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***Full Length Article***

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

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## 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 ($H_2O_2$) 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 $H_2O_2$ 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. 

**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; Jauybon, 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

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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 Samundary, Faisalabad, Pakistan. Ten seeds were sown in each pot filled with 5 kg soil. After germination three uniforms 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 ($H_2O_2$) concentration

The $H_2O_2$ concentration was determined by following the method of Velikova *et al.* (2000). The $H_2O_2$ concentration was determined using a calibration curve constructed using a series (1-50 µM) of analytic reagent grade $H_2O_2$.

### 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 x *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 ($^oC$) | 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 alkalinily (mg/L) | 170 | - | Phosphate (mg/L) | 25 | - |
| DO (mg/L) | 2.1 | 6 | Turbidity (ppm) | 115 | 25NTU |
| BOD (mg/L) | 790 | | Oil and Greece (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 $H_2O_2$ contents in both cultivars. The highest level of SME stress caused maximal accumulation of $H_2O_2$ in both maize cultivars. Relatively higher levels of cellular $H_2O_2$ 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

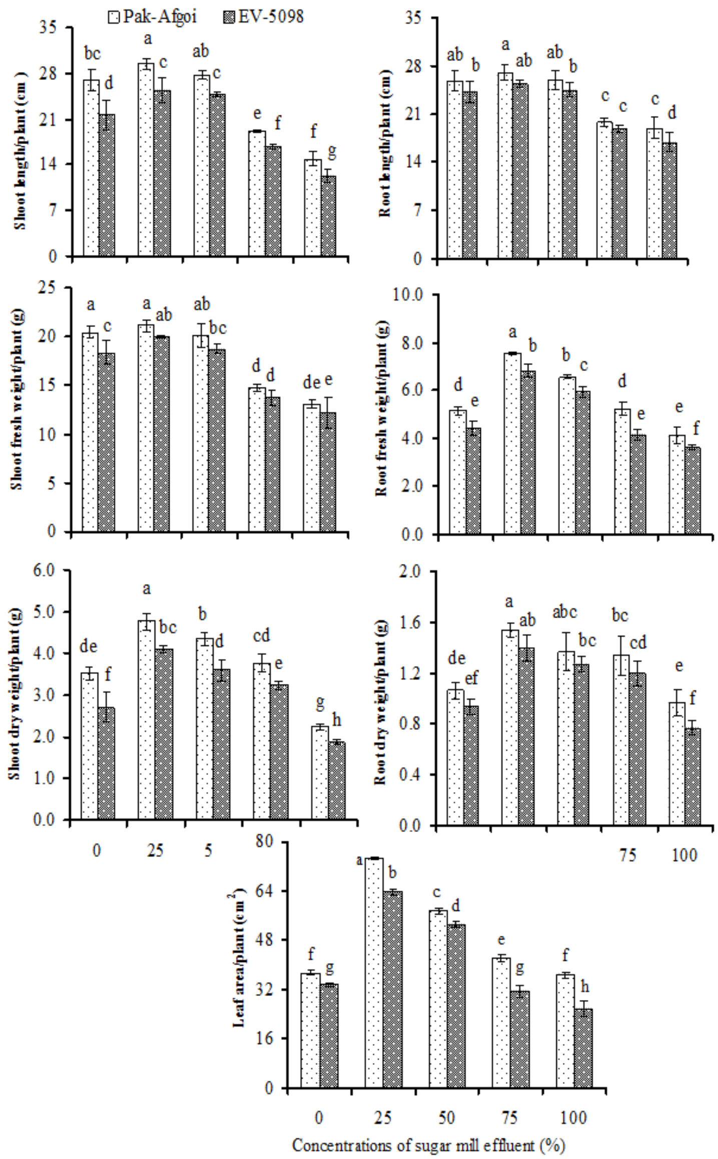


**Fig. 1:** Changes in some growth attributes of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

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

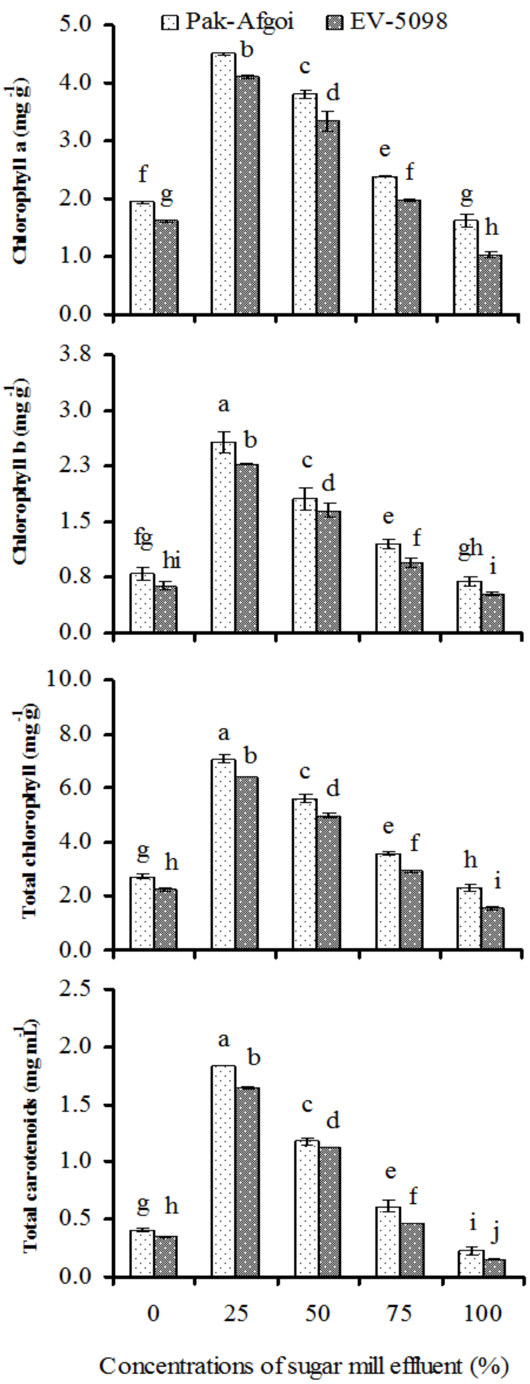


**Fig. 2:** Changes in photosynthetic pigments of maize seedlings exposed to five different levels (0, 25, 50, 75 and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

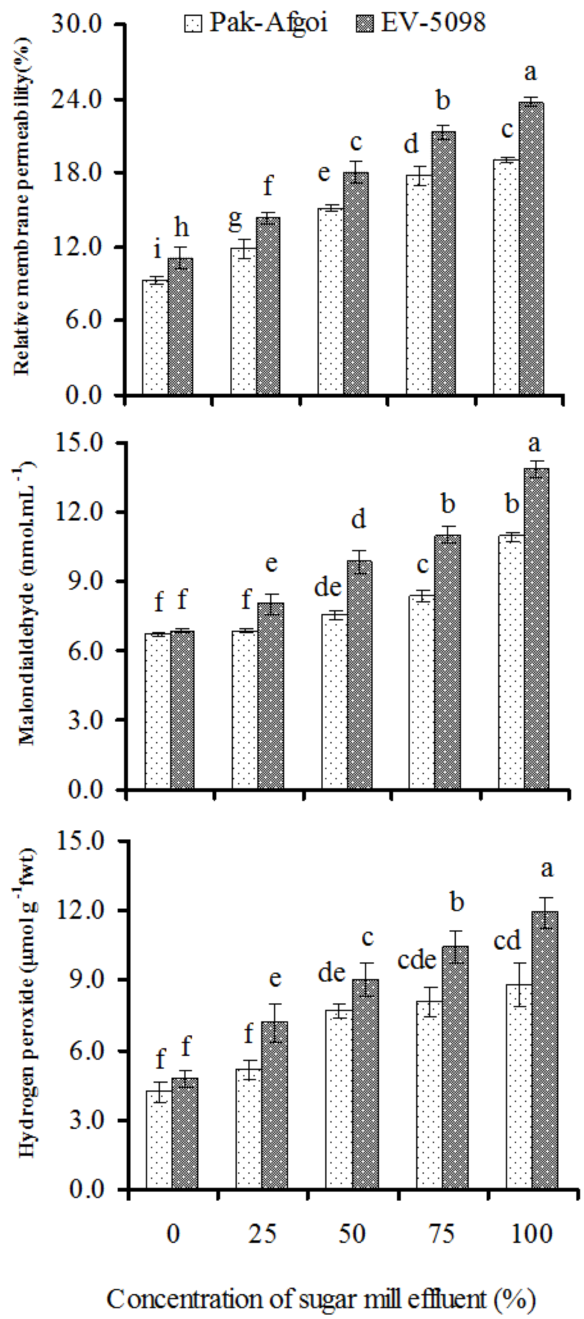


**Fig. 3:** Changes in relative membrane permeability, lipid peroxidation and hydrogen peroxide contents of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

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 (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

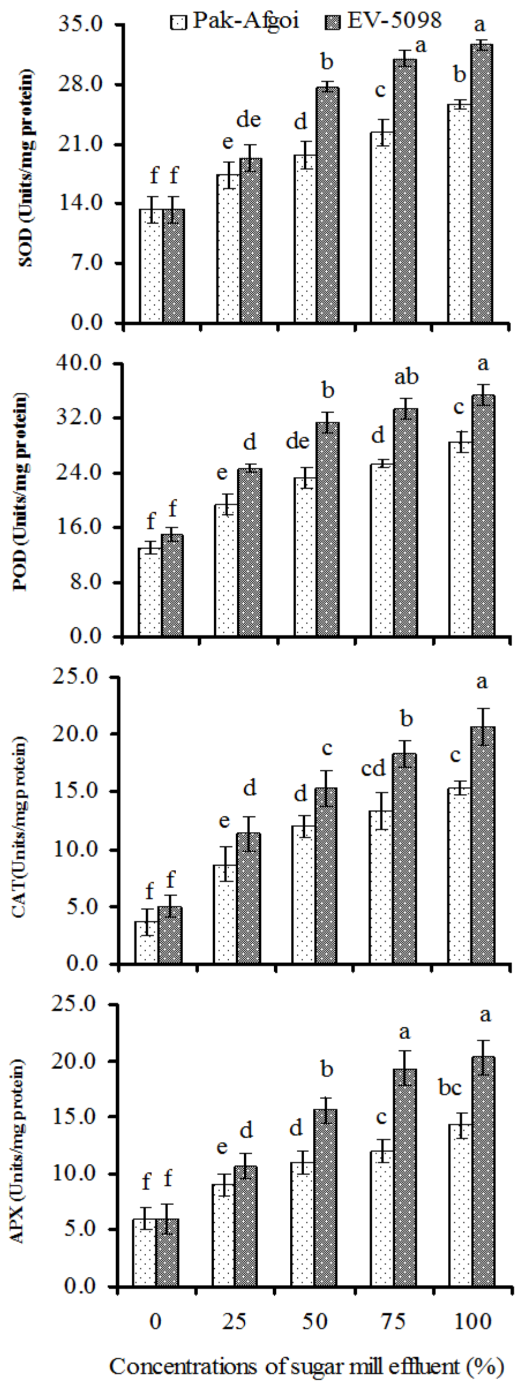


**Fig. 4:** Changes in superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and Ascorbate peroxidase (APX) activity in leaves of maize seedlings exposed to five different levels (0, 25, 50, 75 and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same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

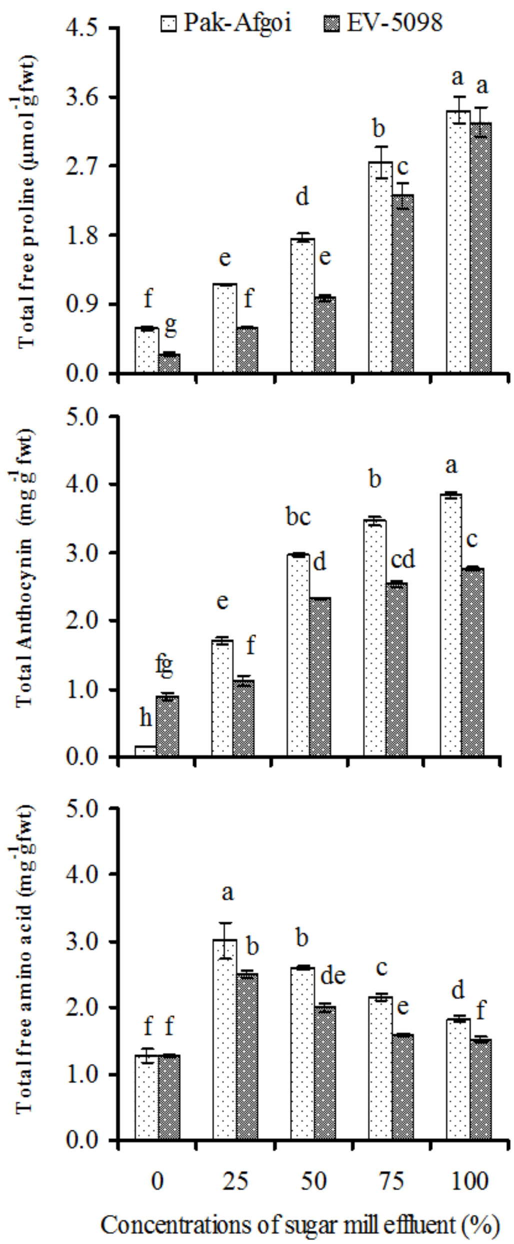


**Fig. 5:** Changes in total free proline, anthocyanin and free amino acids contents in leaves of maize seedlings exposed to five different levels (0%, 25%, 50%, 75% and 100%) of sugar mill effluent. The data presented are means of three replicates. Means sharing same letter differ non-significantly

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 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 | $H_2O_2$ |
|---|---|---|---|
| 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 *a* | -0.385* | -0.497** | -0.619*** |
| Chlorophyll *b* | -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 $H_2O_2$ (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 $H_2O_2$ under SME stress. The $H_2O_2$ concentration was negatively correlated with different growth attributes (Table 2). Lower levels of $H_2O_2$ are involved in different signaling processes (Quan *et al.*, 2008). However, plants tend to accumulate higher levels of $H_2O_2$ 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 $H_2O_2$ 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 $H_2O_2$, 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; Yılmaz 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 $H_2O_2$ 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 acids 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, $H_2O_2$ 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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