Peer Reviewed / ISSN: 2663-1040 *AJ Life Sci. 2020, 3 (2): 164-174* **Research Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.3.2.16

Selenium resistant bacteria enhance Zea mays growth parameters under selenium stress

Ali Rehman[1,*], Anam[2], Hira Saleem[2], Hafsa Tariq[2], Saima Javed[2] and Muhammad Faisal[2]

[1]*Department of Allied Health Sciences Superior University Raiwind Road Lahore, 54000, Lahore, Pakistan*
[2]*Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan.*

Abstract

Heavy metals normally accumulate in the environment and as a result they create various problems to life, especially to plants. In this study two selenium resistant bacteria, *Bacillus pumils* strain CrK08 and *Bacillus licheniformis* strain AsK03 were checked for their effect in enhancing growth of *Zea mays* in the clay pots experiment, under selenium stress. Both strains significantly promoted root length in control and in autoclaved soil. Number of roots from plant base was reduced in control and autoclave soil respectively. Moreover, the fresh weight and dry weight were reduced in control and autoclave soil plants. In selenium treated plants, fresh weight was high than in the non-treated plants. However, Strains also produced an increase in soluble protein content. Indole acetic acid (IAA) content was enhanced to and, while acid phosphatase activity was significantly lower in inoculated plants than the controls and peroxidase content reduced. Selenium content in control plants was high as compared to treated plants. So, these strains promote plant growth under Se stress (17 mg kg^{-1}) in soil.

Key words: Selenium, heavy metal, *Zea mays*, *Bacillus*

Article Info:
Received:
October 29, 2019
Received Revised:
November 29, 2020
Accepted:
December 1, 2020
Available online:
December 31, 2020

**Corresponding Author:*
alirehman.ar11@gmail.com
Ali.rehman@superior.edu.pk

How to cite:
Rehman A, Anam, Saleem H, Tariq H, Javed S, Faisal M. Selenium resistant bacteria enhance Zea mays growth parameters under selenium stress. *Abasyn Journal of Life Sciences 2020; 3(2): 164-174.*

1. INTRODUCTION

Human activities have posed deleterious effects on the natural resources especially land and water resource[1]. Anthropogenic activities have introduced a large number and huge quantities of dangerous chemicals like fertilizers, heavy metals and their salts, pesticides, chlorinated solvents and acids in to air, soil and water[1-2]. Each of these chemicals has its own detrimental effect on life, but the presence of heavy metals in natural resources are of special concern due to their multidimensional damaging activity e.g. carcinogenicity and mutagenicity[3]. Mostly, plants fulfil a part of their metal input needs by process of atmospheric deposition.

Selenium has been considered an important element which is needed in trace quantities, not only for humans but also for animals. Selenium is a metalloid; it's excessive exposure can cause environmental toxicity, water pollution and several problems like deformities and even death to larger animals[4]. Selenium is an important metabolite for life in minute quantities. It acts as cofactor for many important enzymes as reported by Rotruck *et al*. (1973), showed that Selenium is a necessary component of glutathione peroxidase (GSH-Px) enzyme. Selenium has a double type of result on growth which depends on its quantities. It enhances the plant growth by acting as an antioxidant in low quantities. Whereas it reduces plant yield when present in high concentration[5]. Moreover, Selenium also reduces the photo oxidative stress and activates defence mechanisms in potato chloroplasts[5]. Electron microscopic studies have shown that the growth of lettuce plant increases, and higher quantities of starch accumulated when lettuce plants were provided with various dose of selenium[6]. Studies have likewise demonstrated that Selenium ensures the photosynthetic contraption of maize plants from oxidative harm by increasing levels of cancer prevention agents[6-7]. Selenium advances plant development by expanding starch accumulation in the chloroplast[7]. Beside harmful impacts, it has numerous significant capacities in human's body like thyroid hormone, cell reinforcement safeguard framework and in the invulnerable framework. As it is a significant part of selenoprotein, it involved in oxidative harm and in enzymatic responses. In plants, Selenium advances resilience towards oxidative pressure, postpones senescence so helps in higher creation of yield. The World Health Organization (WHO) and USDA prescribed human dietary admission of Se to be 55-200 mg/day. On the off chance that this proportion is upset, at that point distinctive health-related issues may happen[8]. Numerous prototrophic and chemotrophic microbes can lessen oxyanions of selenium Se (IV) and Se (VI), either to essential selenium Se (0), or to an unpredictable structure Se (II)[9]. Microscopic organisms have created numerous components adapt to the overwhelming metal stress.

Several strategies have been discovered through which bacteria tolerate this heavy metal. Some important mechanisms in this regard are conversion of more lethal form to less poisonous form, metallic ions efflux system, accumulation and complex formation inside the cell [9-10]. Freely residing bacteria that are advantageous for the growth of plant also called as growth enhancing rhizobacteria, are usually discovered in roots of many plants[10-11]. Growth enhancing bacteria may enhance plant into a couple of different ways; direct or indirect. Direct growth promotion is through the synthesis of certain growth promoting compounds by that microbe or by increasing nutrient intake by plants through the soil, while indirect growth promotion is either through decreased or complete prevention of deleterious effects of phytopathogenic organisms. They may fix atmospheric nitrogen; produce siderophores; multiple types of hormones of plants including auxins and cytokinin; or may have mechanisms to solubilize minerals like phosphorus; and synthesis of IAA, hydrogen cyanide and ammonia[12]. Indirect development also involves enzymes having potential to amend plant development[12-13-14]. So due to these reasons when seeds treated with plant growth promoting rhizobacteria (PGPRs) or the PGPRs are applied indirectly in to the soil they enhance the growth and yield and decrease heavy metal soil toxicity[14] and increase plant growth and productivity[15]. In this study pot (earthen) experiment is executed in green house for checking, growth promotion effect of *Bacillus pumilus and Bacillus licheniformis* on *Zea mays* under Se stress conditions and growth and biochemical parameters are determined. Selenium content is also checked in plants and soil to glimpse the effect of bacterial inoculums on selenium accumulation by plants.

2. MATERIALS AND METHODS

2.1 Plant-microbe interaction study

Pot experiment was conducted to study plant - microbe interaction. The strains were provided by the environmental microbiology lab for evaluation and testing. Pre-isolated bacterial strains, *Bacillus pumilus*-

CrK08 and *Bacillus licheniformis*-AsK03 were used for the experiment. These strains were isolated from the soil polluted with tannery wastes from Kasur, Pakistan. These strains were also checked for their response to heavy metal stress Both strains were heavy metal resistant. CrK08 is chromium resistant while ask03 is arsenic resistant. Both strains are also selenium resistant and can resist up to 1000 µg ml^{-1} of selenium. The experiment was performed to check the plant growth under selenium stress in presence of bacterial inoculums. The experiment was done under day light and at 30 ± 2°C temperature. Mixed bacterial cultures were directly inoculated into soil.

2.2 Experimental soil

The 30-50 cm upper layer of garden mud was collected from University of the Punjab, Lahore, Pakistan. The characteristic of soil was a loamy and clayish with a pH of 8.2. The collected soil was allowed to dry at 25°C and then filtered through 2.2 mm sieve and then stored for further use in bags. Chemical components of the soil are given in table 1.

Table 1. Chemical analysis of soil sample.

Ingredient	Amount
Sodium	5.43 g kg^{-1}
Potassium	0.12 g kg^{-1}
Calcium	2.16 g kg^{-1}
Magnesium	0.57 g kg^{-1}
Sulfate	2.8 g kg^{-1}
Carbonate	Nil
Bicarbonate	1.22 g kg^{-1}
Organic matter	3.61 %
Phosphate	46.0 g kg^{-1}
Arsenic	Nil
Chromium	Nil
Selenium	50 µg g^{-1}
pH	8.2

2.3 Pot experiment

About 3 kilogram of the soil was filled inside each Pot (15x17cm). For control, pots were filled with soil without autoclaving. For autoclaved treatment, soil was packed in bags of plastic and autoclaved at 121ºC for 15 minutes at fifteen pounds per square inches' pressure. The soil was then set to cool at 25°C for 24 hours after that a second autoclaving was done and again cooled to room temperature. Pots were filled with soil and arranged in setup as shown in table 2.

Table 2. Experimental setup

	Control soil		Autoclaved soil	
Bac	contP1	P1	contP2	P2
AsK03+CrK08	Without Inoculum	AsK03+CrK08	Without Inoculum	AsK03+CrK08

Cont P1, control plant of non-autoclaved/control soil; P1, Control soil with bacterial inoculums; cont P2, autoclaved control; P2, autoclaved soil with bacterial inoculums

2.4 Seeds Sterilization

Seeds of var. NK-6326 by Sygenta (pvt) Ltd. (obtained from National Agriculture Research Centre (NARC), Islamabad, Pakistan) were sterilized from surface by 0.15% $HgCl_2$ for 8 minutes and afterwards were cleaned with autoclaved distilled water for 6 to 8 times before sowing. Seeds were transfer to autoclaved Petri plates. Seed viability was also checked by growing them on filter paper.

2.5 Experimental setup

Soil was brought to water capacity before sowing. Sterilized seeds were planted 1cm deep inside the soil. The pots of plants were arranged in lanes in net house at an average daily temperature. Seed emergence process was observed daily. Initially 12 seeds per plant for maize were sown. After complete germination thinning was done to 9 plants per pot. For the bacterial cultures, bacteria were inoculated and grown in L-Broth overnight at 37ºC. Afterwards bacterial cells were harvested from the broth, and re-suspended in sterilized glass flask having distilled water. The final bacterial density was adjusted to a concentration at around 10^8 CFU ml^{-1}. Bacterial mixed cultures were prepared by mixing equal quantity of single (pure) culture. After thinning, the mixture was directly poured in the respected pots. 50ml of metal solution containing 100 µg ml^{-1} of Na_2SeO_3 was applied to all pots of each bacterial as well as control treatment. With a gap of three days, the pots were again watered with 50 ml of metal solution. Three replicas of each treatment were carried out and the concentration was set at 3.3 mg/kg. Pots were watered at regular basis with measured quantity and allowed to grow. Plant growing and developmental progress were monitored. After 21 weeks the pots were removed with caution from pots and washed for removing soil and other particles.

2.6 Factors for Growth

Various growth parameters of the plants were performed: Shoot length (cm), Root length (cm), total number of leaves, total number of roots, Weight of Fresh plant (g), Dry weight per gram of fresh weight of plant (mg g^{-1}), Dry weight per plant (g).

2.7 Biochemical parameters

Following Mahadevan (1984) auxins were removed from shoots of plants. Solvent proteins were removed through the method of Bhatti et al. (1953), while Lowry et al. (1951) technique was utilized for solvent protein investigation. For the estimation of peroxidases amount David and Murray (1965) strategy was utilized. Iqbal and Rafique's (1987) technique was utilized for the extraction of acid phosphatases.

Estimation of selenium content

The measurement of selenium amount in plants, digestion of plant material was carried out by the procedure of Humphries (1956). Oven dried (80 ºC for 24 hours) plants were removed and dried plants were cut into small pieces. Properly washed and dried conical flasks were taken and labelled according to the treatment. Pieces of weighed plant material were taken in the respective flasks. 10ml conc. of HNO_3 and 2ml of $HClO_4$ was added in each flask. Plant material was dissolved through heating on the water bath. When the white fumes start rising, the digestion was completed. The obtained material was diluted to 10ml and the amount of selenium was analysed with the help of Beckman D2 Spectrophotometer.

Modified Watkinson (1966) method was used to determine selenite contents which involve use of spectrophotometer. Firstly, 1ml of 0.1 M disodium oxalate, 12ml of 0.2 M HCl, 1ml of 0.15M Sodium fluoride (NaF) and 1ml of 0.15M EDTA was dissolved in a glass flask. 260 µl sample was poured followed by 3ml of 0.15% 2, 3-diaminonaphthalenein and 0.2 M HCl was added. Then the tubes were incubated for 45 minutes at 39 ºC and then cooled down to room temperature. 6.5 ml of cyclohexane was used to extract the selenium 2, 3-diaminonaphthalene complex by mixing and treating the tubes vigorously for about 1 minute. Absorbance was recorded at 376 nm of the organic phase. All the process was done in the dark. Calibration curve was made by making solutions of known concentrations of sodium selenite in distilled water.

3. RESULTS AND DISCUSSIONS

3.1 Impact of metal stress on Zea mays

Plant population is one of the most critical factors in crop yield and this also depends upon seed viability. So, before performing experiment in the field seed viability was checked by growing them in petri plates. Seeds have good viability as seed germination occurred after 24 hours. Slight effects of metal toxicity on plant appearance and growth was observed as the lower leaves of all plants dried soon. Purpling of leaves was also observed in all plant of control as well as autoclaved soil that may be because of phosphorus deficiency. More purple colour was observed in plants with control soil as compared to autoclaved soil. Plants in the autoclaved soil looked healthier than the plants in control soil.

3.2 Growth parameters

There were four types of plants that were grown. Control plants of non-autoclaved soil were named as P1(fig 1), Control soil with bacterial inoculums was P2, (Fig 2) autoclaved control P3 and autoclaved soil with bacterial inoculums was given the name P4.

Seed germination began at fifth-day post-planting and finished inside five further days. The rate of seed germination was 80.5 % in charge (un-autoclaved) soil while 87.5 % in autoclaved soil (fig 3). An expansion of 7 % in seed germination was seen in autoclaved soil. There was an expansion of 13% seed germination in control plants P2 having the bacterial inoculum. A critical increment in root length was seen of immunized plants P2 and P4. P4 indicated an additional expansion 51% increment in root length when contrasted with P2. A lessening of 34.9% in root length was seen in charge P3 while no critical abatement in P1(fig 3). To the extent shoot length is concerned blend results were gotten as P2 plants on control soil indicated an expansion in shoot length when contrasted with un-vaccinated P1 plants. In autoclaved soil, there was a reduction in shoot length in P4 vaccinated when contrasted with un-immunized P3 plants. Fresh weights of all inoculated plants were diminished concerning control plants. There was a slight decrease in new weight of P2. Likewise, in autoclaved soil decline in fresh weight was seen in P4 however the thing that matters were not concerning. If there should arise an occurrence of dry weight, increment in dry weight occurred in plants of autoclaved soil when contrasted with control soil (fig 4). In control and autoclaved soil, a drop in dry weight was seen when control plants were contrasted with inoculated one. The inoculated plants of both control and autoclaved soils indicated an expansion in dry load when contrasted with their un-inoculated controls.

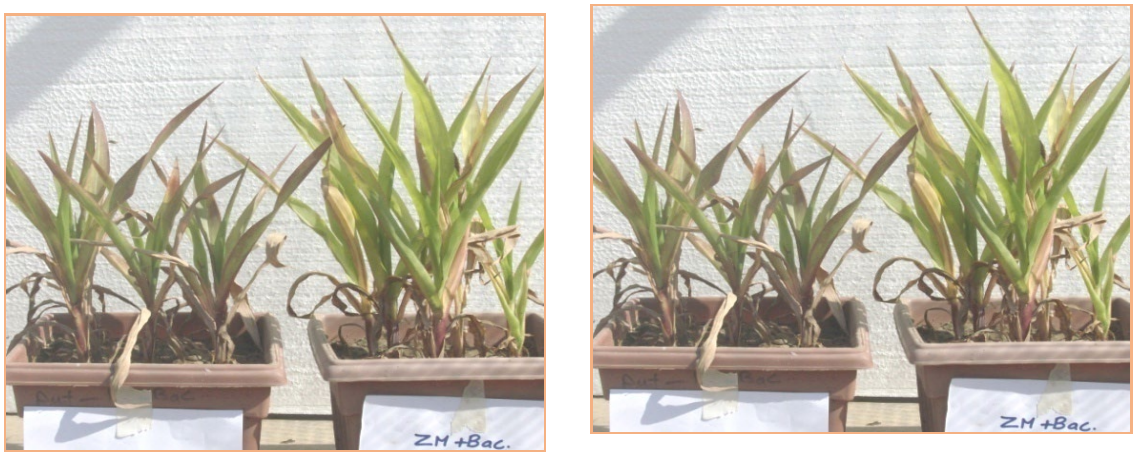
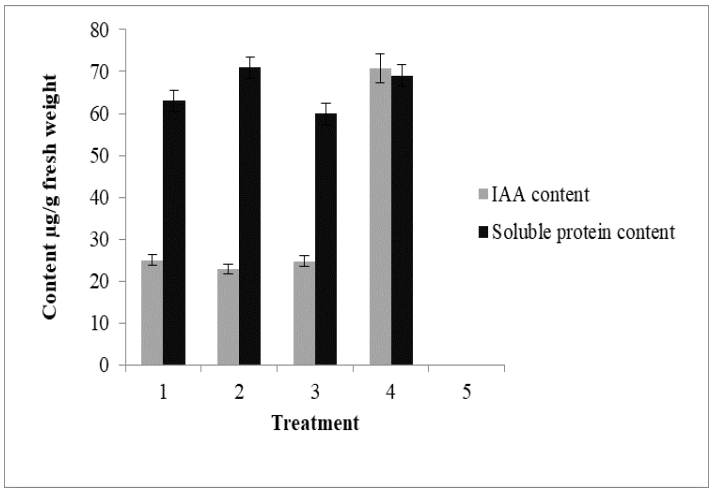
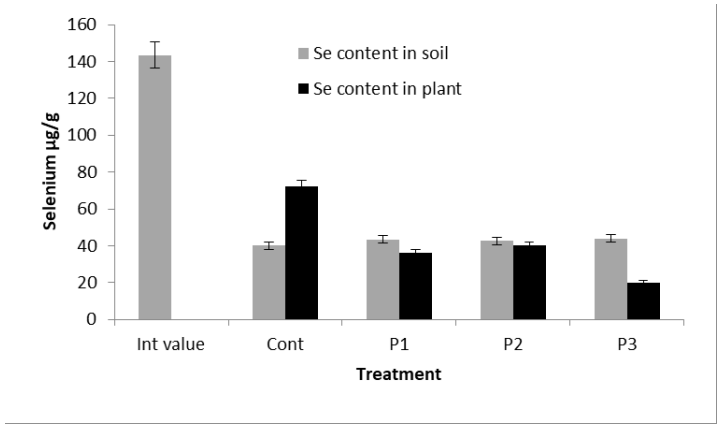


Fig. 1 Comparison of plants in control and autoclaved soil with and without bacterial inoculum. (A) Cont P1: nonautoclaved soil; P1: nonautoclaved soil with bacterial inoculum; (B) Cont P2: autoclaved soil without inoculu; p2: autoclaved soil with bacterical inoculum

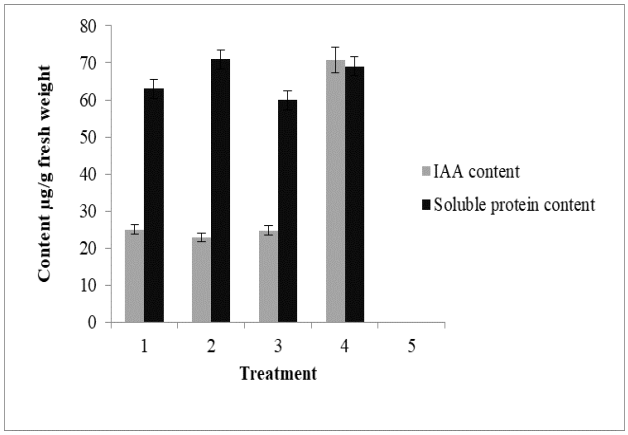


(A)

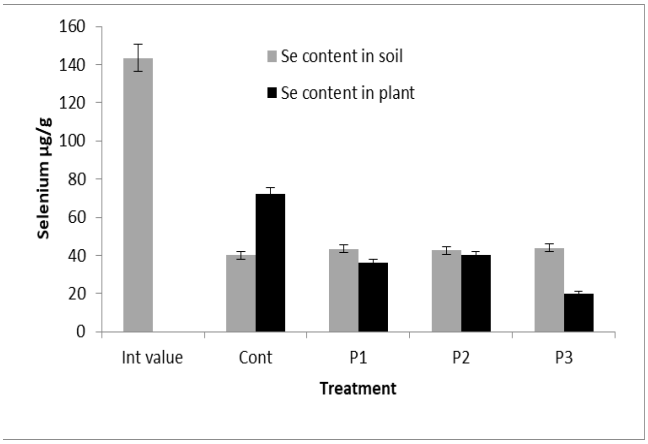


(B)

Fig. 2 Effect of selenium resistant bacteria on (A) root lengths and shoot length (B) fresh weight, dry weight and dry weight µg g^{-1} of fresh weight in *Zea mays*.

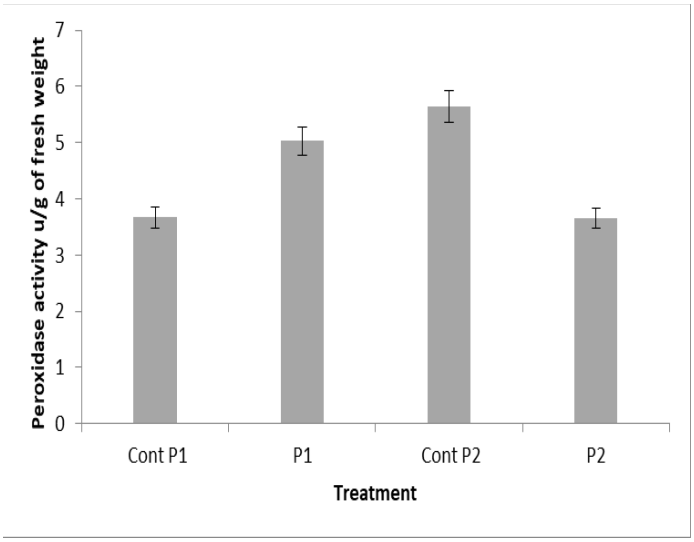


(A)

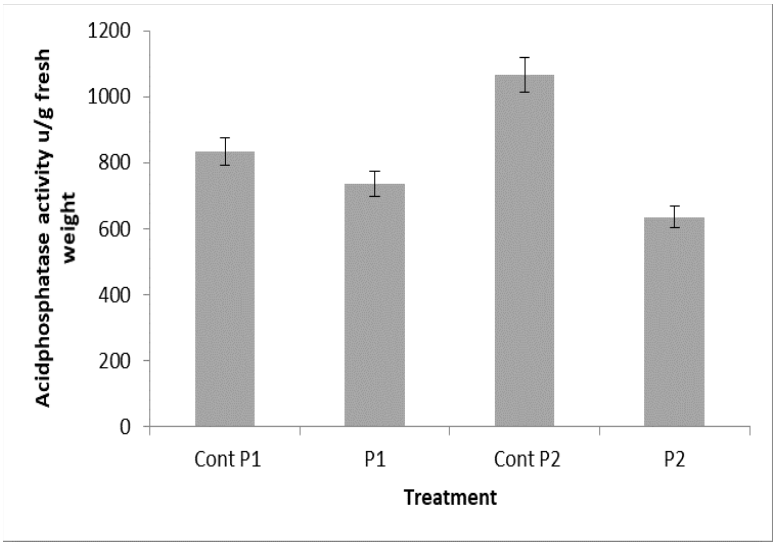


(B)

Fig. 3 Effect of selenium resistant bacteria on (A) Indole acetic acid (IAA) and soluble protein content in plants and (B) selenium content in soil and plants of *Zea mays*.



(A)



(B)

Fig. 4 Effect of selenium resistant bacteria on (A) Peroxidase activity (B) Acid phosphatase activity of *Zea mays*

3.3 Biochemical parameters

There was a decrease in auxin content (10%) in inoculated plant P2 as compared to control plants P1 of non-autoclaved control soil while a marked increase (65%) in inoculated plants P4 as compared to control plants P3 of autoclaved soil. An increase of 9% in protein content was found in inoculated plants of control as well as autoclaved soil when compared to their respective controls. There was an increase in peroxidase activity (0.27%) in inoculated plants of control soil as compared to control plant of this soil while 2% decrease was observed in case of autoclaved soil in which enzyme activity decreased in inoculated plants as compared to control. Acid phosphate activity was 13.3% and 50% less in inoculated plants as compared to controls both in non-autoclaved and autoclaved soil. Selenium contents were maximum in control plants P1 of control soil and were minimum in inoculated plants P4 of autoclaved soil. A significant decrease of up to 50% was observed in inoculated plants in both control and autoclaved soil. Metal content was high in soils of inoculated pots 8% and 1% high as compared to their controls. Metal content in soil in soil analysis was also determined before and after harvesting of plants. There was a marked decrease in the amount of selenium in the soil of all treatments when compared with the initial metal content in soil after the addition of metal. Minimum amount of metal was present in control plant P1 and P3 while the highest metal content was found in soil of autoclaved soil P4 plants in which inoculums was given.

Microscopic organisms are significant for the physical and chemical uniqueness of soil and assume a significant job in soil quality[15-16]. In the present examination, bacterial strains used to inoculate *Zea mays* var. NK 6326 fundamentally influence plant development. It is seen that numerous types of microscopic organisms can invigorate plant development particularly plant development advancing rhizobacteria[17]. Growth promoting rhizobacteria can influence plant development in two different ways either to indirectly protect the plant from metal poisonous effect or to directly deliver the substances that upgrade plant development [18-19]. On account of new weight, dry weight and new weight per gram of dry weight, the expansion in biomass was seen in the case of autoclaved soil. It might be because of the absence of the opposing impact of different microorganisms in the soil. In fact, sterilized soil incites inclination since inoculants are not in competition with indigenous microorganisms and protozoa that are involved in grazing. Previous studies indicated that higher microbial development and endurance are commonly seen under these conditions[20]. ACC deaminase blended by numerous bacterial species like *Pseudomonas brassicacearum, Pseudomonas marginalis, Pseudomonas oryzihabitans, Alcaligenes, Bacillus pumilus*, and *Rhodococcus* have been appeared to build the plant biomass yet not the rate of accumulation of metals by plants [21-22-23]. Hartikainen et al. (2000) demonstrated the development advancing impact of selenium on Ryegrass. Less metal ingestion in plants is described by the assimilation of metal by inoculant microorganisms[24]. In maize decline concentration of selenium in plants show biosorption of metal by *Bacillus pumilus* and *Bacillus licheniformis* as a contrast with the soil without inoculums where metal collected by plants is multiple times higher than without inoculums.

The strains utilized in the investigation tend to upgrade the development of plants within the presence of selenium metal. Development hormones are the substances delivered by plants and among these particular hormones, auxin is the key one, practicing administrative activities over various kinds of plant forms and over huge numbers of different hormones of plants[25-26]. The present strains fundamentally increase the auxin content of plant encouraging its shoot stretching and when utilized in autoclaved soil it can advance root length. Increment in solvent protein goes about as a weakening variable for the metal in plants that is upgraded in plants within the sight of metal and inoculums. In inoculated plants increment in protein, the substance might be because of increment in nutrients and nitrogen obsession as appeared[26]. It has been

reported that inoculation with plant development enhancing microorganisms improve seed nitrogen, protein and phosphorus amount of salicornia species of sunflower[27-28]. Aon and Colaneri (2001) indicated that enzymes have a strong connection and their action assumes a significant impact between physical, chemical and microbial soil properties which enhances plant development. Acid phosphatase from different bacterial species act as virulence factors that help intracellular endurance by hindering the respiratory burst[29-30-31].

Table 3. Effect of selenium resistant bacteria on root and shoot length, fresh weight, dry weight and fresh weight per gram of dry weight of *Zea mays*

STRAINS	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	FRESH WEIGHT (g)	DRY WEIGHT (g)	DRY WEIGHT /FRESH WEIGHT (mg / g)
P1	71.7	24.9	26	4.30	0.17
P2	82.5	27.4	23.74	3.36	0.16
P3	53.12	29	24.58	6.29	0.26
P4	108	24.9	23.30	5.04	0.19

Cont P1, control plant of non-autoclaved/control soil; P2, Control soil with bacterial inoculums; cont P3, autoclaved control; P4, autoclaved soil with bacterial inoculums.

Table 4. Effect of selenium resistant bacteria on growth parameters of *Zea mays*.

STRAINS	Auxin ($\mu g\ ml^{-1}$)	Soluble protein ($\mu g\ ml^{-1}$)	Peroxidase (unit/g)	Acid phosphatase (K.A unit/100ml)
Cont P1	25	63	3.67	834.4
P1	22.8	71	5.03	736
Cont P2	24.8	60	5.64	1066.2
P2	70.8	69	3.66	635.2

Cont P1, control plant of non-autoclaved/control soil; P1, Control soil with bacterial inoculums; cont P2, autoclaved control; P2, autoclaved soil with bacterial inoculums.

4. CONCLUSIONS

It is concluded from the result obtained throughout this study was that *Bacillus pumilus* - CrK08 and *Bacillus licheniformis* - AsK03 have ability to promote different parameters (length of root, shoot and plant height) and biochemical parameters (auxin, soluble protein and peroxidase activity). Hence the strains can be used to help the plants to growth in the soil polluted with selenium. Further the plants can be used as selenium supplements to treat selenium deficiency in animal diet.

ACKNOWLEDGMENT

This research was conducted at University of Punjab Department of Microbiology and Molecular Genetics, Lahore, Pakistan

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Anđelković, M., P. Senapati, V. Ganeshamurthy, C. Uniyal, and M. Radha (2020). Phosphate-solubilizing microbes and biocontrol agent for plant nutrition and protection: Current perspective. Commun. Soil. Sci. Plant. Anal. 51(5): 645-657.
2. Aon, M.A. and Colaneri, A.C (2001). Tamporal and spatial variation of enzymatic activity and physio-chemical properties in an agricultural soil. Appl. Soil. Ecol. 18(3):225-270.
3. Bashan, Y., Moreno, M. and Troyo, E (2000). Growth promotion of sea- water irrigated oil seed halophytes *salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum sp*. Boil. Fertile soil. 32:265-272.
4. Belimov, A.A., Hontzeas, N., Safronova, V.I., Demchinskaya, S.V., Piluzza, G., Bulitta, S and Glick, B.R. (2005). Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. czern.). Soil Biol. Biochem. 37(2): 241-250
5. Bhatti, G.A., Qureshi, N., Qureshi, A and Sultana, K (1993). Studies on heat shock response of wheat seedlings using *E. coli* GroEL antibodies. Pakphyton. 5: 157–166.
6. Biro, B., Koves-Pechy, K., Vorous, I., Takacs, T., Eggenberger, P and Strasser, R.J (2000). Interrelation between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal in the rhizosphere of alfa alfa in sterile, AMF- free or normal soil conditions. Appl. S Ecol. 15(3): 159-168.
7. David, R. and Murray, E (1965). Protein synthesis in dark- brown leaves. Can. J. Bot. 43(7):817-824.
8. Esringu, A., Ekinci, M., Usta, S., Turan, M., Dursun, A., Ercisli, S. and Yildirim, E (2015). Selenium supplementation affects the growth, yield and selenium accumulation in lettuce (*Lactuca sativa* L.). Sciences Agraires. 68(6):801-810.
9. Evrim, E., Öktem, H.A. (2020). Inorganic Cadmium detection using a fluorescent whole-cell bacterial bioreporter. Analytical Letters. 53(17): 2715-2733.
10. Gamalero, E., Lingua, G., Berta, G. and Glick, B.R (2009). Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can. J. Microbiol. 55(5): 501-514.
11. Gupta, A.K and Sinha, S. (2004). Chemical fractionation and heavy metals accumulation in the plants of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: selection of single extractants. *Chemos.,* 64(1):161–173.
12. Hartikainen, H., Xue, T. and Piironen, V (2000). Selenium as an anti-oxidant and pro-oxidant in rye grass. Plant Soil. 225: 193–200.
13. Hughes, D. J., Fedirko, V., Jenab, M., Schomburg, L., Meplan, C., et al. (2015). Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort. Int. J. Cancer. 136(5) 1149–1161.
14. Humphiries, E.C. (1956). Mineral components and ash analysis. In: modern methods of plant analysis (peach, k and Traley, M.Y), Springer Verlong, Berlin, Gothingen Heildlberg.
15. Iqbal, J. and Rafique, N. (1987). Toxic effects of $BaCl_2$ on germination, early seedling growth, soluble protein and acid phosphatasein *Zea mays* (L). Pak. J. Bot. 19:1-8
16. Kessi, J. and Hanselmann, K.W. (2004). Similarities between the abiotic reduction of selenite with glutathione and the dissimilatory reaction mediated by *Rhodospirillum rubrum* and *Escherichia coli.* J. Biol. Chem. 279(49): 50662-50669.
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L and Randall, R.J (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 1993:265-275.

18. Lugtenberg, B. and Kamilova, F (2009). Plant-growth-promoting rhizobacteria. Annu. Rev. micro. 63: 541-556.
19. Mahadevan, A. (1984). Growth regulatory microorganisms and diseased plants. Oxford and IBHP Publishing Company; India. 31 p.
20. Yasin, M., El-Mehdawi, A.F., Anwar, A., Pilon-Smits, E.A., Faisal, M (2015). Microbial-enhanced Selenium and Iron Biofortification of Wheat (*Triticum aestivum* L.)-Applications in Phytoremediation and Biofortification. Int J Phytoremediation. 17(1-6):341-347.
21. Outten, F.W., Outten, C.E. and O'Halloran, T.V (2000). Metalloregulatory systems at the interface between bacterial metal homeostasis and resistance, Bacterial Stress Responses. In G. Storz (Ed.), *Bacterial Stress Responses.* ASM Press. 145-157 p.
22. Pennanen, A., Xue, T.L and Hartikanien H (2002). Protective role of selenium in plants subjected to severe UV irradiation stress. J. Appl. Bot. 76(1-2): 66–76.
23. Rayman M.P (2012). Selenium and human health. The Lancet. 379(9822):1256–1268.
24. Rajkumar, M., Nagendran, R., Lee, K.J., Lee, W.H and Kim, S.Z. (2006). Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemos. 62(5):741–748.
25. Sanmartin, C., Plano, D., Sharma, A.K., Palop, J.A (2012). Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. Int J Mol Sci. 13(8):9649–9672.
26. Schloter, M., Dilly, O and Munch, J.C (2003). Indicators for evaluating soil quality. Agriculture, Ecosystems & Environment. 98(1-3): 255-262.
27. Seppänen, M., Turakainen, M and Hartikainen, H. (2003). Selenium effects on oxidative stress in potato. Plant Sci., 165(2): 311– 319.
28. Shaukat, S., Afrasayab, S and Hasnain, S. (2006). Growth response of helianthus annuus to plant growh promoting rhizobacteria used as biofertilizers. Intl. J.Agri. Res., 1(6) 573-581.
29. Wadgaonkar, S.L., Nancharaiah, Y.V., Esposito, G and Lens, P.N.L. (2018): Environmental impact and bioremediation of seleniferous soils and sediments, Critical Reviews in Biotechnology. *38(6): 941-956*.
30. Siddique, T., Zhang, Y., Okeke, B.C and Frankenberger J., W.T. (2006). Characterization of sediment bacteria involved in selenium reduction. Bioresour. Technol. 97(8):1041-1049
31. Stolz, J.F and Oremland, R.S. (1999). Bacterial respiration of arsenic and selenium. FEMS Microbiol. Rev. 23(5): 615-627.
32. Lebeau, T., Braud, A and Jézéquel, K (2008). A review of Performance of bioaugmentatin-assisted phytoextraction applied to metal contaminated soils. J. Environ. Poll. 153(3):497-522
33. Turgut, C. (2003). The contamination with organochlorine pesticides and heavy metals in surface water in Kucuk Menderes River in Turkey. Environ. Int. 29(1):29–32.
34. Van Breusegem, F., Slooten, L., Stassart, J.M., Mones, T., Botterman, J., Van Montago, M and Inze. D. (1999). Over production of Arabidopsis thaliana FESOD confers oxidative stress tolerance to transgenic maize. Plant cell physiol. 40(5): 512-523.
35. Watkinson, J.H (1966). Semi-automated fluorometric determination of selenium in biological materials with 2, 3 diaminonaphthalene. Anal. Chem. 38(1): 92-97.