ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Human Journals
Research Article
October 2022 Vol.:25, Issue:3

Growth and Biochemical Tolerance of *Sesuvium portulacastrum* L. as a Potential Species for Phytoremediation of Pharmaceutical Effluents

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Vinayak H. Lokhande

Department of Botany, J.T.S.S.P.M. Shri Shiv Chattrapati College of Arts, Commerce and Science, Bodkenagar, Junnar, Pune, Maharashtra, India – 410 502.

Submitted: 28 July 2022
Accepted: 18 September 2022
Published: 30 October 2022

HUMAN JOURNALS

ijppr.humanjournals.com

Keywords: Antioxidant enzymes, Osmolytes, Pharmaceutical effluents, *Sesuvium portulacastrum*

ABSTRACT

Sesuvium portulacastrum is a facultative halophyte with an enormous application in the execution of various abiotic stresses. Industrial effluent is the ever-growing serious dilemma of environmental pollution. Given developing the effective strategies for detoxification of such pollutant, present investigation has been focused on understanding the tolerance potential of *Sesuvium portulacastrum* to pharmaceutical effluents concerning its growth, physiological and biochemical responses. Results on physico-chemical analyses of the effluents revealed higher values for pH, EC, BOD, COD, TDS, sulphates, chlorides, and dissolved phosphates. Phyto-toxicity analysis demonstrated complete inhibition or deprived rate of seed germination and seedling growth of glycophytes (mustard and wheat); though, the halophyte, *Sesuvium* has shown superior survival at the cost of reduced growth rate (shoot and root length), tissue water content and increased shoot dry matter content. *Sesuvium* exposed to the effluent treatment revealed a significant increase in total chlorophyll and carotenoid content in comparison to control. Proline, total soluble sugars and total phenolic content was significantly higher in shoot parts than roots of the stressed plant as compared to control. In response to increased malondialdehyde content, the superoxide dismutase (SOD) activity revealed a significant increase for the detoxification of superoxide radicals. The activities of catalase (CAT), ascorbate peroxidase (APX), guaicol peroxidase (GPX) and glutathione reductase (GR) were astonishingly higher in the roots of stressed plants than in the shoots. The outcome of the present study suggests an effective strategy for successful remediation of pharmaceutical effluents using potent abiotic stress-tolerant halophyte *S. portulacastrum*

INTRODUCTION

Pharmacologically active substances, also called antibiotics have been used intensively for the treatment and prevention of various kinds of diseases and illnesses[1]. Production of these antibiotics by the pharmaceutical industries has led to its waste discharge into drinking water bodies either in partially processed or unprocessed form, thus contaminating the natural water sources to severe extent and responsible to cause sever water pollution[2,3]. When such contaminated water is used for the irrigation of crop plants, it could possibly impact the vegetation growth, development and productivity of the crops as well as soil microbial activity [4]. Besides, the crops and food products when grown in the area contaminated with such kind of pharmaceutical effluents possesses rich content of these hazardous chemical residues and when pass through the food chain causes serious health problems to human. Although, the discharge of effluents from pharmaceutical industries into the water bodies is very high and its toxicity on vegetable, crop systems and human have been reported, but not much attention has been given on the remediation of these effluents. Different technologies such as ozonation, reverse osmosis, oxidation processes and many other techniques have been used widely to reduce the discharge of pharmaceutical effluents into receiving water bodies[5,6]; however, the cost of these processes is not affordable. Therefore, the exploitation of plants, as a natural and readily available source for the purification of contaminated water bodies is an emerging, highly cost-effective, and eco-friendly technology to reduce the toxicity of effluents.

One of the efficient methods to detect the toxic effects of unknown substances in complex mixtures or the possible synergistic effects among compounds in effluents is toxicity testing[7]. In recent years, the chemical phytotoxicity has been assessed using seed germination and plant growth test which becomes a routine technique to measure the toxicity of the industrial effluents. However, very little information is available on phytotoxicity effect of antibiotics such as sulphadimethoxine, enrofloxacin and oxytetracycline to crop plants. Available data demonstrated that the effects of antibiotics on plants in soils were found different between compounds and plant species. Therefore, the studies commenced on understanding the mechanism of these harmful pharmaceutical effluents on the growth, development, physiological and biochemical processes of plants will help to apply the eco-friendly strategies for the remediation of the contaminated environment at large scale.

Sesuvium portulacastrum is one of the important facultative halophytes showing diversified tolerance to various abiotic stress conditions such as salinity, drought, and heavy metals[8,9,10,11]. In the present investigation, the effluents from industrial area of pharmaceutical companies were collected to study their impact on tolerance potential of *S. portulacastrum* by assessing its growth, physiological and biochemical responses. The phytotoxicity was assayed using a seed germination test for two glycophytes namely wheat and mustard; whereas, phytotoxicity of *S. portulacastrum* was performed to assess its tolerance potential with the help of effect on its growth, biomass accumulation, and physiological and biochemical characteristics. Physico-chemical analyses of the effluents were carried out to support the evidences of effluent toxicity.

MATERIALS AND METHODS

Source of plant material and pharmaceutical effluents

The plant material of *S. portulacastrum* was collected from the coastal estuary located at Navi Mumbai, Maharashtra, India. The nodal segments (~ 4.0 cm) with single pre-existing axillary bud and two opposite leaves were planted in the small plastic pots (15 x 7 cm) containing fine sand and watered with tap water at an interval of 3 days for 30 days. Additionally, the plants received half-strength MS[12] solution devoid of vitamins and sucrose at ten days interval. The plants were maintained in the polyhouse with day/night temperature of 28/25^{o}C and relative humidity of 75±5%.

Collection of pharmaceutical effluent

The pharmaceutical industries discharging the weak stream effluent in the drainage system was collected from the Industrial area of Solapur, Maharashtra, India from three different locations and used for the present study.

Physico-chemical analyses of the effluents

Physico-chemical parameters such as pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD), Chloride, Sulphate, and dissolved phosphates, were analyzed as per the standard methods[13,14,15].

pH: pH of the electroplating effluent was measured using pH meter.

Electrical conductivity: For electrical conductivity, 50 ml effluent was subjected to a conductivity meter and expressed in terms of mS min^{-1}. NaCl (100 mM) was used as standard.

Total phosphates: About 100 ml effluent sample was taken into beaker, to this activated charcoal (1 gm) was added and incubated on the shaker for 6 hrs. At the end of incubation period, the solution was filtered through Whatman filter paper No. 1. The filtrate produced was the colourless solution. For total phosphate estimation, 50 ml filtrate was taken into a conical flask, to this 2.0 ml ammonium molybdate and 2.0 ml ANSA reagent was added and mixed and readings were taken.

Total sulphates: About 100 ml effluent sample was taken into beaker, to this activated charcoal (1 gm) was added and incubated on the shaker for 6 hrs. At the end of incubation period, the solution was filtered through Whatman filter paper No. 1. The filtrate produced was colourless solution. The turbidity of the solution was observed with the help of a pinch of Barium chloride. Based on the intensity of turbidity, filtrate was diluted and used for sulphate estimation.

Chlorides: The chlorides in the effluent were measured using the arginometric method. The pH of the effluent was adjusted ranged between 7 to 10 before the test. The sample was taken for analysis based on the colour, pH and appearance of the effluent. To the pH adjusted effluent ranged between 7 to 10, 1.0 ml $K_2Cr_2O_4$ solution was added as an indicator and the effluent was titrated against silver nitrate (0.041 N) till the formation of pinkish yellow colour as an endpoint.

Total dissolved solutes (TDS): 100 ml distilled water was filtered through Whatman filter paper No. 1 followed by drying the paper for 1 hr in a hot air oven at $80^{O}C$ and the initial weight of the paper was recorded. Then, 100 ml effluent was filtered through the same filter paper, dried in hot air oven and the final weight was recorded. Based on the initial and final weight of filter paper, the amount of total dissolved solutes was calculated using formula: TDS = Final weight – initial weight.

Suspended solids (SS): The initial weight of the empty porcelain dish was recorded, and the filtered effluent solution was transferred to the dish and kept on hot water bath for evaporation till total dryness. After drying, the final weight of porcelain dish was recorded.

The soluble salts were calculated using the formula: SS = Final weight of porcelain dish – Initial weight of dish.

Biological Oxygen Demand (BOD): BOD measurements were recorded at 20^{O}C in 5 days which was 70-80 % of the ultimate BOD for domestic waste water. Now a day, in order to facilitate BOD determination in less time, BOD is determined at 27^{O}C in 3 days. Thus BOD at 27^{O}C in 3 days was considered to be equivalent to BOD at 20^{O}C in 5 days.

Chemical Oxygen Demand (COD): The chemical oxygen demand test (COD) determines, the oxygen required for the chemical oxidation of organic matter with the help of strong chemical oxidant. The COD is a test used to measure pollution of domestic and industrial waste. The waste is measured in terms of equality of oxygen required for oxidation of organic matter to produce CO_2 and water. It is a fact that all organic compounds with a few exceptions can be oxidizing agents under the acidic condition. COD test is useful in pinpointing toxic condition and presence of biologically resistant substances. For COD determination samples were preserved using H_2SO_4 and processed for COD determination after the entire sampling operation was complete.

Phytotoxicity study

Seed germination test

The effect of pharmaceutical effluents on the percent seed germination of two glycophyte crops viz., wheat and mustard was carried out using the filter paper method. The seeds were surface sterilized with 30% ethanol for 3 min, washed thoroughly to remove any traces of ethanol and distributed in four different sets of both crops. Three sets of both the crop seeds were pre-treated by soaking in effluent solutions (200 seeds/50 ml solution) for six hours and the remaining one set was soaked in distilled water to serve as a control. After pre-treatment with the effluent, the seeds of wheat and mustard were placed on a filter paper (9 cm diameter) kept in each Petri dish (10 cm diameter) with 30 seeds per Petri dish and the filter papers in Petri dishes were treated with 10 ml of effluent solution. The Petri dishes were covered and incubated under conditions such as temperature 25^{O}C, 75% humidity and darkness for the first 48 hours and thereafter exposure to 16/8 hr light/dark photoperiod using cool white fluorescent light (Philips India, Ltd., 40 µmol m^{-2} s^{-1} photon flux density) up to ten days. The seed germination was evaluated considering the emergence of 2-mm long radicle as a marker for germination. The treatment was carried out in five replicates. At the end of the treatment, observations were recorded for percent seed germination.

Effluent treatments to *Sesuvium* in hydroponics

One-month-old *Sesuvium* plants grown in small plastic pots were uprooted and washed five times with distilled water to remove the remnants of sand particles. The plants were grouped into four sets each with 15 replicates and placed in the hydroponics supplemented with half MS nutrient medium devoid of vitamins and sugars for five days to acclimatize the plants to hydroponic conditions. Thereafter, the three sets of plants were exposed to pharmaceutical effluents and the remaining one set received distilled water which served as control. The plants were maintained for 5 days under hydroponic conditions in the culture room with a temperature 25^{O}C and 16/8 hr light/dark photoperiod. At the end of the treatment, the third leaf and roots of the stressed and control plants were harvested separately and used for physiological and biochemical analyses.

Growth, biomass and tissue water content

The growth and biomass accumulation was recorded in the form of fresh weight (FW) and dry weight (DW) of shoots and roots (g plant^{-1}). The FW of shoots and roots was recorded immediately after harvesting the plants; however, DW was recorded after drying the tissue at 80^{O}C in the hot air oven for 48 h till the constant weight. The FW and DW of both shoots and roots obtained from each treatment were used to determine the water status and expressed in the form of percent tissue water content (TWC %) calculated using equation [(FW-DW) / FW] x 100.

Physiological and biochemical analyses

The physiological and biochemical analyses such as accumulation of proline, and total soluble sugars were carried out following the methods described by Bates et al.[16], and Watanabe et al.[17], respectively. Oxidative damage to the membrane in terms of malondialdehyde (MDA) content was measured according to protocol of Heath and Packer[18]. Antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaicol peroxidase (GPX) and glutathione reductase (GR) were evaluated according to protocol of Becana et al.[19], Cakmak and Marschner[20], Nakano and Asada[21], Hemeda and Klein[22] and Smith et al.[23], respectively. The protein content of the enzyme extract was measured following the protocol described by Lowry et al.[24].

Data analysis

Data obtained was analysed using General Linear Model procedure in SPSS software (SPSS 10.0 for Windows), employing one-way analysis of variance (ANOVA). The significance of the differences between mean values was compared using Duncan's multiple range test (DMRT) and determined with post hoc least significant difference (LSD) test at 5 % probability. Data were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Physico-chemical characteristics of the effluent

The physico-chemical parameters of the effluents were recorded and presented in the Table 1. The weak stream effluents collected from three different localities of the pharmaceutical industrial area have shown significantly higher values and indicated toxicity as per the upper permissible limits given by the pollution control board and WHO standards[3].

Table 1: Physico-chemical properties of pharmaceutical effluents.

Parameters	Concentration*
pH	8.94 ± 0.21
Electrical Conductivity (mS min^{-1})	15.92 ± 2.54
Total dissolved solutes (g lit^{-1})	1.371 ± 0.30
Suspended solids (g lit^{-1})	0.038 ± 0.007
BOD (ppm)	4216.67 ± 508.9
COD (ppm)	1750.67 ± 246.24
Chlorides (ppm)	3858.33 ± 570.10
Sulphates (ppm)	721.35 ± 101.54
Dissolved phosphates	3.23 ± 0.98

*Values in the table indicates Mean ± SE of three independent replicates.

The pH of the effluent was significantly alkaline showing its incompatibility for the optimum growth of the microorganisms as well as the crop systems. The alkaline nature of the effluent was observed due to significantly higher values for electrical conductivity, dissolved solutes, sulfate and phosphate. This has revealed drastically the higher demand of dissolved oxygen for the living organisms growing in the vicinity of the effluent discharging area. The physico-chemical characteristics of the effluent indicates the pollution load of the effluent and reveal more polluted conditions. Our investigation was in concurrent with Mehrarad et al.[25] and Hussain et al.[3] who had revealed the toxic concentrations of pollutants in the pharmaceutical effluents whereas, Malik and Ravindran[26] have shown the toxic characteristics of effluent obtained from paper mill.

Effect of pharmaceutical effluents on seed germination

Seed germination is the most critical stage in the life cycle of the plant for the optimum growth and productivity. However, most of the abiotic stress conditions significantly affected the seed germination process and emergence of the seedling due to presence of toxic chemicals and heavy metals and hazardous suspended solids which decreases the water potential of the germination medium i.e. water. The pure water without the presence of any contaminants help in the germination of seeds relatively at higher speed in comparison to seeds germinating in the contaminated water. In the present investigation, two glycophyte systems namely wheat and mustard exposed to pharmaceutical effluent treatments revealed complete inhibition in seed germination. Similarly, significant reduction in percent seed germination and seedling vigour was recorded in *Amaranthus hybridus* exposed to pharmaceutical effluent treatments[27]. This has been correlated with the disturbed water potential of the growing medium (effluent) and the higher levels of toxic pollutants such as sulfate, chloride, and phosphate associated with higher pH values might have interfered with the physiological process of seed germination[27].

Growth, biomass, and tissue water content of *Sesuvium* to effluent treatment

The findings have revealed that glycophyte systems were not able to cope up with the adverse toxicity of the effluent as revealed by inhibited seed germination responses as well as death of the seedlings exposed to effluent treatments (data not shown). Interestingly, halophytic system, *Sesuvium* has shown significant growth tolerance potential to these adverse environmental pollutants i.e. pharmaceutical effluent. Halophytes have been identified and proved as the most evolved plant species in comparison to glycophytes with the ability to grow on soil contaminated with salinity, drought, heavy metals and effluents from different industrial sources[28,29]. In the present investigation, the *Sesuvium* exposed to pharmaceutical effluent treatments revealed survival under the adverse conditions in comparison to glycophytes at the cost of reduced growth rate and biomass accumulation (Fig. 1a-c). Shoot length of the effluent treated plants was significantly decreased in comparison to the control, however, no significant change in root length was recorded (Fig. 1a). Similarly, the relative tissue water content of the shoots was significantly decreased without showing differences in the tissue water content of the roots (Fig. 1b). Although, relative tissue water content of the effluent treated plants was significantly decreased but the dry biomass in terms of dry weight was significantly higher in comparison to control and found to be lower in the roots (Fig. 1c). The decrease in relative water content of the tissue was correlated with higher values for dry weight. Thus, the reduced growth rate and relative water content with increased dry matter of the *Sesuvium* revealed its survival even under the adverse conditions of pharmaceutical effluent exposure. The results are in concurrent with the growth responses studied for carrot[3] and maize[4] under the influence of pharmaceutical effluents. In addition, the toxic levels of sulfate, chloride and phosphate might be responsible to restrict the entry of the water inside the plant which thus decreases the turgor pressure of the root and shoot and resulted in the reduced water content, and showing direct effect on shoot and root elongation and dry weight accumulation. Further, the limited availability of the essential nutrients changes the normal physiological and biochemical processes of the plants, and more energy is transferred to tolerate the adverse conditions through the synthesis of organic osmolytes at the cost of maximum energy utilization rather than energy consumption in the growth and development of plant These are the possible reasons that can reduce the growth of the *Sesuvium* under effluent treatment. Similarly, various other workers showed adverse effect on growth, water content and dry biomass accumulation of *Sesuvium* at *in vitro* and *ex vitro* levels under various abiotic stress conditions such as salinity, drought and heavy metals[9,10,11,30,31]. Besides, Malik and Ravindran[26] had shown the decreased growth rate of the halophyte *Suaeda maritima* exposed to paper mill effluent.

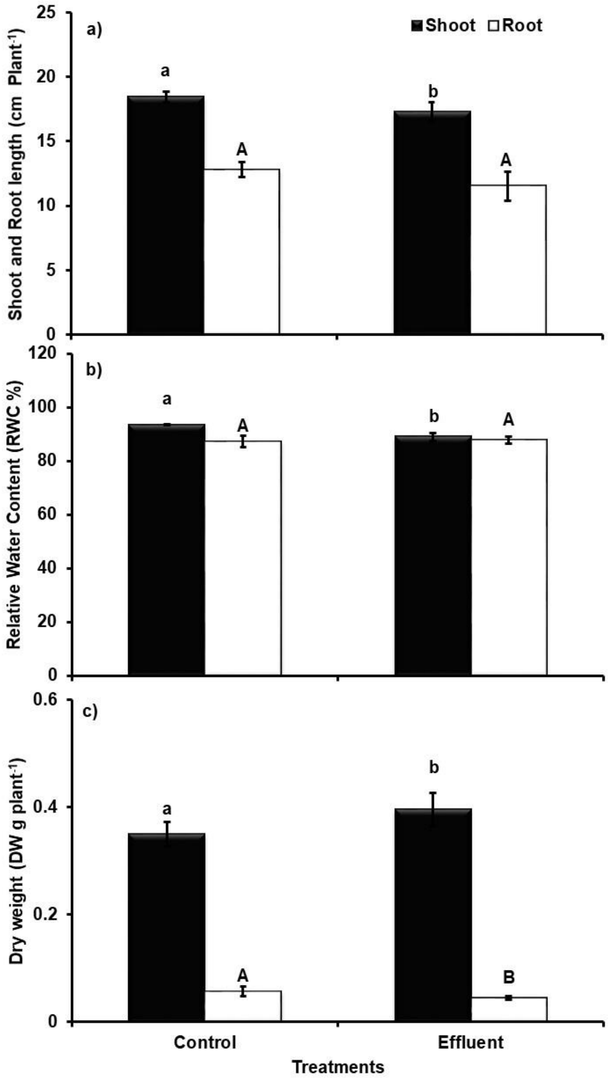

Fig.1: Effect of pharmaceutical effluent on growth characteristics of *Sesuvium.* a) - shoot and root length, b) – percent relative water content, and c) - dry weight of shoot and root.

Effect of effluent treatment on osmolytes accumulation

Generally, plants under abiotic stress conditions are stressed by an increase in the osmotic potential of rooting medium and by the toxic effect of high concentration of toxic ions[33]. Osmotic adjustment under abiotic stress is an adaptation mechanism operated by both halophytes and glycophyte maintains their water balance[33]. The best characterized biochemical response of plant cells to osmotic stress is accumulation of some inorganic ions such as sodium (Na^+) and compatible organic solutes like proline, glycine betaine (GB), and soluble sugars[33]. These compatible solutes can accumulate to high levels without disturbing intracellular biochemistry[34], protecting sub-cellular structures, mitigating oxidative damage caused by free radicals[35], and maintaining the enzyme activities under salt stress[36].

In the present investigation, accumulation of proline, and total soluble sugars has been estimated in the plants exposed to pharmaceutical effluents (Fig. 2a-b).

Proline accumulation

The plants exposed to effluent treatment revealed significantly higher accumulation of proline in shoot as well as root parts as compared to control (Fig. 2a). Effluent-treated plants revealed an almost two-fold increase in proline accumulation in comparison to control (Fig. 2a). Accumulation of proline under stress conditions is an indication of oxidative damage which provides protection to cytosol from dehydration through the development of compatible cytoplasmic osmoticum. Proline act as osmotica in the presence of low water content and plays a major role in the maintenance of osmotic balance, which was found to be higher in tolerant species (halophytes) than in sensitive species[37]. Proline also play role in the protection of enzymes and stabilizing proteins. The increase in proline content of *Sesuvium* in response to various abiotic stress conditions has also been demonstrated by various workers[9,10,11,30,31]. An increase in free proline content has also been demonstrated in carrot plants exposed to pharmaceutical effluent treatments[3]. Similarly, *Suaeda maritima* revealed an increasing trend of proline accumulation in response to paper mill effluent treatment[26].

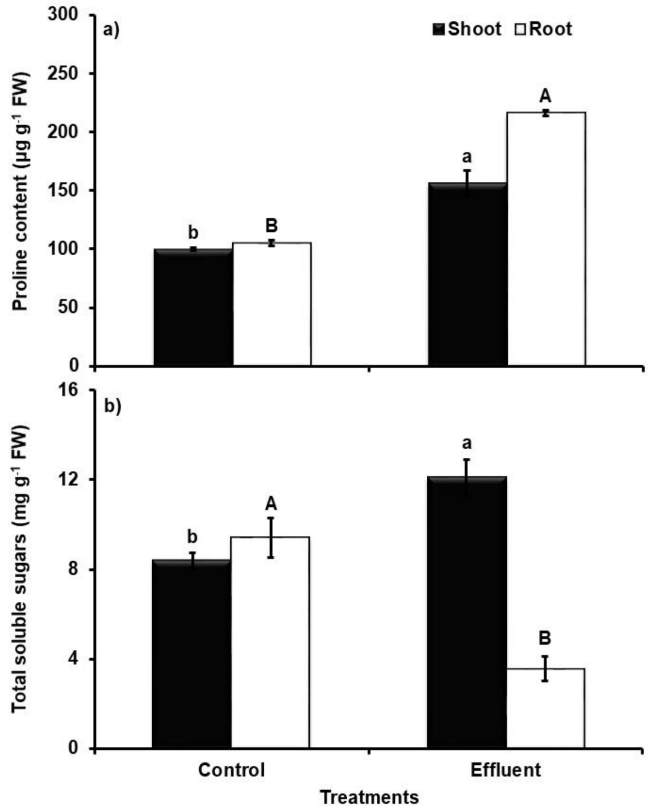

Fig. 2: Effect of pharmaceutical effluent on osmolytes accumulation in the shoot and roots of *Sesuvium.* a) – accumulation of proline content and b) – total soluble sugar content.

Total soluble sugars accumulation

In general, modulations in the carbon metabolism and the levels of carbohydrates (sugars) are seen due to changes occurring in the process of photosynthesis and carbon partitioning of the plant at organ level and in whole plants exposed to abiotic stresses. Soluble sugars function as metabolic resources and structural constituents of cells, besides acting as signals regulating various processes associated with plant growth and development. The accumulation of soluble sugars has been attributed as an important parameter of osmotic adjustment in the halophytes exposed to a variety of stresses[9,10,11]. A variety of sugar compounds such as sucrose, glucose, mannose, maltose, trehalose, and many other sugar alcohols have been studied in response to abiotic stresses[38]. In the present investigation, *Sesuvium* plants exposed to effluent treatment revealed significantly higher accumulation of total soluble sugars in shoot parts than roots as compared to the control (Fig. 2b). Our results are in concurrent with the accumulation of total soluble sugar content in the carrot exposed to pharmaceutical effluents[3]. Previously, the accumulation of TSS has also been observed under variable growth conditions in the *Sesuvium* after exposure to salinity and drought stress[9,10,11].

Effect of effluents on lipid peroxidation

Oxidative stress due to effluents disturbed the physiology of plants and affected the essential molecules of plant cell like proteins, lipids and nucleic acids. The measurement of lipid peroxidation in terms of MDA content due to the production of reactive oxygen species under abiotic stress conditions has frequently been used as an indicator of oxidative stress. The extent of damage to the membrane in terms of MDA content demonstrates the toxicity of abiotic stress in plants. In the present investigation, plants exposed to effluent treatment showed an increase in MDA content of the shoots and roots in comparison to the control (Fig. 3a). A similar increase in membrane damage in the *Sesuvium* calli exposed to salt stress or iso-osmotic salt and PEG stress has been observed by Lokhande et al.[10,11]. In correlation to the results of the present investigation, pharmaceutical effluents significantly increased the MDA content in the *Typha*, and carrot plants[3,5].

Effect of effluents on antioxidant enzymes

The halophytic plants display a cascade of events upon exposure to environmental stresses leading to metabolic disturbance. The cascade of events includes physiological water deficit-abscisic acid regulated stomatal closure in leaves, limited CO_2 availability, over-reduction of electron transport chain in the chloroplast and mitochondria and finally generation of reactive oxygen species (ROS). These ROS are highly toxic and in the absence of a protective mechanism in the plant can cause oxidative damage to proteins, DNA and lipids[38]. Additionally, this may also lead to alteration in the redox state resulting in further damage to the cell[40]. To regulate the ROS levels, plant cells evolved with complex enzymatic and non-enzymatic antioxidant defense mechanisms, which together help to control the cellular redox state under changing environmental conditions. Superoxide dismutase (SOD) constitutes the first line of defense converting superoxide radicals ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2), which is further reduced to water and oxygen by ascorbate peroxidase (APX) and catalase (CAT). Monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), Glutathione reductase (GR) and glutathione peroxidase (GPX) are an important enzymes involved in the regeneration of ascorbate and GSH for the proper functioning of ASC-GSH cycle[39,41].

Halophytes have evolved various mechanisms of adaptations of which increased antioxidant enzyme activities was found one of an important mechanism of stress tolerance. In the present investigation, plants exposed to effluent treatments responded better in terms of various antioxidant enzyme activities such as SOD, CAT, APX, GPX and GR (Fig. 3b-f). SOD activity showed a significant increase in shoot and root parts of the plants exposed to effluent treatments in comparison to control (Fig. 3b). CAT activity was found to be significantly higher in roots than shoots of the plants exposed to effluent treatments (Fig. 3c). However, the APX and GPX activities were significantly higher in both shoot and root parts as compared to control (Fig. 3d,e).

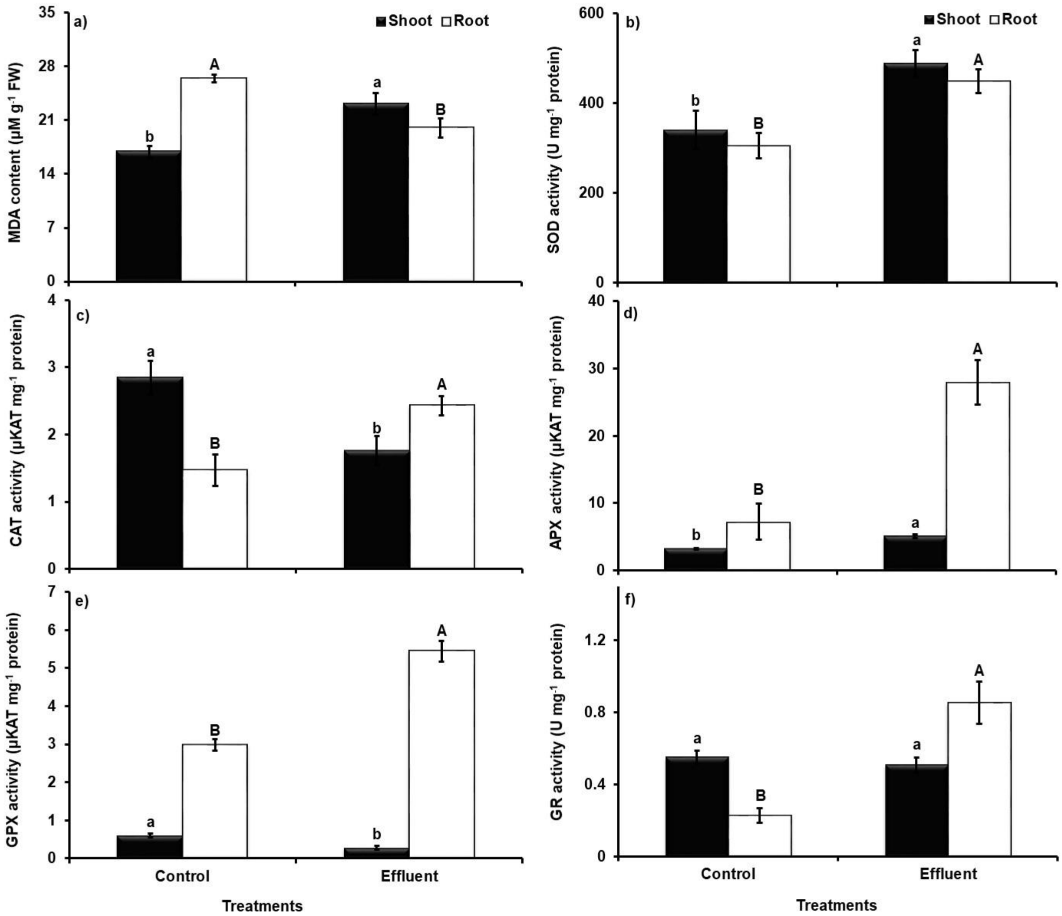

Fig. 3: Effect of pharmaceutical effluent on MDA content formation and antioxidant enzyme activities in the shoots and roots of *Sesuvium.* a) – MDA content; b) SOD; c) CAT; d) APX; e) GPX and f) GR activities.

GR activity revealed a significant increase in roots with no change observed in shoot parts in comparison to control (Fig. 3f). The results revealed that the SOD activity was correspondingly higher in both shoot and root parts of effluent-treated plants which is taking important role in dismutation of superoxide radical to form H_2O_2. Further degradation of H_2O_2 was carried out by enhanced activities of CAT, APX, and GPX. However, GR showed its role in regeneration of reduced glutathione (GSH) for the proper functioning of water-water cycle. The results of the present investigation suggest that, antioxidant enzymes are efficiently playing the important role in the detoxification of ROS generated due to oxidative stress formed by toxic chemical composition of pharmaceutical effluents. Similarly, Lokhande et al.[10] found the effective role of enzymatic and non-enzymatic antioxidants in protecting the plants from oxidative stress generated by salt stress and other abiotic stress conditions. Our results are in agreement with the Iori et al.[2], Hussain et al.[3], Baskaran et al.[42].

CONCLUSION

It is inferred that, glycophyte being the sensitive plant systems are not able to cope the adverse anthropogenic and climatic conditions caused due to abiotic stresses like pharmaceutical effluents, salinity, drought and heavy metals contamination in the soil and water bodies. On the other side, halophytes with the innate ability to overcome any kind of adverse growth conditions develops very strong defence system of mechanism against diversified abiotic stresses by synthesizing the osmolytes to greater extent and removing the excessively produced reactive oxygen species due to the effective mechanism of antioxidant enzymes. In the present investigation, *Sesuvium portulacastrum* has significantly proved the tolerance mechanism against very toxic pharmaceutical effluents at the cost of reduced growth rate and synthesis as well as accumulation of osmolytes (proline and total soluble sugars). Besides, the superoxide dismutase (SOD) enzyme has developed the first line of defence mechanism to remove the superoxide radical and make the journey of catalyzing enzyme smooth to break down the hydrogen peroxide. The outcome of the research suggests that, *Sesuvium* could effectively be useful strategy for successful remediation of pharmaceutical effluents in the regimes of pharmaceutical industrial areas.

REFERENCES

1. Liu F, Ying GG, Tao R, Zhao JL, Yang JF, Zhao LF (2009) Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ Poll. 157: 1636-1642.
2. Iori V, Pietrini F, Zacchini M (2012) Assessment of ibuprofen tolerance and removal capability in *Populus nigra* L. by *in vitro* culture. J. Haz. Mat. 229-230: 217-223.
3. Hussain I, Rehman K, Ashraf MA, Rasheed R, Gul J, Akash MSH, Bashir R (2021) Effect of pharmaceutical effluents on growth, oxidative defense, secondary metabolism, and Ion homeostasis in Carrot. Dose-Response: An Int J (Jan-March): 1-11.
4. Osaigbovo AM, Orhue ER (2006) Influence of pharmaceutical effluent on some soil chemical properties and early growth of maize (*Zea mays* L). Afri. J. Biotechnol. 5(12): 1612-1617.
5. Dordio AV, Belo M, Teixeira DM, Palace Carvalho AJ, Dias CMB, IPico Y, Pinto AP (2010) Evaluation of carbamazepine uptake and metabolization by *Typha* spp., a plant with potential use in phytotreatment. Biores Technol 102: 7827-7834.
6. Zhang D, Gersberg RM, Ng WJ, Tan SK (2014) Removal of pharmaceutical and personal care products in aquatic plant-based systems: A review. Environ. Poll. 184: 620-639.
7. Sacan MT, Balcioglu IA (2006) A case study of algal response to raw and treated effluents from an Aluminum plating plant and a pharmaceutical plant. Ecotox. Env. Saf. 64: 234-243.
8. Lokhande VH, Nikam TD, Suprasanna P (2009) *Sesuvium portulacastrum* (L.) L., a promising halophyte: cultivation, utilization, and distribution in India. Genet. Resour. Crop Evol. 56:741-747.
9. Lokhande VH, Nikam TD, Suprasanna P (2010) Biochemical, physiological and growth changes in response to salinity in callus cultures of *Sesuvium portulacastrum* L. Plant Cell Tiss Org. Cult. 102:17-25.
10. Lokhande VH, Srivastava S, Patade VY, Dwivedi S, Tripathi RD, Nikam TD, Suprasanna P(2011) Investigation of arsenic accumulation and tolerance potential of Sesuvium portulacastrum (L.) L. Chemosphere. 82:529–534.
11. Lokhande VH, Patade VY, Srivastava S, Suprasanna P, Shrivastava M, Awasthi G (2020) Copper accumulation and biochemical response of *Sesuvium portulacastrum* (L.). Mat. Today: Proceed. 31: 679-684.
12. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497.
13. FAO .1985. Water Quality for Agriculture, 1985: Recommendations of the FAO (Food and Agriculture Organization of the United Nations) for the quality of water used for irrigation purposes. http://www.fao.org/DOCREP/003/T0234E/T0234E00.htm.
14. Fu W, Franco A, Trapp S (2009) "Methods for estimating the bioconcentration factor of ionizable organic chemicals. Environ Toxicol Chem 28: 1372-1379.
15. Ziarati, P., Ziarati, N.N., Nazeri, S., Saber-Germi, M (2015) Phytoextraction of heavy metals by two *Sorghum* spices in treated soil using black tea residue for cleaning-up the contaminated soil. Orient J Chem 31: 317-26.
16. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205-208.
17. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I– kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophy125:189-198.
18. Watanabe S, Kojima K, Ide Y, Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in Populus euphratica in vitro. Plant Cell Tiss Organ Cult 63:199-206.
19. Becana M, Moran JF, Iturbe-Ormaetxe I (1986) Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. Plant Soil 201:137-147.
20. Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol 98:1222-1227.
21. Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867-880.
22. Hemeda HM, Klein BP (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J Food Sci 55: 184-185, 192.

23. Smith IK, Vierheller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5’-Dithio-bis(2-nitrobenzoic acid). Anal Biochem 175:408-413.
24. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265-275.
25. Mehrarad F, Ziarathi P, Mousavi Z (2016) Removing heavy metals from pharmaceutical effluent by *Pelargonium grandiflorum*. Biomed. Pharma. J. 9(1): 151-161.
26. Malik ZH, Ravindran KC (2018) Biochemical tolerance of *Suaeda maritima* L. (Dumort) as a potential species for phytoextracting heavy metals and salt in paper mill effluent contaminated soil.
27. Nwadinigwe AO, Amadi CC, Amujiri AN, Nwadinigwe CC, Ozokolie CB (2019) Effects of pharmaceutical effluents on germination, growth and development of *Amaranthus hybridus* L. NJB 32(2):1-10.
28. Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. Ann Rev Plant Physiol 28:89-121.
29. Munns R, Tester M (2008) Mechanism of salinity tolerance. Annu Rev Plant Biol 59:651-581.
30. Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel MH, Abdelly C (2007) Effect of Cd^{2+} on K^{+}, Ca^{+} and N uptake in two halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: Consequences on growth. Chemosphere 67:72-79.
31. Slama I, Messedi D, Ghnaya T, Savouŕe A, Abdelly C (2006) Effects of water-deficit on growth and proline metabolism in *Sesuvium portulacastrum.* Environ Expt Bot 56:231-238.
32. Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239-250.
33. Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945-963.
34. Bohnert HJ, Jensen RG (1996) Strategies for engineering water stress tolerance in plants. Trends Biotechnol 14:89-97.
35. Attipali R.R., Kolluru V.C., Munusamy V. (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189-1202.
36. Yokoi S, Bressan RA, Hasegawa PM (2002) The Japan International Centre for Agricultural Sciences (JIRCAS) Working Report No. 23. In Iwanaga M (ed) Genetic engineering of crop plants for abiotic stress. Salt stress tolerance of plants. Tsukuba: Japan International Centre for Agricultural Sciences Publishing, pp 25-33.
37. Zhang F, Yang YL, He WL, Zhao X, Zhang LX (2004) Effects of salinity on growth and compatible solutes of callus induced from *Populus euphratica*. In vitro Cell Dev Biol-Plant 40:491-494.
38. Yuanyuan, M., Yali, Z., Jiang, L., and Hongbo, S. 2009. Roles of plant soluble sugars and their responses to plant cold stress. Afri. J. Plant Biotechnol. 8:2004-2010.
39. Mittler R (2002) Oxidative stress, antioxidants, and stress tolerance. Trends in Plant Sci 7:405-410.
40. Mittler R, Vanderauwera S, Gollery M, Breusegem FV (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490-498.
41. Noctor G, Foyer CH (1998) Ascorbate and Glutathione. Keeping active oxygen under control. Ann Rev Plant Physiol Mol Biol 49: 249-279.
42. Baskaran L, Chidambaram AA, Sundaramoorthy P, Unnikannan P. Bioreclamation of Sugar Mill Effluent Polluted Soil Using Earth-worm and its Responses on Green gram (*Vigna radiata* L.). J Phytol. 2009;1(1):07.

Author -1

Author Name – Dr. Lokhande Vinayak Haribhau
Author Affiliation: Corresponding author.
Author Address/Institute Address:
Department of Botany, J.T.S.S.P.M. Shri Shiv Chattrapati College of Arts, Commerce and Science, Bodkenagar, Junnar, Pune, Maharashtra, India – 410 502.