



Full Length Article

Effect of Sugar Mill Effluent on Growth and Antioxidative Potential of Maize Seedling

Iqbal Hussain^{1*}, Muhammad Iqbal¹, Muhammad Nawaz¹, Rizwan Rasheed¹, Abida Perveen¹, Saqib Mahmood^{1*}, Azra Yasmeen² and Abdul Wahid³

¹Department of Botany, Government College University, Faisalabad-38000, Pakistan

²Department of Agronomy, Bahauddin Zakariya University Multan, Pakistan

³Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan

*For correspondence: iqbalbotanist1@yahoo.com; drsaqibm@yahoo.com

Abstract

The effects of sugar mill effluent (SME) on plant growth and biochemical attributes of two maize cultivars (Pak-Afgoi and EV-5098) were evaluated in a pot experiment. The maize kernels were sown in soil-filled pots. After 15 days of germination, maize seedlings were irrigated with four different concentrations (25%, 50%, 75% and 100% v/v) of SME. The seedlings irrigated with half strength of Hoaglands' nutrient solution (0% SME) served as control. The data for various attributes were collected after 15 days of treatments. Although high concentrations of SME decreased the growth, the low concentration (25%) of SME was very effective in increasing the growth of both maize cultivars when compared with control. The increase in growth was associated with increased photosynthetic pigments, carotenoids, anthocyanins, total free proline and total free amino acids in both cultivars. The results indicated that the increase in SME concentration caused a marked increase in relative membrane permeability (RMP), malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents, activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) in both cultivars. The increase in antioxidative potential enabled maize plants to withstand SME-induced oxidative stress. However, cv. Pak-Afgoi was found to be more SME tolerant as compared with cv. EV-5098 because it exhibited lower RMP and had low H₂O₂ and MDA contents. The better growth of maize plants irrigated with SME (25%) suggested that diluted SME could be used for irrigation in nutrients deprived environments. © 2013 Friends Science Publishers

Keywords: Antioxidant enzymes; Proline; Lipid peroxidation; Photosynthetic pigments; Sugar mill effluent; Maize

Introduction

Sugar mill is one of the most important agro-based industries in Pakistan. There are 84 sugar mills in Pakistan (Pakistan Sugar Annual Report, 2009), sharing 16% in the agricultural economy of our country (Khan and Jamil, 2004). Sugar mill discharged effluents into the soil and water are causing a major water pollution problem (Mahar *et al.*, 2012). The effluents contained various amounts of inorganic, organic pollutant and heavy metals that could alter the quality of water and affect the aquatic flora and fauna (Panday and Srivastava, 2002). Sugar mill effluents when disposed of in to the surroundings cause a severe hazard to the rural and semi-urban populations (Barman *et al.*, 2000; Kisku *et al.*, 2000). Sugar factory effluent has an obnoxious odour and unpleasant color when released into the environment without proper treatment. The pollutants like chloride, sulfate, phosphate, magnesium and nitrate are released with the effluent (Saranraj and Stella, 2012). Higher concentration of SME could inhibit seed germination and seedling growth and eventually yield in

some crops such as green gram (Baskran *et al.*, 2009), sorghum (Doke *et al.*, 2011), peanut (Siva Santhi and Suja Pandian, 2012).

Having high biological oxygen demand (BOD), chemical oxygen demand (COD), total hardness and total dissolved salts, sugar mill effluent is one of the abiotic stresses that affects the whole life cycle of plants (Doke *et al.*, 2011). It inhibits seed germination, photosynthesis, respiration, transpiration rate, enzymatic activities, uptake and distribution of micro- and macro-nutrients in plant tissues and disturbs plant water-relationships, ionic relations, and induces free radical formation (Yildirim *et al.*, 2006; Jaubyon, 2012). Oxidative stress in terms of production of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydrogen peroxides and hydroxyl radicals (Sharma *et al.*, 2012) could cause cell death by lipid peroxidation, oxidation of proteins and inducing substantial damage to DNA (Gill and Tuteja, 2010). In addition, ROS is shown to alter the expression of many genes including the genes for the synthesis of enzymatic and non-enzymatic antioxidants (Parida and Das, 2005). However, plants make

use of antioxidant defense machinery comprising of both enzymatic and non-enzymatic antioxidants to cope with ROS (Weisany *et al.*, 2012). The levels of lipid peroxidation measured in terms of MDA contents are considered as a potential indicator of salt and heavy metal-induced oxidation in cell membranes and could be used as a potential selection criterion to characterize the cultivars for stress tolerance (Jaffel *et al.*, 2011).

Maize (*Zea mays* L.) is one of the most important agricultural crops cultivated worldwide. Being a rich source of nutrition (4.8% edible oil, 8.5% fiber, 10% protein and 72% starch), maize is one of the major sources of human food, animal and poultry feed (Okoruwa, 1995). In the present study, an attempt has been made to find out the effects of different concentrations (0-100%) of SME on the growth and antioxidative potential of maize.

Materials and Methods

Plant Materials, Collection of Effluent and Treatment

Kernels of two maize cultivars (Pak-Afgoi and EV-5098) were obtained from Maize and Millets Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. The experiment was conducted at the Department of Botany, Government College University, Faisalabad (latitude 29°30 N, longitude 72°11 E and altitude 214 m). The soil used in this experiment was sandy loam and the pH of the soil was 7.1. The SME was collected in plastic containers from disposal point of the Shamim sugar mill located in Samunday, Faisalabad, Pakistan. Ten seeds were sown in each pot filled with 5 kg soil. After germination three uniform and healthy seedlings were retained for the determination of growth and biochemical attributes. Fifteen days old seedlings were irrigated with five different concentrations (0%, 25%, 50%, 75% and 100%) of SME on every three day intervals. The control plants were irrigated with half strength Hoaglands' nutrient solution. The experiment was laid-out in a completely randomized design with three replications per treatment. Following data for various attributes were recorded after 15 days of the treatment at the seedling stage.

Measurement of Physico-chemical Properties of Effluent

The physico-chemical properties of the effluent such as temperature, colour, taste, odour, pH, BOD, COD, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chloride, alkalinity, total hardness, calcium, magnesium, sulfate, phosphate and total nitrate, were measured using the standard methods (APHA, 1998) and are shown in Table 1.

Growth Attributes

After harvest, shoot and root fresh and dry weights were recorded. Leaf area of intact plants was determined as

maximum leaf length × maximum leaf width and multiplied by 0.68 (correction factor) per plant.

Determination of Chlorophyll and Total Carotenoids Contents

Chlorophyll (Chl *a* and *b*) and total chlorophyll contents were determined as described by Yoshida *et al.* (1976). The total carotenoids were determined following the method of Davies (1976).

Electrolyte Leakage Analysis

Electrolyte leakage (%) of fresh leaf tissues was determined as described earlier (Yang *et al.*, 1996).

Determination of Malondialdehyde (MDA) Contents

The byproduct of lipid peroxidation, MDA was determined as described earlier (Dhindsa *et al.*, 1981). The absorbance of the supernatant was recorded at 450 nm, 532 nm and 600 nm on UV-VIS spectrophotometer (Hitachi U-2910). The MDA contents were calculated according to the given formula: $[MDA] = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

Determination of Hydrogen Peroxide (H₂O₂) concentration

The H₂O₂ concentration was determined by following the method of Velikova *et al.* (2000). The H₂O₂ concentration was determined using a calibration curve constructed using a series (1-50 μM) of analytic reagent grade H₂O₂.

Antioxidant Enzyme Assays

Enzyme extraction: For extracting antioxidant enzymes, fresh leaves (0.5 g) were ground in 5 mL of 50 mM cooled potassium phosphate buffer (pH 7.8) placed in an ice bath. The homogenate was centrifuged at 15000 × *g* for 20 min at 4°C. The supernatant was used for the determination of superoxide dismutase (Gong *et al.* (2005), catalase (Cakmark *et al.*, 1993) and ascorbate peroxidase (Krivosheeva *et al.*, 1996) activities.

Determination of Free Proline Contents

Free proline was determined by using the method of Bates *et al.* (1973). The amount of free proline was calculated from the standard curve and expressed as μmol g⁻¹ fresh weight.

Determination of Total Anthocyanin Contents

Total anthocyanin contents were determined by the method of Hodges and Nozzolillo (1996). The absorbance of the supernatant was measured at 540 nm and 600 nm by using

Table 1: Physico-chemical parameters of sugar factory effluent

Parameters	Raw effluent	WHO standard	Parameters	Raw effluent	WHO standard
Temperature (°C)	24	25	COD	4140	-
Color	Light grey	-	Total Sulphate (mg/L)	398.85	400
Odor	Decaying smell	Odorless	Total nitrates (mg/L)	41	50
pH	6.35	6.50-8.50	Total dissolved salt (mg/L)	2368	1500
EC (µS/cm)	758	1000	Total calcium (mg/L)	452	200
Total coliform	Present	absent	Total Chloride (mg/L)	825	600
Total hardness (mg/L)	980	<500	Mg (mg/L)	440	150
Total alkalinity (mg/L)	170	-	Phosphate (mg/L)	25	-
DO (mg/L)	2.1	6	Turbidity (ppm)	115	25NTU
BOD (mg/L)	790	-	Oil and Grease (mg/L)	18	10

EC= Electrical conductivity; DO = Dissolved oxygen; COD = Chemical oxygen demand, BOD = Biological oxygen demand

UV-VIS spectrophotometer (Hitachi U-2910). The acidified methanol was used as blank. The amount of total anthocyanin contents was expressed as mg g⁻¹ fresh leaves.

Total Free Amino Acid Contents

Total free amino acids were determined as described earlier (Hamilton and Van-Slyke, 1943). The amount of total free amino acids was calculated from the standard curve of Lucine at 570 nm and expressed as mg g⁻¹ fresh weight.

Statistical Analysis

The data collected was subjected to analysis of variance technique (ANOVA) by using a computer software CoStat version 6.2, CoHort Software, 2003, Monterey, CA, USA. The least significant difference among means was computed. The data in the figures are represented as means ±SD (*n* = 3) for each parameter.

Results

Physico-chemical parameters of the effluent were found to be above the acceptable level according to WHO Standards (Table 1). The analysis of SME showed that it is basic in nature with gray in colour. It contained large amounts of dissolved solids and suspended particles. The effluent had higher value of BOD and COD, substantial amounts of chloride, fluoride, calcium, magnesium, nitrate and sulfate.

The SME (25%) cause a marked increase ($P \leq 0.001$) in shoot and root lengths, fresh and dry weights and leaf area in two maize cultivars (Pak-Afgoi; EV-5098). The effect of SME on different growth attributes was dose dependent. Maximum decline was observed at 75% and 100% of SME in both maize cultivars. The response of both maize cultivars was differential under varying SME levels. Cultivar Pak-Afgoi exhibited lesser decline in different growth attributes as compared with cv. EV-5098 (Fig. 1).

Addition of SME to the growth medium caused a substantial increase ($P \leq 0.001$) in photosynthetic pigments (chl. a, b) at 25% level. Increase in photosynthetic pigments was more pronounced in cv. Pak-Afgoi as compared with cv. EV-5098, except for slightly higher degradation of photosynthetic pigments at higher levels of SME (50, 75 and 100%) in cv. EV-5098. A similar trend was observed

for total chlorophyll contents in the two cultivars (Fig. 2). Effluent stress caused a marked ($P \leq 0.001$) decrease in total carotenoids in both cultivars at higher levels of SME. However, the increase in this attribute was greater in cv. Pak-Afgoi than that in cv. EV-5098 (Fig. 2).

Relative membrane permeability increased ($P \leq 0.001$) with increasing levels of SME in the growth medium of two maize cultivars. Greater RMP was recorded in cv. EV-5098 as compared with cv. Pak-Afgoi at all levels of SME. The differences in two maize cultivars with respect to this attribute were indiscriminate (Fig. 3). Addition of SME to the growth medium of both maize cultivars resulted in a substantial increase ($P \leq 0.001$) in MDA contents. The response of the cultivars was dose dependent, being maximal at 100% level of effluent with respect to this attribute. Cultivar EV-5098 accumulated higher MDA as compared with cv. Pak-Afgoi under all levels of SME (Fig. 3).

SME caused a considerable increase ($P \leq 0.001$) in cellular H₂O₂ contents in both cultivars. The highest level of SME stress caused maximal accumulation of H₂O₂ in both maize cultivars. Relatively higher levels of cellular H₂O₂ were recorded in cv. EV-5098 compared with cv. Pak-Afgoi under SME stress (Fig. 3).

Exposure of maize cultivars to different levels of SME resulted in a significant ($P \leq 0.001$) increase in the activity of SOD. However, the increase in the activity of SOD was more in cv. EV-5098 than that in cv. Pak-Afgoi. The SME-induced increase in this attribute was dose dependent, being maximal at 100% level of SME (Fig. 4). Addition of SME to the growth medium caused a consistent increase ($P \leq 0.001$) in the activities of both types of peroxidases (APX and POD). Higher activities of APX and POD were recorded in cv. EV-5098 when compared with cv. Pak-Afgoi (Fig. 4). Effluent-induced consistent increase ($P \leq 0.001$) in the activity of CAT was observed in both cultivars.

A substantial increase ($P \leq 0.001$) in leaf total free proline and total anthocyanin contents were recorded in both cultivars under SME stress. However, SME-induced accumulation of total free proline and anthocyanin contents in cv. Pak-Afgoi as compared with cv. EV-5098 (Fig. 5). The maximal values of total free proline and anthocyanin contents were recorded in cv. Pak-Afgoi as compared with

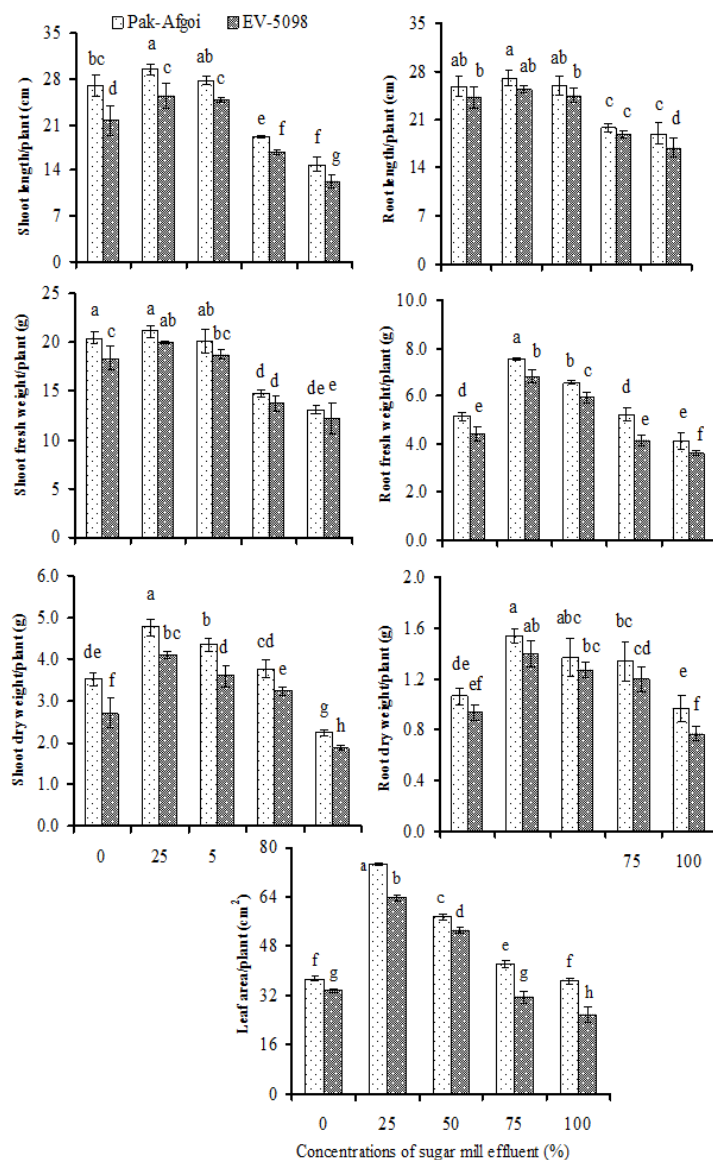


Fig. 1: Changes in some growth attributes of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

cv. EV-5098 at 75% and 100% levels of SME. However, low level of SME increased, while higher concentrations of SME relatively decreased total free amino acids in both maize cultivars (Fig. 5).

Discussion

The sugar mill plays a major role in producing large amounts of water pollution because its effluent contained large amounts of chemical elements, total hardness, TDS, BOD, COD, sodium, sulfate, calcium and magnesium. In addition, any appreciable amount of metals such as iron, magnesium, zinc, copper and lead are also present in the

SME (Table 1). The physico-chemical properties of SME are in accordance with the earlier findings (Borole and Patil, 2004; Vijayaragavan *et al.*, 2011).

Sugar mill effluent is known to be one of the abiotic stresses that limit plant vigor and productivity (Ayyasamy *et al.*, 2008; Kazemi and Eskandari, 2011). However, in the present study, shoot and root lengths and fresh and dry masses of both maize cultivars increased at lower and decreased at higher concentration of the SME. The lower concentration of SME contained optimum levels of nutrients, which might have increased the growth attributes of plants (Thamizhiniyan *et al.*, 2009; Vijayaragavan *et al.*, 2011). However, deleterious effects of SME on different

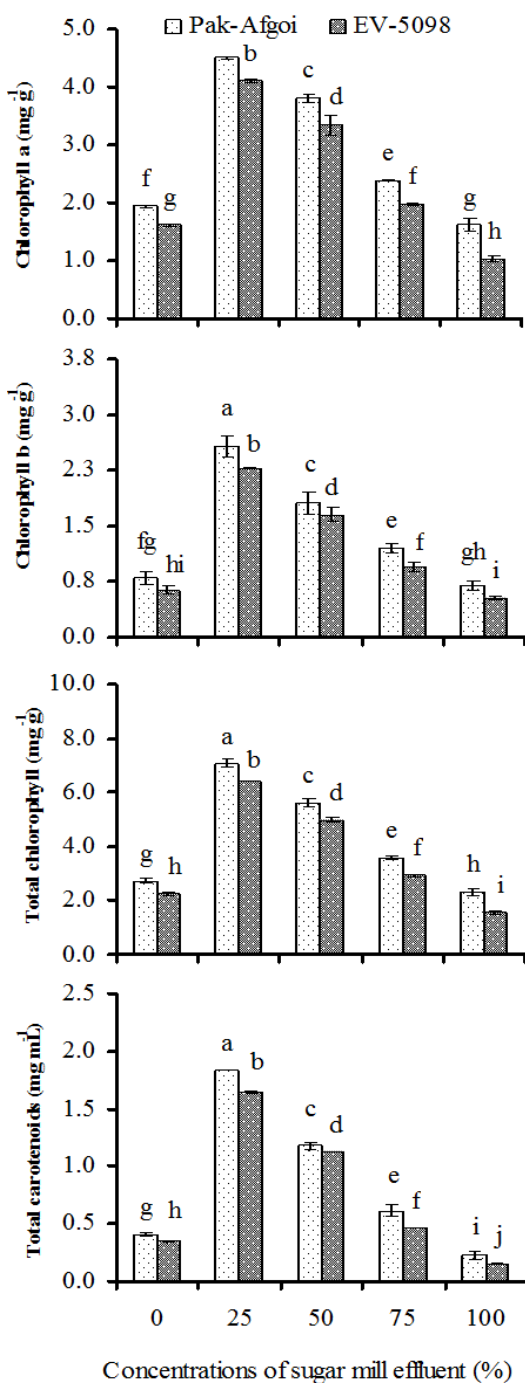


Fig. 2: Changes in photosynthetic pigments of maize seedlings exposed to five different levels (0, 25, 50, 75 and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

growth parameters were more evident in cv. EV-5098 than in cv. Pak-Afgoi. Effluent-induced reduction in different growth attributes has been reported in a number of crop plants, e.g., green gram (Baskran *et al.*, 2009), wheat

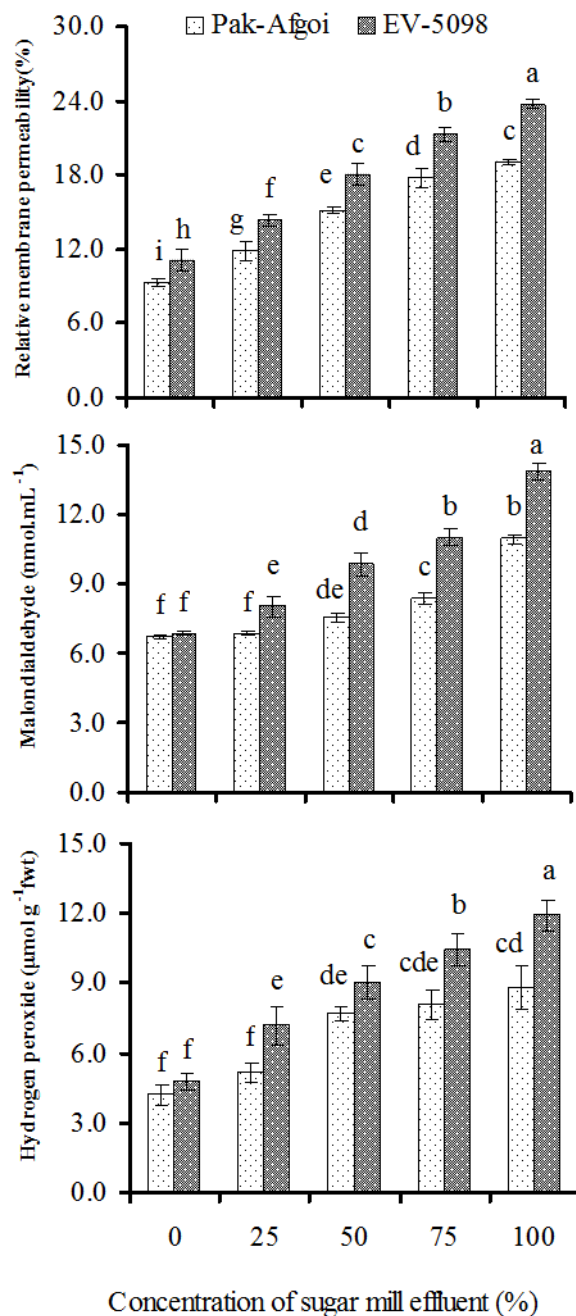


Fig. 3: Changes in relative membrane permeability, lipid peroxidation and hydrogen peroxide contents of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

(Kaushik *et al.*, 2004), green gram and maize (Ayyasamy *et al.*, 2008), maize (Ezhilvannan *et al.*, 2011) and radish (Vijayaragavan *et al.*, 2011). There are a number of plausible explanations for this reduction in growth including disturbed osmotic relations due to the presence of calcium

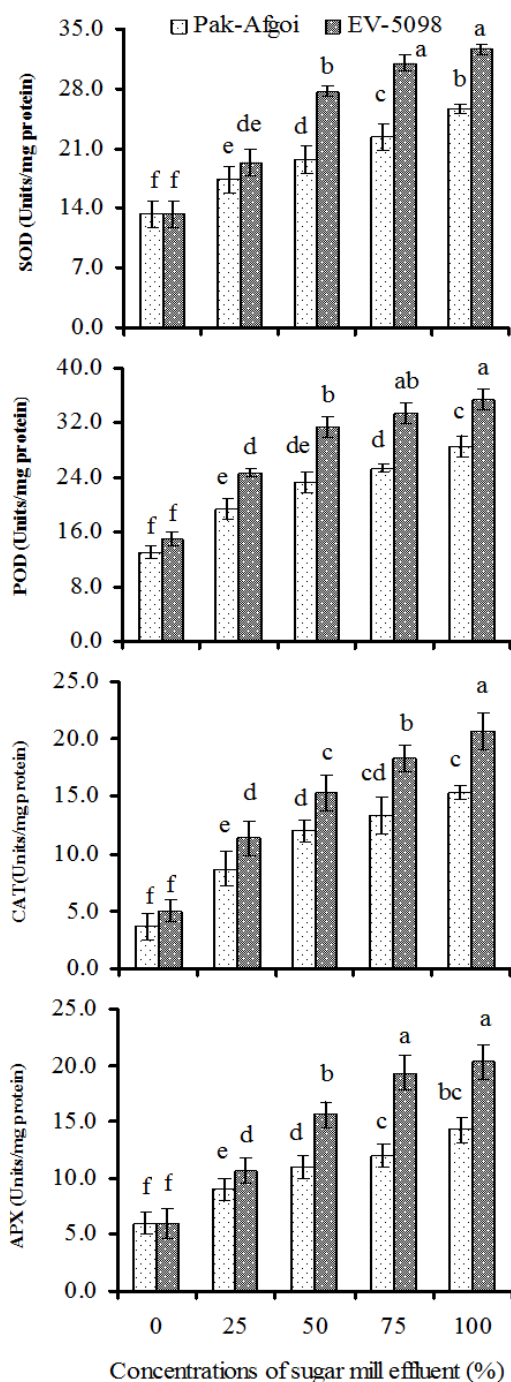


Fig. 4: Changes in superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and Ascorbate peroxidase (APX) activity in leaves of maize seedlings exposed to five different levels (0, 25, 50, 75 and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

and magnesium resulting in the wilting of seedlings (Gomathi and Oblisami, 1992). In our study, the growth of cultivars was highly affected due to the excess amount of

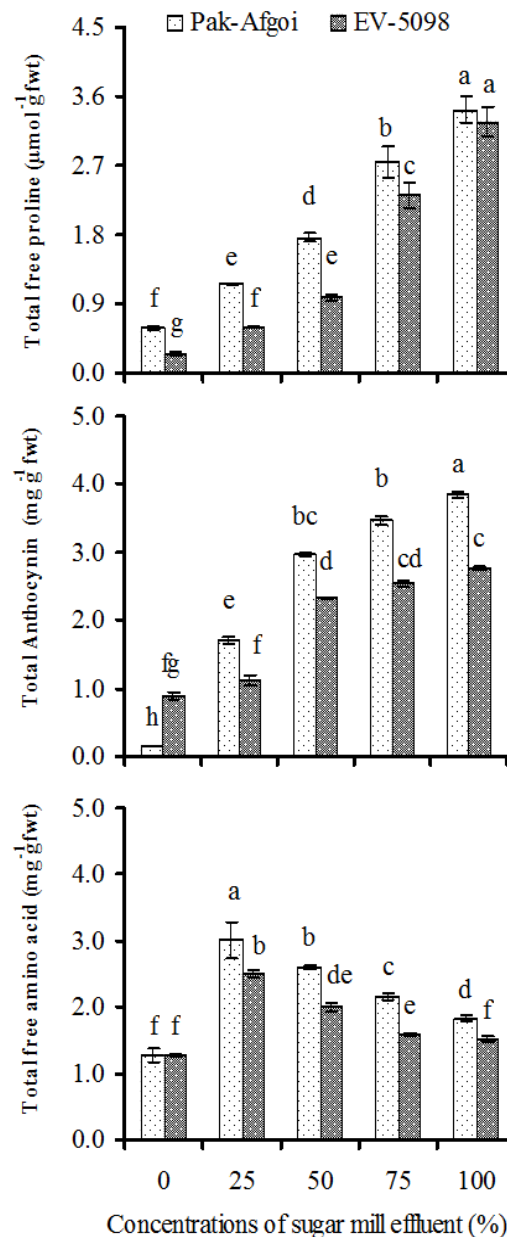


Fig. 5: Changes in total free proline, anthocyanin and free amino acids contents in leaves of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

total chloride, alkalinity, hardness, magnesium, calcium, sulfate and phosphate in the SME.

Lower concentration of SME increased chlorophyll and carotenoids contents in maize plants. This might be due to the presence of nitrogen and other inorganic and organic elements present in the SME (Nagajyoti *et al.*, 2008; Vijayaragavan *et al.*, 2011). In addition, effluents contain magnesium, potassium, iron, copper and zinc those are

Table 2: The correlation between growth and biochemical attributes of maize grown under sugar mill effluent

Attributes	RMP	MDA	H ₂ O ₂
Shoot length	-0.786***	-0.787***	-0.693***
Root length	-0.828***	-0.765***	-0.744***
Shoot fresh weight	-0.832***	-0.778***	-0.741***
Root fresh weight	-0.522**	-0.598***	-0.468*
Shoot dry weight	-0.518**	-0.656***	-0.461*
Root dry weight	-0.311ns	-0.546**	-0.268ns
Leaf area	-0.440*	-0.530**	-0.386*
Chlorophyll <i>a</i>	-0.385*	-0.497**	-0.619***
Chlorophyll <i>b</i>	-0.330ns	-0.439*	-0.569**
Tot-Chlorophyll	-0.366ns	-0.478**	-0.507**
Carotenoid	-0.374*	-0.464*	-0.507**
Proline	0.804***	0.709***	0.738***
Anthocyanin	-0.745***	-0.547**	-0.680***
Total amino acids	-0.156ns	-0.309ns	-0.125ns
Superoxide dismutase	0.964***	0.874***	0.931***
Peroxidase	0.922***	0.768***	0.917***
Catalase	0.962***	0.829***	0.949***
Ascorbate peroxidase	0.950***	0.859***	0.948***
Relative membrane permeability (RMP)		0.892***	0.963***
Malondialdehyde (MDA)			0.841***

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively; ns= non-significant.

important for chlorophyll synthesis (Subramaniam *et al.*, 1999; Vijayaragavan *et al.*, 2011). In the present investigation, SME stress resulted in a marked degradation of chlorophyll contents in both cultivars. The SME irrigation is known to cause significant degradation of chlorophyll pigments in some crops (Nagajyoti *et al.*, 2008; Baskran *et al.*, 2009; Vijayaragavan *et al.*, 2011; Munir *et al.*, 2013). However, SME-induced degradation of chlorophyll pigments was more in cv. EV-5098 than that in Pak-Afgoi. The degradation of chlorophyll pigments under SME-stress could be linked with increased activity of chlorophyllase or reduced *de novo* synthesis of chlorophyll (Saravanamoorthy and Kumari, 2005; Nagajyoti *et al.*, 2008).

Plants under different environmental stresses exhibit changes in RMP that ultimately lead to the loss of membrane integrity (Valentovič *et al.*, 2006; Farkhondeh *et al.*, 2012). Therefore, the ability of the cell membranes to control movement of ions across the cell was taken as an indicator of damage induced by effluent stress (Farkhondeh *et al.*, 2012). In the present study, increasing effluent levels increased RMP, particularly in Pak-Afgoi cultivar. The effluent-induced increased RMP is already reported in rice (Siringam *et al.*, 2011), wheat (Wahid *et al.*, 2007), sugar beet (Farkhondeh *et al.*, 2012) and sunflower (Heidari *et al.*, 2011). The RMP exhibited a negative correlation with different growth attributes (Table 2). Jamil *et al.* (2012) found a negative correlation between RMP and growth attributes in sugar beet. The inhibitory effects of effluent on membranes are largely due to the elevated levels of toxic salts and metals in the growth medium (Parida and Das, 2005).

The membrane permeability and MDA contents are used as biomarkers for lipid peroxidation (Kukreja *et al.*, 2005). In our study, effluent stress caused a substantial

increase in MDA levels in both maize cultivars. Effluent-induced increase in MDA has been reported in maize (Hussain *et al.*, 2012), *Pisum sativum* (Ahmada *et al.*, 2008) and soybean (Weisany *et al.*, 2012). A strong negative association of MDA with various growth attributes was observed (Table 2). This shows that oxidative stress was main cause of growth reduction in effluent treated maize plants. Plants grown under effluent stress are prone to oxidative damage induced by the elevated levels of ROS such as H₂O₂ (Sharma *et al.*, 2012). Hydrogen peroxide is one of the lethal ROS as it inhibits Calvin cycle and consequently reduces the carbon fixation. In the present investigation, both cultivars accumulated higher levels of H₂O₂ under SME stress. The H₂O₂ concentration was negatively correlated with different growth attributes (Table 2). Lower levels of H₂O₂ are involved in different signaling processes (Quan *et al.*, 2008). However, plants tend to accumulate higher levels of H₂O₂ under effluent stress that causes substantial degradation of photosynthetic pigments (Sharma *et al.*, 2012).

Plants exposed to effluent exhibit higher activities of antioxidant enzymes to counteract the oxidative damage due to ROS (Baskran *et al.*, 2009; Nouman *et al.*, 2012). In the present investigation, a significant SME-induced increase in SOD, APX, POD and CAT activities was observed in maize cultivars. Baskran *et al.* (2009) reported effluent-induced increase in the activity of SOD in green gram and *Clarias gariepinus*, respectively (Aina *et al.*, 2012). Comparatively lower levels of MDA and H₂O₂ in cv. Pak-Afgoi than that in cv. EV-5098 could have been due to higher activities of antioxidant enzymes. In addition, low degradation of photosynthetic pigments in cv. Pak-Afgoi could be attributed to lower levels of H₂O₂, since ROS are known to induce substantial damage to pigments (Sharma *et al.*, 2012).

Exposure of plants to SME promoted the

accumulation of proline. The accumulation of proline has been frequently used as a biochemical marker for stress tolerance in plants (Matysik *et al.*, 2002; Ramasubramanian *et al.*, 2006). Aside from acting as a metal chelator and osmolyte, proline has been reported to scavenge hydroxyl radicals and singlet oxygen, thus providing protection against ROS-induced cell damage (Alia and Saradhi, 1991; Yilmaz and Parlak, 2011). Anthocyanins are involved in photoprotection induced by various abiotic stresses (Merzlyak *et al.*, 2008). In the present investigation, a significant increase in anthocyanin contents was observed in both maize cultivars at 75% and 100% levels of SME. We observed a negative association between anthocyanins and H₂O₂ contents. Flavonoids act as non-enzymatic antioxidants and protect the plants against ROS-induced oxidative stress. Moreover, loss of membrane integrity in terms of lipid peroxidation has been reported in anthocyanin deficient mutant of *Arabidopsis* (Agati *et al.*, 2007). The results suggested that maize plants tolerated to high levels of effluents by accumulating more anthocyanins. Exposure of plants to effluent resulted in reduction of amino acid contents. Reduction in total free amino acid contents has been reported due to the presence of higher magnesium concentrations and the acidic pH of the effluent. Magnesium-fed plants had lower free amino acids and soluble protein contents in their leaves (Lasa *et al.*, 2000). The decrease in free amino acid contents at high concentration of SME can be attributed to the inhibitory effect of the effluent on protease activity. The reduction in amino acids has already been reported in green gram (Baskran *et al.*, 2009), maize (Ezhilvannan *et al.*, 2011) and radish (Vijayaragavan *et al.*, 2011).

It can be concluded from the study that physico-chemical parameters of the effluent such as pH, EC, COD, calcium, magnesium, chloride, sulfate, hardness and TDS were relatively elevated in the SME and severely affected the plant growth. However, the effluent stimulated the growth at lower levels. The effluent nominally decreased the photosynthetic pigments in both maize cultivars. The results indicated that the SME-induced a marked increase in MDA, H₂O₂ contents and increased RMP, activities of antioxidant enzymes (SOD, POD, CAT and APX). Furthermore, it enhanced the accumulation of free proline and anthocyanin contents. Diluted effluent (25%) increased the growth parameters, pigments and amino acid contents in both cultivars. Overall, the cultivar Pak-Afgoi was more tolerant than cv. EV-5098. At low concentration (25%), SME served as a liquid fertilizer and enhanced growth of maize plants. The results suggested that diluted SME ($\leq 25\%$) could be used for irrigation of maize crop.

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References

- Agati, G., P. Matteini, A. Goti and M. Tattini, 2007. Chloroplast-located flavonoids can scavenge singlet oxygen. *New Phytol.*, 174: 77–89
- Ahmada, P., R. Johnb, M. Sarwatc and S. Umard, 2008. Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. *Int. J. Plant Prod.*, 2: 1735–8043
- Aina, O.A., M.O. Ielabayo, R.I. Oju, V.C. Azubuike, A.A. Isaac and O.F. Ebenezer, 2012. Long-term exposure to industrial effluent induces oxidative stress and affects growth in *Clarias gariepinus*. *Res. J. Environ. Earth Sci.*, 4: 738–746
- Alia, A. and P.P. Saradhi, 1991. Proline accumulation under heavy metal stress. *J. Plant Physiol.*, 138: 554–558
- APHA, 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association, Water Pollution Control Federation. Washington DC, USA
- Ayyasamy, P.M., R. Yasodha, S. Rajakumar, P. Lakshmanaperumalsamy, P.K.S.M. Rahman and S. Lee, 2008. Impact of sugar factory effluent on the growth and biochemical characteristics of terrestrial and aquatic plants. *Bull. Environ. Contam. Toxicol.*, 81: 449–454
- Barman, S.C., R.K. Sahu, S.K. Bhargava and C. Chatterjee, 2000. Distribution of heavy metals in wheat, mustard, and grown in field irrigated with industrial effluent. *Bull. Environ. Contam. Toxicol.*, 64: 489–496
- Baskran, L., P. Sundaramoorthy, A.L.A. Chidambaram and K. Sankar Ganesh, 2009. growth and physiological Activity of green gram (*Vigna radiata* L.) under effluent stress. *Bot. Res. Int.*, 2: 107–114
- Bates, I.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205–207
- Borele, D.D. and P.R. Patil, 2004. Studies on physicochemical parameters and concentration of heavy metals in sugar industry. *Pollut. Res.*, 23: 83–89
- Cakmark, I., D. Strbac and H. Marschner, 1993. Activities of hydrogen peroxide scavenging enzymes in germinated wheat seeds. *J. Exp. Bot.*, 44: 127–132
- Davies, B. H., 1976. Carotenoids. In: *Chemistry and Biochemistry of Plant Pigments*, pp: 138–165. Goodwin, T.W. (ed.). Academic Press London, UK
- Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe, 1981. Leaf senescence correlated with increase leaves of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93–101
- Doke, K.M., M.E. Khan, J. Rapolu and A. Shaikh, 2011. Physico-chemical analysis of sugar industry effluent and its effects on seed germination of *Vigna anularis*, *Vigna cylindrical* and *Sorghum cernum*. *Ann. Environ. Sci.*, 5: 7–11
- Ezhilvannan, D., P.S. Sharavanan and M. Vijayaragavan, 2011. Effect of sugar mills effluent on changes of growth and amino acids and protein contents of maize (*Zea mays* L.) plants. *J. Ecobiotechnol.*, 3: 26–29
- Farkhondeh, R., E. Nabizadeh and N. Jalilnezhad, 2012. Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. *Int. J. Agric. Sci.*, 2: 385–392
- Gill, S.S. and N. Tuteja, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48: 909–930
- Gomathi, V. and G. Oblisami, 1992. Effect of pulp and paper mill effluent on germination of tree species. *Ind. J. Environ. Health*, 34: 326–328
- Gong, H., X. Zhu, K. Chen, S. Wang and C. Zhang, 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.*, 169: 313–321
- Hamilton, P.B. and D.D. Van-Slyke, 1943. Amino acid determination with ninhydrin. *J. Biol. Chem.*, 150: 231–233
- Heidari, A., M. Toorchi, A. Bandehagh and M.R. Shakiba, 2011. Effect of NaCl Stress on growth, water relations, organic and inorganic osmolytes accumulation in sunflower (*Helianthus annuus* L.) lines. *Int. J. Environ. Sci. Technol.*, 1: 351–362

- Hodges, D.M. and C. Nozzolillo, 1996. Anthocyanin and anthocyanoplast content of cruciferous seedlings subjected to mineral nutrient deficiencies. *J. Plant Physiol.*, 147: 749–754
- Hussain, I., M. Iqbal, S. Qurat-Ul-Ain, R. Rasheed, S. Mahmood, A. Perveen and A. Wahid, 2012. Cadmium dose and exposure-time dependent alterations in growth and physiology of maize (*Zea mays*) *Int. J. Agric. Biol.*, 14: 959–964
- Jaffel, K., S. Sai, N.K. Bouraoui, R.B. Ammar, L. Legendre, M. Lachâal and B. Marzouk, 2011. Influence of salt stress on growth, lipid peroxidation and antioxidative enzyme activity in borage (*Borago officinalis* L.). *Plant Biosyst.*, 145: 362–369
- Jamil, M., M. Ashraf, S. Rehman, M. Ahmad and E.S. Rha, 2012. Salinity induced changes in cell membrane stability, protein and RNA contents. *Afr. J. Biotechnol.*, 11: 6476–6483
- Jauybon, Z., 2012. The effects of salt stress on plant growth. *Tech. J. Engin. App. Sci.*, 2: 7–10
- Kaushik, A., B.R. Kadyan and C.P. Kaushik, 2004. Sugar mill effluent effects on growth, photosynthetic pigments and nutrient uptake in wheat seedlings in aqueous vs. soil medium. *Water Air Soil Poll.*, 87: 39–46
- Kazemi, K. and H. Eskandari, 2011. Effects of salt stress on germination and early seedling growth of rice (*Oryza sativa*) cultivars in Iran. *Afr. J. Biotechnol.*, 10: 17789–17792
- Khan, I. and M. Jamil, 2004. Sugar crops and sugar policy of Pakistan. *Sci. Vision*, 9: 1–8
- Kisku, G.C., S.C. Barman and S.K. Bhargava, 2000. Contamination of soil and plants potentially toxic elements irrigated with mixed industrial effluent and impact in the environment. *Water Air Soil Pollut.*, 120: 121–137
- Krivosheeva, A., D.L. Tao, C. Ottander, G. Wingsle, S.L. Dube and G. Oquist, 1996. Cold acclimation and photoinhibition of photosynthesis in Scots pine. *Planta*, 200: 296–305
- Kukreja, S., A.S. Nandwal, N. Kumar, S.K. Sharma, V. Unvi and P.K. Sharma, 2005. Plant water status, H₂O₂, scavenging enzymes, ethylene evaluation and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biol. Plant.*, 49: 305–308
- Lasa, B., S. Frechilla, M. Aleu, B. Gonzalez-Moro, C. Lamsfus and P.M. Aparicio-Tejo, 2000. Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. *Plant Soil*, 225: 167–174
- Mahar, M.T., M.Y. Kuhuwar, M.A. Baloch and T.M. Jahangir, 2012. Effects of spent wash of ethanol industry on groundwater: A case study of Rahimyar Khan district, Pakistan. *J. Environ. Sci. Water Resour.*, 1: 85–94
- Matysik, A.J., B. Bhalu and P. Mohanty, 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.*, 82: 525–532
- Merzlyak, M.N., O.B. Chivkunova, A.E. Solovchenko and K.R. Naqvi, 2008. Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. *J. Exp. Bot.*, 59: 3903–3911
- Munir, S., H.I. Siddiqi, K.H. Bhatti, K. Nawaz, K. Hussain, R. Rashid and I. Hussain, 2013. Assessment of inter-cultivar variations for salinity tolerance in winter radish (*Raphanus sativus* L.) using photosynthetic attributes as effective selection criteria. *World App. Sci. J.*, 21: 384–388
- Nagajyoti, P.C., N. Dinakar, T.N.V.K.V. Prasad, C. Suresh and T. Damodharam, 2008. Heavy metal toxicity: Industrial Effluent Effect on Groundnut (*Arachis hypogaea* L.) Seedlings. *J. Appl. Sci. Res.*, 4: 110–121
- Nouman, W., M.T. Siddiqui, S.M.A. Basra, R.A. Khan T. Gull, M.E. Olson and H. Munir, 2012. Response of *Moringa oleifera* to saline conditions. *Int. J. Agric. Biol.*, 14: 757–762
- Okoruwa, A.E., 1995. *Utilization and Processing of Maize*, p: 27. IITA research guide 35. Training institute of tropical agriculture (IITA), Ibadan, Nigeria
- Pakistan Sugar Annual Report, 2009. <http://www.thebioenergysite.com/articles/335/pakistan-sugar-annual-report-2009>
- Panday, S. and Srivastava, 2002. Heavy metals accumulation in industrial solid waste amended soils. *Nat. Environ. Pollut. Technol.*, 1: 73–75
- Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Safety*, 60: 324–349
- Quan, L.J., B. Zhang, W.W. Shi and H.Y. Li, 2008. Hydrogen Peroxide in Plants: A Versatile Molecule of Reactive Oxygen Species Network. *J. Integr. Plant Biol.*, 50: 2–18
- Ramasubramanian, V., R. Jeyaprakash and T.N. Ramasamy, 2006. Assessment of physico-chemical parameters in three industrial effluents. *Ind. J. Environ. Protect.*, 26: 1090–1092
- Saranraj, P. and D. Stella, 2012. Effect of bacterial isolates on reduction of physico – chemical characteristics in sugar mill effluent. *Int. J. Pharmaceut. Biol. Arch.*, 3: 1121–1128
- Saravanamoorthy, M.D. and B.D.R. Kumari, 2005. Effect of cotton yarn dye effluent on physiological and biochemical contents of peanut and green gram. *Biochem. Cell Arch.*, 5: 113–117
- Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli, 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.*, 2012: Article ID 217037
- Siringam, K., N. Juntawong, S. Cha-um and C. Kirdmanee, 2011. Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. *indica*) roots under iso-osmotic conditions. *Afr. J. Biotechnol.*, 10: 1340–1346
- Siva Santhi, K. and R. Suja Pandian, 2012. Effect of sugar mill effluent on seed germination of peanut (*Arachis Hypogaea*) and green gram (*Vigna Radiata*). *Int. J. Pharm. Chem. Sci.*, 1: 804–806
- Subramaniam, A., E.J. Carpenter, D. Karentz and P.G. Falkowski, 1999. Bio-optical properties of the marine diazotrophic cyanobacteria *Trichodesmium* spp. I. Absorption and photosynthetic action spectra. *Limnol Oceanogr.*, 44: 608–617
- Thamizhiniyan, P., P.V. Sivakumar, M. Lenin, M. Sivaraman, 2009. Sugar mill effluent toxicity in crop plants. *J. Phytol.*, 1: 68–74
- Valentovič, P., M. Luxová, L. Kolarovič and O. Gašpariková, 2006. Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environ.*, 52: 186–191
- Velikova, V., I. Yordanov and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. *Plant Sci.*, 151: 59–66
- Vijayaragavan, M., C. Prabhakar, J. Sureshkumar, A. Natarajan, P. Vijayarangan and S. Sharavan, 2011. Soil irrigation effect of sugar mill effluent on changes of growth and biochemical contents of *Raphanus sativus* L. *Curr. Bot.*, 2: 09–13
- Wahid, A., M. Perveen, S. Gelani and S.M.A. Basra, 2007. Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.*, 164: 283–294
- Weisany, W., Y. Sohrabi, G. Heidari, A. Siosemardeh and K.G. Golezani, 2012. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics J.*, 5: 60–67
- Yang, G., D. Rhodes and R.J. Joly, 1996. Effect of high temperature on membrane stability and chlorophyll fluorescence in glycine betaine-deficient and glycinebetaine containing maize lines. *Aust. J. Plant Physiol.*, 23: 437–443
- Yildirim, E., A. G. Taylor and T.D. Spittler, 2006. Ameliorative effects of biological treatments on growth of squash plants under salt stress. *Sci. Hort.*, 111: 1–6
- Yilmaz, D.D. and K.U. Parlak, 2011. Changes in proline accumulation and antioxidative enzyme activities in *Groenlandia densa* under cadmium stress. *Ecol. Indic.*, 11: 417–423
- Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*. IRRI, Los Banos, Philippines

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