							
W.W	44.25	60.80	34.63	801.19	149.65	61.00	45.45
W.Ca	-23.08	81.87	3.65	472.00	-91.11	16.66	101.45
NFM-12-7							
W.W	-12.33	48.34	16.80	161.37	599.00	53.00	54.41
W. Ca	-51.70	69.39	-24.30	-27.80	0.00	17.66	97.17

4. Discussion

During current work, the effect of textile industrial wastewater on 48 h harvested mung bean seedlings was detected. Textile wastewater decreased seedling length as compared to control. Decrease in the growth and biomass of *Cicer arietium* was observed by distillery effluent, reported by Srivastava and Sahai (1987). These findings were supported by Khan *et al*., (2011), reported that the tomato biomass was significantly affected by different treatments of wastewater. The heavy metals contaminated soil are usually lack of long term vegetation (Xia 2004; Rotkittikhum *et al.*, 2007) due to adverse soil properties and lack of nutrients and toxicity from residual heavy metals (Bradshaw 1997). Current results also exhibited that by the addition of calcium in wastewater significantly improved growth of mung bean seedlings as compared to wastewater alone. This finding is supported by the work of Danh *et al.,(*2011), who observed growth and survival of plants cultivated on heavy metals contaminated soils had be improved by soil amendments of $CaCO_3$. Wastewater may contain various heavy metals which can reduce number of nutrients thus disturb physiological processes and hence growth, however the application of calcium make plant more adaptable to adverse effect of wastewater. Khan *et al.,(*2010) reported that addition of calcium in the NaCl and Ni containing medium, enhanced plant growth.

The results of this study indicated that the wastewater possessed inhibitory effect on fresh weight of mung bean seedlings and the calcium treatment showed improvement in all genotypes but with some variations. The influence of wastewater on the growth, yield and quality of forage from *alfafa* and *Bermuda* has been studied and found decrease in fresh weight (Day *et al*., 1984). Similarly Bhardwaj *et al.,* 2009 reported the decrease in fresh weigh of *P. vulgaris* when exposed to lead and cadmium. The negative effect of wastewater was reduced by the addition of calcium, supported by Suzuki 2005, reported that calcium ions significantly alleviate the inhibitory effect of cadmium on growth and physiological processes.

During the current study, results demonstrated that the textile industrial wastewater promote dry weight of seedling, calcium also showed promotion in dry weight but less than in wastewater alone with the exception in genotype NFM-12-7, which showed inhibitory effect on dry weight in calcium treatment. Faizan *et al*., 2011 reported decrease in chickpea leaf dry mass when treated with cadmium. Textile waste water application increased plant dry weight in two varieties of peanut (Saravanamoorthy and Ranjitha, 2007). Liu *et al*., (2011) also observed increase in dry weight of different tissues of *L. japonica* after cadmium stress. Present results represented that the textile wastewater showed decrease in moisture content of mung bean seedlings except for genotype Ramzan, indicating that seedlings were under stress during treatments. Gill *et al*., 2001 also reported decrease in moisture in shoots and roots of sorghum seedlings under some abiotic stresses. Prado *et al*., 2000 found decrease in water content in drought and salt treated *Chinopodium quinoa* wild seeds.

Our results indicated that textile wastewater had effect on soluble proteins in mung bean genotypes with varietals variations. All genotypes represented promontory effect on soluble proteins except NFM-12-7 which showed inhibitory effect on soluble proteins. The concentration of soluble proteins decreased when plants were exposed to high concentration of the heavy metals (Zheng *et al*., 2009).The results of the present study suggest that there was not always a simple relationship between accumulation of metals and toxicity, as evidence by the increase in protein content, which may be due to the effect of organic matter along with presence of trace metals reported in the tannery sludge (Tudunwada *et al*., 2007). Calcium added in wastewater reduced total proteins as compared to wastewater alone for all genotypes except in NM-5-63-19.

Lipid Peroxidation was measured by the amount of thiobarbituric acid reactive substance (TBARS), and it was determined as textile wastewater significantly increased its amount in all genotypes of mung bean seedling, while decreased in the presence of calcium. 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. These results are in agreement with the findings of Singh and Agrawal (2010), reported increase in MDA content when *Beta vulgaris* was treated with municipal waste water. The peroxidation of lipids in cell membrane is one of the most damaging cellular responses observed in responses to water stress (Thankamani *et al*., 2003). The amount of lipid peroxidation has also long been considered as one of the factors, which indicate the severity of stress experienced by a plant (Chowdhury and Chowdhury, 1985). Inhibition of MDA content may be due to the role of calcium in controlling membrane structure and function by binding to phospholipids that stabilizes lipid bilayers and thus provides structural integrity to cellular membranes (Hirschi, 2004). Furthermore, there could be the involvement of oxidative signal transduction concomitant with the regulation of antioxidant enzymes under stress (Khan *et al*., 2010)

APX is thought to play the most essential role in scanvenging ROS and protecting cells in higher plants, algae, euglena and other organisms. Present results demonstrated that textile waste water significantly increased APX activity, while showed reduction in APX activity in the presence of calcium in all genotypes except Ramzan. Hsu and Kao (2007) reported that pretreatment of *O.sativa* seedlings with H_2O_2 under non heat shock condition resulted in an increase in APX activity and protected rice seedlings from the subsequent Cd stress. Enhanced activity of APX was also found in salt stress *A.doliolum* (Srivastava *et al*., 2005). Significant activities was noted under water stress in pre cultivars of *P.vulgaris* (Zlatev *et al*., 2006). Sharma and Dubey (2005), found that mild drought stress plant and higher chloroplastic APX activity then control grown plants. Growth media containing $CaCl_2$ decreased the Cd concentration, activity of antioxidant enzymes, and reactive oxygen species accumulation in the *Matricaria chamomilla* L plants treated with different $CdCl_2$, while increased the growth parameters. The beneficial effects of $CaCl_2$ in ameliorating $CdCl_2$ toxicity can be attributed to the Ca-induced reduction of Cd concentration, by reducing the cell-surface negativity and competing for Cd^{2+} ion influx, activity enhancement of antioxidant enzymes, and biomass accumulation (Farzadfar *et al*., 2013).

Our results evaluated increase activity of GPX in waste water stress, and reduced activity was observed when remediate by calcium. The activity of GPX varies considerably depending upon plant species and stress conditions. It increased in Cd-exposed plants of *T. aestivum* (Milone *et al.*, 2003), *A. thaliana* (Cho and Seo, 2005) and *C. demersum* (Arvind and Prasad, 2003). Radotic *et al,* (2000), noted an initial increase in GPX activity in spruce needles subjected to Cd stress and subsequent Cd treatments caused a decline in the activity. A concomitant increase in GPX activity in both the leaf and root tissues of *Vigna radiate* (Panda, 2001) and in *O. sativa* (Koji *et al., 2009)* has also been reported under salinity stress. Pre-treatment with CaCl2, reduced GPX activity, particularly during the later phase of heating, and plants treated with CaCl2 had lower CAT activity than their control plants prior to heating and within 48 h of heat stress (Larkindale and Huang,2004)

CAT activity increased in waste water stress in all genotypes, and became decreased than the control when seedlings exposed with textile waste water along with calcium, suggested calcium acted as stress inhibitor activity increased in *O. sativa* (Hsu and Kao, 2004), *B. juncea* (Mobin and Khan, 2007), *T. aestivum* (Khan, *et al.*, 2007), *C. arietinum* (Hasan *et al.*, 2008) and *V. mungo* roots (Singh *et al.*, 2008) under Cd stress. Eyidogan and Oz (2005) reported a significant increase in CAT activity in *C. arietinum* leaves under salt treatment. Similarly, increase in CAT activity in *C. arietinum* roots following salinity stress was noted by Kukreja *et al.* (2005). Salt stressed angar plants, calcium decreased CAT and APX activity (Hernández et al.,2003)

During the research study it has been observed that, the seedling treated with textile wastewater showed high proline accumulation, however decreased level in calcium treated samples. It is indicating that accumulation of proline in plants is considered to be responsible for stress tolerance mechanism. It is suggested to act as an osmolyte as well as a source of nitrogen during recovery from stress. Proline accumulation was correlated with improved plant performance under salt stress.

From the phytochemical studies, there was significantly high amount of tannin in the extract of mung bean seedlings treated with wastewater, which was increased further in presence of calcium. Lavid *et al.,2000* reported tannin content were high in *Nymphae* plant grown in the presence of cadmium. The finding of Lummerzheim *et al.,* (1995) reported high tannin accumulation in *A.thaliana* grown on lead toxicity and bacterial infection.

5. Conclusion

Industrial waste which contains various chemicals and compounds including heavy metals is extensively used to irrigate different crops in our country as it is considered as the cheapest source of fertilizer. Present study observed the negative effect of wastewater, on growth and biochemical parameters of mung bean seedlings. However the application of calcium improves seedling growth and altered various enzymes. Therefore it is suggested that by the addition of calcium in the wastewater before irrigating crops, plant performance can be improved.

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