Punjab Univ. J. Zool., Vol. 28 (2), pp. 55-60, 2013

Original Article

Beneficial impact of selenium resistant bacteria on selenium contaminated soil and plant growth

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(**Article history:** Received: August 03, 2013; Revised: November 17, 2013)

Abstract
Present study deals with the inoculation of two selenium resistant bacterial strains *Bacillus pumilus* and *Bacillus licheniformis* on various growth parameters of *Helianthus annuus* (sunflower) in pot trials. For pot experiment natural garden soil was used but in one experiment the soil was used as sterilized. Bacterial inoculations caused a reduction (14%) in seed germination as compared to un-inoculated (control). Maximum root length (103 cm) was observed in inoculated sunflower plants in sterilized garden soil while maximum shoot length (28 cm) was seen in plants growing in sterilized soil without inoculums. Maximum number of roots was observed in case of un-inoculated control plants grown in sterilized soil. Inoculated plants grown in sterilized soil exhibits highest auxin content (72%) as compared to other treatments where it range from 22 to 25%. Acid phosphatase and peroxidase activity was optimum in case of control plants grown in sterilized garden soil.
Key words: Selenium, phytoremediation, bacteria, plant growth, auxin.

To cite this article: ANAM, HUSSAIN, A. AND FAISAL, M., 2013. Beneficial impact of selenium resistant bacteria on selenium contaminated soil and plant growth. *Punjab Univ. J. Zool.,* **28**(2): 55-60.

INTRODUCTION

Most important component of environment (water and land) is valuable natural assets and mankind survival is greatly dependant on these resources. Unfortunately, these resources have been utilized to maximum which severely damaged or polluted due to such action (Islam *et al.,* 2007). The major component (heavy metals, pesticides and chlorinated solvents) of this polluted ecosystem have been extensively reported in literature (Menezes-Oliveira *et al.,* 2013). Each of these components is very harmful to the living organisms including microorganisms, plants, animals and humans. The heavy metals accumulated in wastewater and soil is of special concern because of their low solubility in biota and their carcinogenic and mutagenic impact (Akbar *et al*., 2011; Saeedi and Shokrzadeh, 2013). Selenium (Se) is metalloid and ubiquitous in the environment. In most soils its concentration varies from 0.01 to 2.0 mg kg^{-1}, and usually 0.4 mg kg^{-1}; however, sometimes its concentrations (>10 mg kg^{-1}) may be present in seleniferous areas. Beside its natural occurrence selenium is also used in many industries such as petroleum refining and coal carbonization (Berrow and Ure, 1989). Although, Se is not considered an essential element for plant growth but it is beneficial to plants capable of accumulating it (Bañuelos *et al*., 2012). More selenium is accumulated in shoot and leaf than in roots (Zwolak and Zaporowska, 2012). Bacteria have been found capable of reducing oxyanions of selenium Se(IV) and Se(VI), either to elemental selenium Se(0) which is less soluble and less toxic form or to a volatile form Se(II) (Yasin and Faisal, 2013). The reduction of soluble oxyanions to insoluble red selenium precipitates occurs both under aerobic and anaerobic conditions by indigenous soil bacteria (Ikram and Faisal, 2010). The objectives of the present study was to evaluate the role of selenium resistant bacteria in improving growth of cash crop (sunflower) under metal (Selenium) stress conditions under natural day light and temperature.

MATERIALS AND METHODS

Pot experiments were conducted to evaluate the role of selenium resistant bacteria in improving growth of plants and accumulation

0079-8045/13/0055-0060 $ 03.00/0

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of selenium in plants under metal stress conditions and natural day light and temperature. This experiment was designed to study the effect of direct inoculations of soil with mixed bacterial cultures on plant growth (sunflower) under metal stress conditions. For pot experiments, mixed cultures of *Bacillus pumilus* and *Bacillus licheniformis* were used.

Normal field soil was collected and 3.2 Kg soil was filled in each earthen pot (15x17cm). The soil had a pH of 8.5; E.C. 1.92; T. C. 20.4; S.A.R. 4.47; organic matter 0.156. Soil was sieved with 2mm sieve. For soil sterilization, weighed soil was packed in polyethylene bags and sterilized at 121° C for 15 min at 15 lb pressure. After 24 hours, same process was repeated and soil was cooled to room temperature overnight. The experiment was designed as follow. P1, Un-inoculated control sunflower in natural garden soil; P2, Inoculated sunflower plants in natural garden soil; P3, Un-inoculated control sunflower in sterilized garden soil and P4, Inoculated sunflower plants in sterilized garden soil. Certified seeds of *Helianthus annuus* var. Hysun-33 (a hybrid imported from Australia) were collected from National Agriculture Research Center (NARC), Islamabad, Pakistan and they were surface sterilized with 0.1% $HgCl_2$ for 6 min and then washed with sterilized distilled water for four to five times before sowing. At maturity (21 weeks after sowing) plants were carefully removed, washed with tap water to remove any attached particles. Various growth parameters and chemical analysis of harvested plants were performed. Along with test control experiment was also run parallel.

RESULTS

Percentage seed germination

After sowing plants were daily observed to count the number of seeds germinated per day. Seed germination started after 5 days of sowing and completed in 15 days. The seed germination observed was 86% for P1, 75% for P2, 97% for P3 and 78% for P4 (Fig. 1).

Measurement of growth parameters

After harvest different growth parameters like root length, shoot length, number of roots, number of leaves, fresh weight of plant, dry weight of plant and dry weight per gram of fresh weight were measured and results were noted.

Maximum root length (103 cm) was observed in P4 where inoculums was given in sterilized garden soil whereas maximum shoot length (28 cm) was seen in sterilized soil without inoculums (Fig. 1). Plants grown in soil without inoculums gave highest number of the leaves and maximum fresh weight whereas plants germinated in sterilized soil gave maximum number of roots to the plants and had high dry weight. Un-inoculated sterilized soil showed more dry weight per gram of fresh weight as compared to others treatments (Fig. 2).

Estimation of indole acetic acid and soluble protein content

In both treatments *i.e.,* control and sterilized soil, an increase in indole acetic acid (IAA) and soluble protein content was observed in those plants in which inoculum is present (Fig. 3).

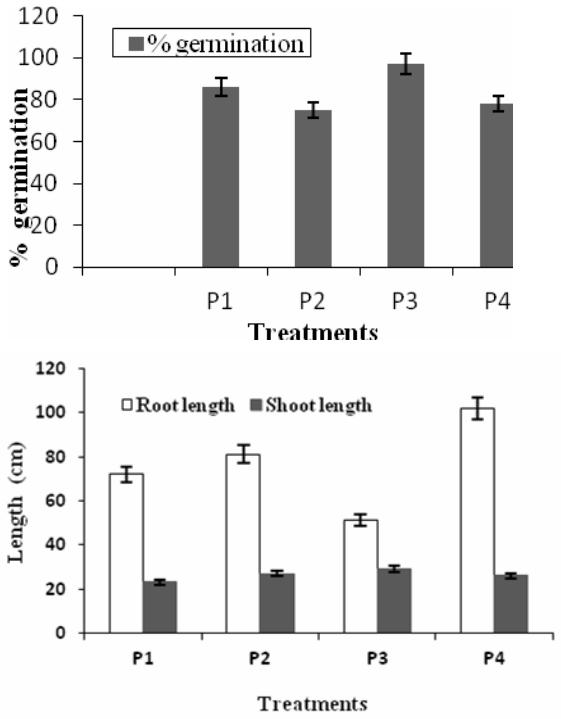

Figure 1 Effect of bacterial inoculation on seed germination, root and shoot length of sunflower plants grown in natural and sterilized garden soil. P1; Un-inoculated control sunflower in natural garden soil. P2; Inoculated sunflower plants in natural garden soil. P3; Un-inoculated control sunflower in sterilized garden soil. P4; Inoculated sunflower plants in sterilized garden soil.

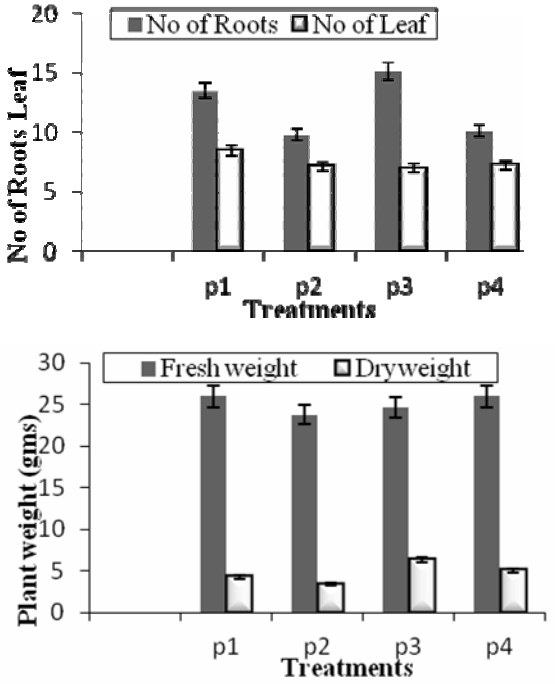

Figure 2 **Effect of bacterial inoculation on number of roots, number of leaves, fresh weight (g) and dry weight (g) of sunflower plants.**

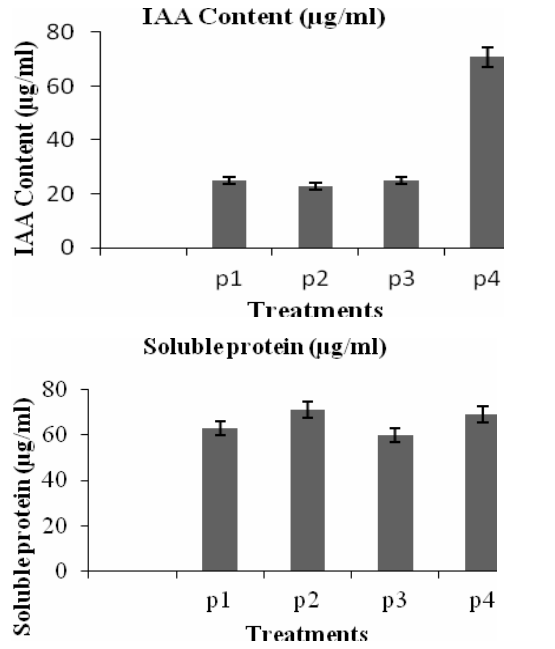

Figure 3 **Effect of bacterial inoculation on indole acetic acid and soluble protein content of sunflower plants grown in natural and sterilized garden soil.**

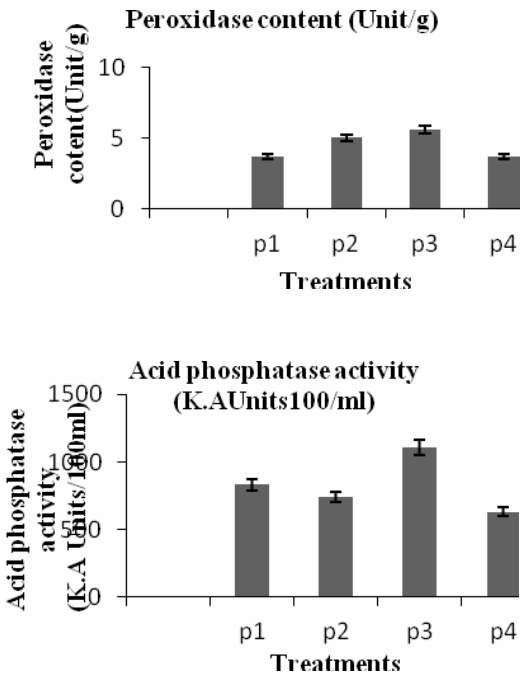

Figure 4 **Effect of bacterial inoculation on peroxidase and acid phosphatase content of sunflower plants grown in natural and sterilized garden soil.**

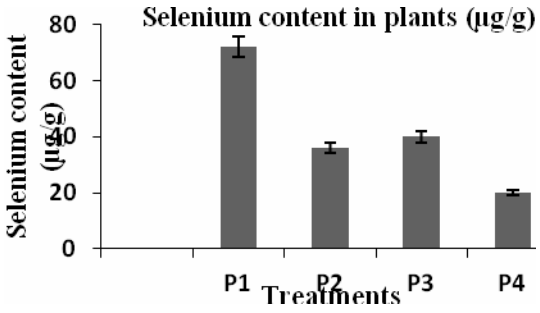

Figure 5 **Effect of bacterial inoculation selenite content of sunflower plants grown in natural and sterilized garden soil.**

Peroxidase content

Peroxidase content was determined using frozen plant material. An increase in peroxidase content was observed in P2 and P3 while no significant difference was observed in P1and P4 (Fig. 4).

Estimation of acid phosphatase

Acid phosphatase content was determined by using frozen plant material.

Decrease in acid phosphatase content was observed in P2 and P4 and some incensement in P3 (Fig. 4).

Determination of selenium content in plants and soil

A decrease in metal content of plant was observed in P2, P3 and P4. In case of selenium uptake, marked decrease was observed in all treatments, maximum decrease was observed in P4 (Fig. 5).

DISCUSSION

Selenium is an important metalloid required for the well being of many organisms including humans, animals and plants in small concentrations. Humans and animals mainly get Se from dietary plant sources and plants accumulate selenium according to the Se concentrations and chemical species in the soil. Selenium creates serious problems in both high and low concentrations. Selenium can be readily accumulated or volatilized by certain plants which serve as significant potentials for phytoremediation (Pilon-Smits and LeDuc, 2009). High amounts of Se accumulated in soils released as a result of various human activities are highly toxic and efforts should be done to remediate this serious issue. Microbial activities are considered as the primary means by which, highly toxic, soluble and bioactive forms of selenium can be converted to inert states.

The present study suggests that the addition of bacterial cultures promoted seed germination in both sterilized and non-sterilized soil samples. In the study conducted by Rajkumar and Freitas (2011), the addition of plant growth promoting bacteria which showed resistance to metal improved the plant growth under metal stress conditions. Rhizobacteria not only play role in metal detoxification but they also facilitate plant growth by producing siderophores and other growth promoters. Among the various parameters used to estimate the effect of treatments on plants growth, root lengths revealed that seeds grown in sterilized inoculated soil produced longest roots followed by those grown in soil with inoculums, soil without inoculums and sterilized soil without inoculums. So, in this case bacteria might have enhanced the plant growth by producing hormones leading to roots elongation. However, shoot length grew at maximum in sterilized soil without inoculums followed by P2 whereas, P1 and P4 showed equal shoot length. Ramos *et al.* (2003) observed an increase in root lengths of Alder plant when the seedlings were germinated in the soils inoculated with *Bacillus licheniformis* an important example of plant growth promoting rhizobacteria (PGPR). Riaz *et al.* (2010) also demonstrated that PGPR mostly stimulate plants growth by producing auxin, gibberellins and cytokinins along with many other factors. P3 treatment (sterilized soil without inoculum) produced maximum no. of leaves whereas P1 treatment (soil without bacteria) was reported as giving maximum no. of roots. Minimum roots and leaves were found in treatments P2 and P3 respectively. So the results of the present study depicted that the number of roots and leaves of germinating plants were not enhanced under the influence of rhizobia inoculums. However, Lucy *et al.* (2004) demonstrated that rhizobacteria can be used to enhance plants leaves in order to use them for forest regeneration purposes and also to phytoremediation the contaminated sites. Fresh and dry weights of plants germinated in the present study also revealed independence on the presence of bacterial inoculums in the soils. P3 treatment showed greatest ratio for dry weight per gram of fresh weight. In this study, plants grown in P4 soil with inoculum gave maximum values for indole acetic acid (IAA) production. Many types of bacteria such as Rhizobacteria along with higher plants and fungi tend to produce IAA in the soil either in the presence or absence of the physiological precursors like tryptophan. Exogenous IAA has profound impact on the plant growth and development particularly enhancing the root length shoots length and formation of adventitious roots (Shahab *et al.,* 2009). In the current study, soluble protein content of plants grown in soil with bacteria was highest among all of the treatments. Therefore, these results show that bacterial inoculums protected plants from having any decline in their soluble protein content that might occur due to their exposure to heavy metal stress. Bacteria might have reduced the toxicity of metals in the surrounding environment and thus protects plants from protein deprivation. John *et al.* (2009) observed that protein content of *Brassica juncea* was reduced from 87.3% to 77.4% by Cd and Pb respectively which were present in the surrounding environment.

The present study showed that peroxidase and acid phosphatase content was highest among plants grown in sterilized soil having no bacterial inoculums. So, bacteria did

not contribute significantly towards these parameters. Shahab *et al.* (2009) stated that those bacteria which are capable of phosphate solubilization improve plant growth by making Pi available to them. And in this case bacteria are not solubilizing phosphate available in various bound forms in the soil. Kawano (2003) revealed that exogenous plant peroxidase catalyze the production of reactive forms of oxygen along with the oxidation of plant growth hormones such as IAA and defense related compounds such as salicylic acid, aromatic monoamines and oligosaccharides. So, these enzymes play important roles in generating various components of plant growth promoting or signaling pathways.

In the present study, a decrease in metal content of plant was observed in P2, P3 and P4. In case of soil, marked decrease was observed in all treatments, maximum decrease was observed in P1. Jing *et al.* (2007) declared that the microbial populations in the soil affect the availability and mobility of heavy metals and metalloids to the plants by various mechanisms that may include the release of chelating agents, acidification, phosphate solubilization and redox changes. All of these may facilitate the plant protection from toxic effects of these metals and promote plant growth by releasing growth factors.

Acknowledgements
This research work was funded by University of the Punjab, Lahore, Pakistan.

REFERENCES

AKBAR, M., BREWER, J.M. AND GRANT, M.H., 2011. *Effect of chromium and cobalt ions on primary human lymphocytes in vitro. J. Immunotoxicol.,* **8**: 140.

BAÑUELOS, G.S., STUSHNOFF, C., WALSE, S., ZUBER, T., YANG, S.I., PICKERING, I.J. AND FREEMAN, J.L., 2012. Biofortified, selenium enriched, fruit and cladode from three Opuntia Cactus pear cultivars grown on agricultural drainage sediment for use in nutraceutical foods. *Food Chem.,* **135**: 9-16.

BERROW, M.L. AND URE, A.M., 1989. Geological materials and soils. In: *Occurrence and Distribution of Selenium* (ed. Ihnat, M.). CRC Press, Inc., Boca Raton, FL, USA, pp. 213-242.

IKRAM, M. AND FAISAL, M., 2010. Comparative assessment of selenite (SeIV) detoxification to elemental selenium (Se0) by *Bacillus* sp. *Biotechnol*. *Lett*., **32**: 1255-1259.

ISLAM, E.E., YANG, X., HE, Z.L, AND MAHMOOD, Q. 2007. Assessing potential dietary to toxicity of heavy metals in selected vegetables and food crops. *J.Zhejiang University Sci. B.,* **8**: 1-13.

JING, Y., HE, Z. AND YANG. X., 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. Biomed. *Life Sci*., **8**: 192-207.

JOHN, R., AHMAD. P., GADGIL. K. AND SHARMA. S., 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *Int. J. Plant Prot*., **3**: 65-75.

KAWANO, T., 2003. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Biomed. Life Sci*., **21**: 829-837.

LUCY, M., REED. E. AND GLICK. B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek*., **86**: 1-25.

MENEZES-OLIVEIRA,V.B., SCOTT-FORDSMD, J.J., SOARES, A.M.V.M. AND AMORIM, M.J.B., 2013. Effects of temperature and copper *pollution* on soil community-extreme temperature events can lead to community extinction**.** *Environ. Toxicol. Chem*., **32**: 2678-2685.

PILON-SMITS, E.A.H. AND LEDUC, D.L., 2009. Phytoremediation of selenium using transgenic plants. *Food Biotechnology/ Plant Biotechnology*., **20**: 207-212.

RAJKUMAR, M. AND FREITAS. H., 2011. Influence of metal resistant-plant growth promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. *Chemosphere*., **71**: 834-842.

RAMOS, B., GARCIA. J.A.L., PROBANZA. A., BARRIENTOS. M.L. AND MAÑERO.

F.J.G., 2003. Alterations in the rhizobacterial community associated with European alder growth when inoculated with PGPR strain *Bacillus licheniformis. Environ. Exp. Bot*., **49**: 61-68.

RIAZ, S, FAISAL, M. AND HASNAIN, S., 2010. *Cicer arietinum* growth promotion by *Ochrobactrum intermedium* and *Bacillus cereus* in the presence of $CrCl_3$ and K_2CrO_4.. *Ann. Microbiol*., **60**: 729-733.

SAEEDI, S.S. AND SHOKRZADEH, M., 2013. Heavy metals contamination in water and three species of most consumed fish sampled from Caspian Sea, 2011**.** *Environ. Monit. Assess*., 185: 10333-10337.

SHAHAB, S., AHMAD, N. AND KHAN. N.S., 2009. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *Afr. J. Agr. Res*., **4**: 1312-1316.

YASIN, M. AND FAISAL, M. 2013. Assessing the phytotoxicity of tanneries waste contaminated soil on *Zea mays* (Lin) growth. *Pol. J. Environ. Stud*., **22**:1871-1876.

ZWOLAK, I. AND ZAPOROWSKA, H., 2012. Selenium interactions and toxicity: a review: selenium interactions and toxicity. *Cell Biol. Toxicol.*, **28**: 31-46.