



Retrieval Effect of Microbial Fertilizers in *Pennisetum americanum* L. Seedlings under Aqueous Nickel (II) Ions Stress

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Article Info

Abstract

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The retrieval effect of microbial fertilizers in Nickel chloride treated *Pennisetum americanum* L. seedlings were grown in pots filled with mixture of red, black and sandy soil. Simultaneously, control (water) and control (6mM Nickel chloride) plants were also maintained. The effect of microbial fertilizers treatments accelerated to restore the growth of the plants. On the fresh weight basis, the addition of adsorbents caused more accumulation of chlorophyll a, b, carotenoid, and class of accessory pigments were also high in microbial fertilizers treated seedlings than the Nickel chloride alone treated control seedlings. The impact of microbial fertilizers treatments on the biochemical constituents in terms of free amino acid, soluble proteins and glucose was studied. The level of free amino acid content was high in treated seedlings. The accumulation of soluble protein in leaves is considered to be an indication of efficient metabolic status than its counter parts. Further, the scavenging enzymatic activities were decreased in increased concentration of microbial fertilizers application reveals the stress relieving nature of plants. Thus an overall assessment of plant in terms of growth, pigment composition, biochemical constituents and enzymatic activities has exhibit the retrieval efficacy of microbial fertilizers from the Nickel chloride treated *Pennisetum americanum* L. seedlings.

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Introduction

Heavy metal pollution in water is a major environmental problem facing the modern world. The global heavy metal pollution is increasing in the environment is due to increase in number of industries. Much industrial waste water contains heavy metals like cadmium, lead, zinc, cobalt and chromium. Water is the lifeline of Earth and it is the foundation of the rich environmental cycle as it is responsible for the great diversity and abundance of life on earth. Water supports all living things, but its quality is damaging due to the

rapid increase in population, urbanization and industrialization.

Water pollution is the introduction of unwanted substances that alter the quality of water and impairs its usefulness and disposed into water bodies, altering their usefulness and quality. Heavy metal pollution and its toxicity to the environment is one of a major problem faced by several developed and under developing countries, because of the rapid increase in population and industries in and around cities. Pollution refers to the addition of foreign material to our surroundings or

changes in physical and chemical constituents of nature which may directly or indirectly affect the environment either immediately or after sometime.

Nowadays we use chemical fertilizers in great quantities to compensate the deficiency of nutrient in soil. It is observed that the abundance use of chemical fertilizers affects soil and plants in due course. Therefore, the current trend is to expose the possibility of supplementing chemical fertilizers with organic manures particularly the seaweeds. In India many industries are using heavy metals in their process and exiled out without proper treatment. Metals are released into the environment leads wide. Spectrum of anthropogenic activities are smelting of metallic ores, industrial fabrication and commercial application of metals, which are polluting our aquatic bodies.

Nickel is a common pollutant arising from industries such as electroplating, metal processing and paint formulations. Up to that acceptance doses, nickel poisoning causes cyanosis, cancer in lungs, nose and bones. Thus, it is necessary to remove them from industrial waste water. Several methods such as chemical precipitations, coagulations, ion exchange and adsorption are generally used. Owing to high and expensive cost, adsorption was reported as an efficient and economic feasible option.

Biosorption can be defined as a non-directed Physico chemical interaction that may occur between metal species and plant cells. It is a biological method of environmental control and can be an alternative to conventional contaminated water treatment facilities. It is also offers several advantages over conventional treatment methods including cost effectiveness, efficiency, minimization of chemical or biological sludge, the requirement of additional nutrients and regeneration of biosorbent with possibility of metal recovery.

Azospirillum is a key plant growth-promoting rhizo bacterium, primarily colonizing plant root surfaces. It attaches to plant roots in two steps using a polar flagellum and an unidentified surface polysaccharide. Nitrogen fixation is regulated by conserved *nif* genes, with *NifA* playing a key role in controlling nitrogenase activity. Oxygen and nitrogen levels influence this process. *Azospirillum* regulates nitrogenase activity through ADP-ribosylation under conditions of ammonium or low oxygen, using enzymes DraT and

DraG. *Azospirillum* produces the plant growth hormone indole-3-acetic acid (IAA) through three pathways, including a unique Trp-independent route. Its ability to fix nitrogen, produce hormones, and efficiently interact with plant roots makes significant for enhancing plant growth and productivity. In the present study was aimed to assess the Retrieval effect of microbial fertilizers such as *Azospirillum*, *Phosphobacterium* and *Azotobacter* against Ni(II)ions treated *Pennisetum americanum* L seedlings.

Materials and Methods

Seeds of *Pennisetum americanum* (L.) were obtained from local seed centre and healthy and uniform seeds of *Pennisetum americanum* (L.) were surface sterilized with 0.1% mercuric chloride for 1 minute and then it was washed 3 times with distilled water.

Microbial Fertilizers Used

Azospirillum (Sample 01)

Azospirillum is a genus of free-living, nitrogen-fixing bacteria that belongs to the family *Azospirillaceae*. These bacteria are known for their ability to promote plant growth and are widely used in agriculture as biofertilizers. They are Gram-negative, micro aerophilic, and rod-shaped. *Azospirillum* species are commonly found in soil, particularly in association with the rhizosphere (root zone) of plants.

Phosphobacterium (Sample 02)

Phosphobacterium refers to a group of phosphate-solubilizing bacteria that play a vital role in enhancing soil fertility and promoting plant growth. These microorganisms are capable of converting insoluble forms of phosphorus into soluble forms, making it accessible to plants. *Phosphobacterium* is widely used in agriculture as a biofertilizer to improve crop productivity.

Azotobacter (Sample 03)

Azotobacter, also known as *Azotobacterium*, is a genus of free-living, nitrogen-fixing bacteria that plays a critical role in sustainable agriculture. These microorganisms are Gram-negative, aerobic, and motile rods or cocci, known for their large cell size and ability to survive in diverse soil environments. *Azotobacter*

contributes significantly to soil fertility and plant growth by fixing atmospheric nitrogen without forming symbiotic relationships with plants.

Preparation of Microbial Fertilizers

The experiments were carried out using the above microbial fertilizers were transferred to plastic troughs containing the 25 ml of 6Mm (Optimal conc) nickel chloride solution for ten days. Later it was filtered and added to soil bed of experimental seedling.

Experimental Plant

Seedlings of *Pennisetum americanum*, (L), procured from local seed centre was surface sterilized in 0.1% mercuric chloride and were depleted of micro and macro nutrients. It was grown in different plastic troughs containing the mixture of red, black and sandy soil in the ratio of 1:1:1. After a period of 3days, the seedlings were subjected to treatment for 10 days.

Control was also maintained with water and 6mM Nickel chloride (Optimal concentration) alone treated plants. After 10 days of treatment, Growth parameters such as seedling length, Fresh weight, dry weight and leaf area, photosynthetic pigments (Wellburn & Lichtenthaler, 1984) Glucose (Jayaraman, 1981) Protein (Lowry *et al.*, 1951) Amino acid (Jayaraman, 1981) Proline (Bates *et al.*, 1973) Leaf nitrate (Cataldo *et al.*, 1978) and enzymatic activities such as Nitrate reductase (Jaworski *et al.*, 1971) Catalase (Kar and Mishra, 1976) and Peroxidase (Addy and Goodman, 1972) were analyzed.

Results and Discussion

The result obtained on the present study indicated that increase in concentration of Nickel chloride results in decrease in the shoot length and root length (Table-1). Foy *et al.*, (1978) and Alam and Adams (1979) proved that the dry matter yield of tops and roots of oats decreased with increased zinc chloride level.

Zinc chloride reduces the possibility of successful seedlings establishment was observed in germinating grass seeds during the revegetation in acid soil in which concentration of zinc and other metals very high (Winter halder, 1985).

In the present study highest total leaf area was observed S4 treated nickel chloride. Promotion of greater leaf

area might have resulted in higher yield of plants. The rapid adsorption and removal of nickel by various microbial fertilizer adsorbents indicated a high affinity of available surface groups on adsorbents for heavy metals. The highest value of accumulation and removal of heavy metals was observed in the leaf and root of the test crop (Michael Kumi *et al.*, 2013).

In the present study, both the leaf area and total chlorophyll are found to increase with the various adsorbents (Table-2 & Fig-1-3). This indicated that, the various adsorbents enhanced not only the leaf area but also the content of photosynthetic pigments which finally led to increase in the plant biomass. An increase in leaf area and pigment content has been reported after adsorbents application by Sekar *et al.*, (1995). The various adsorbents caused restoration of the photosynthetic pigment in *Pennisetum americanum*, L seedlings than the Nickel chloride alone treated plants. Their increase in chlorophyll content may be due to a decrease in chlorophyll degradation. Yield enhancement effects due to improved chlorophyll content.

The result obtained on the protein content also revealed an increase in protein content in plants treated with various adsorbents (Fig-4). The same result was observed by Ghilidiyal *et al.*, (1986) in Linn seeds. The elevation in protein may be due to interference of adsorbents with the enzyme activity, pigments synthesis, photo synthesis and nitrogen metabolism (Beanford *et al.*, 1977). As the major soluble protein in the leaf is only RUBP case, the enzyme involved in photosynthesis and any inhibition in the photosynthesis will also affected the leaf protein. Hence it can be related with various adsorbents act as inducer.

Proline accumulates in the leaves of any plants, when the plants are subjected to stress (Paleg and Aspinal, 1981). It was observed in the present study also. The enhanced leaf proline content *viz.*, glutamate, through the activity of glutamate of protein (Greenway and Munns, 1980). The accumulation of proline is also considered as on adaptive response to stress (Stewart and Lee, 1974). In the present study, the proline content was decreased with various adsorbents treatment indicating the stress relieving nature of it.

In the present study, an enhanced peroxidase activity with the increase in the concentration of Nickel chloride was observed (Table-3 & Fig-5-8). Whereas, during various adsorbents treatment, activity of peroxide was

reduced and it is revealed that rectify the stressful effect caused by Nickel chloride in experimental plants. Catalase is the enzyme which catalyzes the accumulation hydrogen peroxide in the system and thus acts as a scavenger to remove toxic hydrogen peroxide (Bowler *et al.*, 1992). Increased concentration of catalase in nickel treated plants indicated the operation of different mechanism

against oxidative stress. After application of various microbial fertilizer adsorbents, activities of catalase and peroxidase were decreased which in turn indicate the adsorption potential of the microbial fertilizer retrieved from the stressful nature of these plants which was treated by Nickel chloride alone.

Fig.1 Microbial fertilizers treated Nickel chloride on chlorophyll 'a' in *P. americanum*

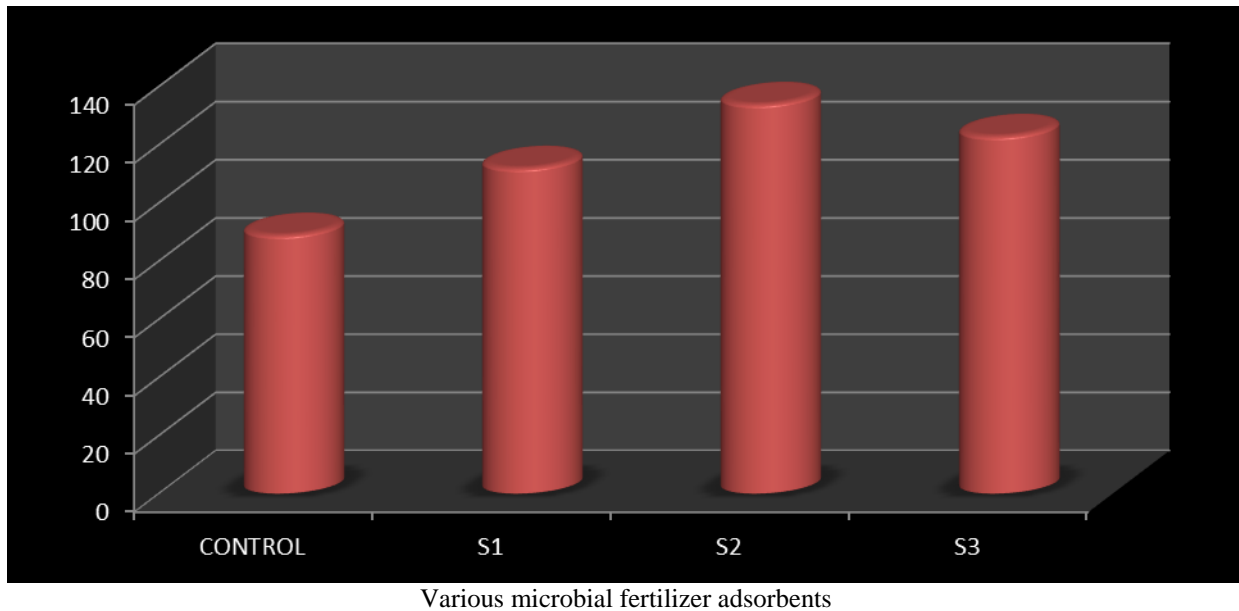


Fig.2 Microbial fertilizers treated Nickel chloride on chlorophyll 'b' in *P. americanum*

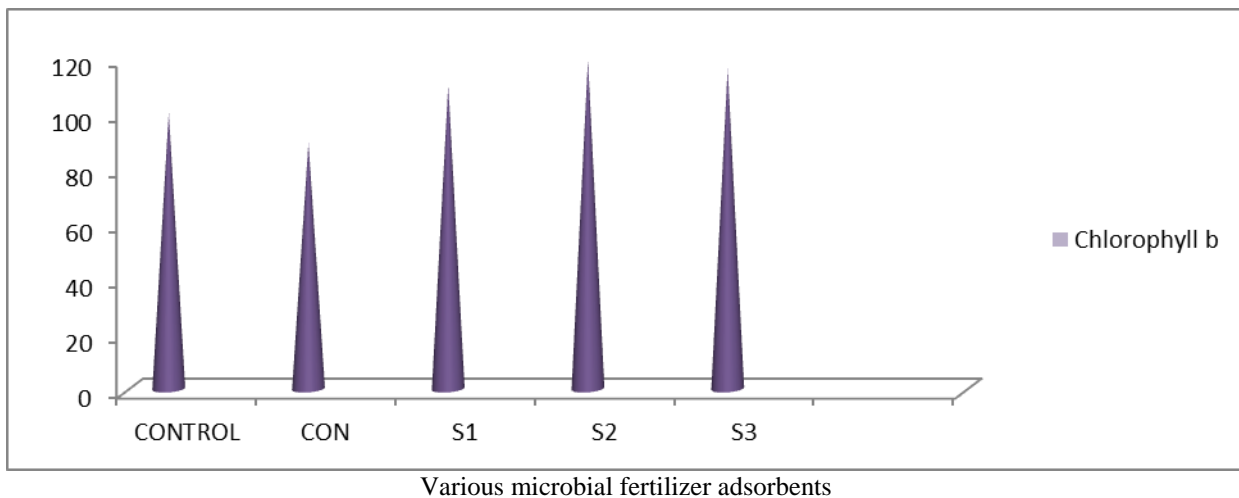


Fig.3 Microbial fertilizers treated Nickel chloride on total chlorophyll in *P. americanum*

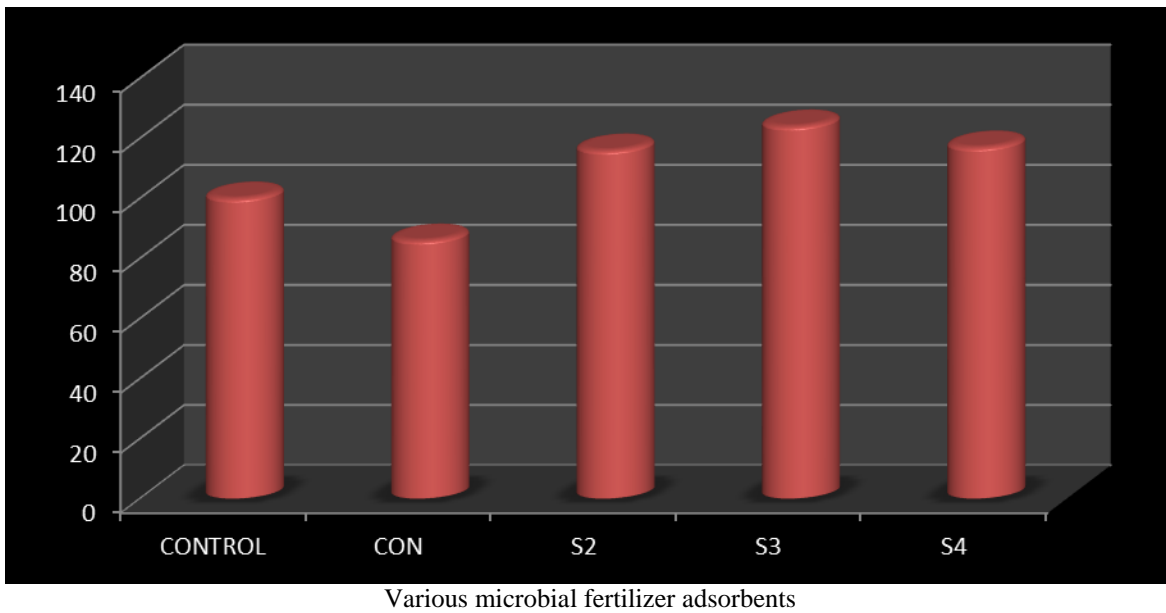


Fig.4 Microbial fertilizers treated Nickel chloride on Carotenoids in *P. americanum*

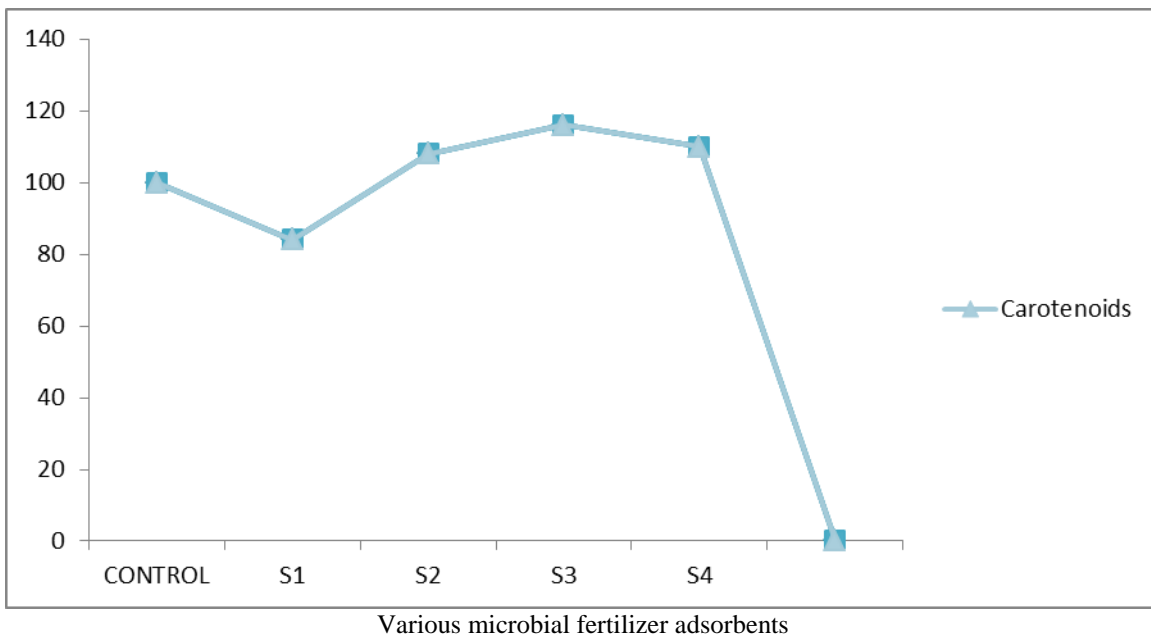


Fig.5 Microbial fertilizers treated Nickel chloride on Catalase Activity in *P. americanum*

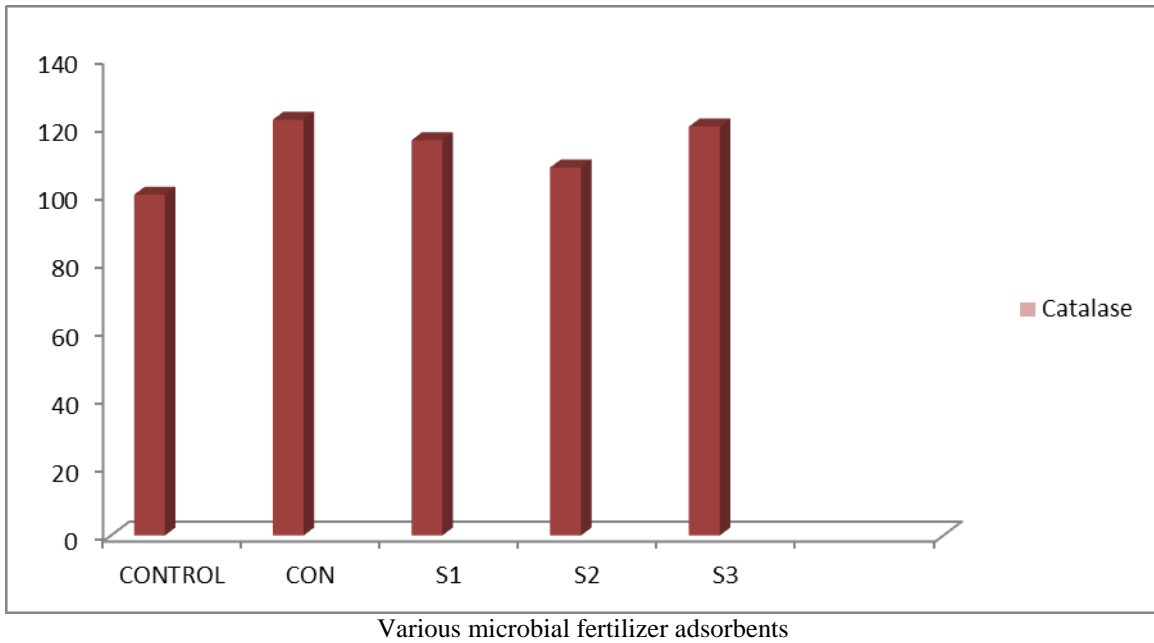


Fig.6 Microbial fertilizers treated Nickel chloride on Peroxidase Activity in *P. americanum*

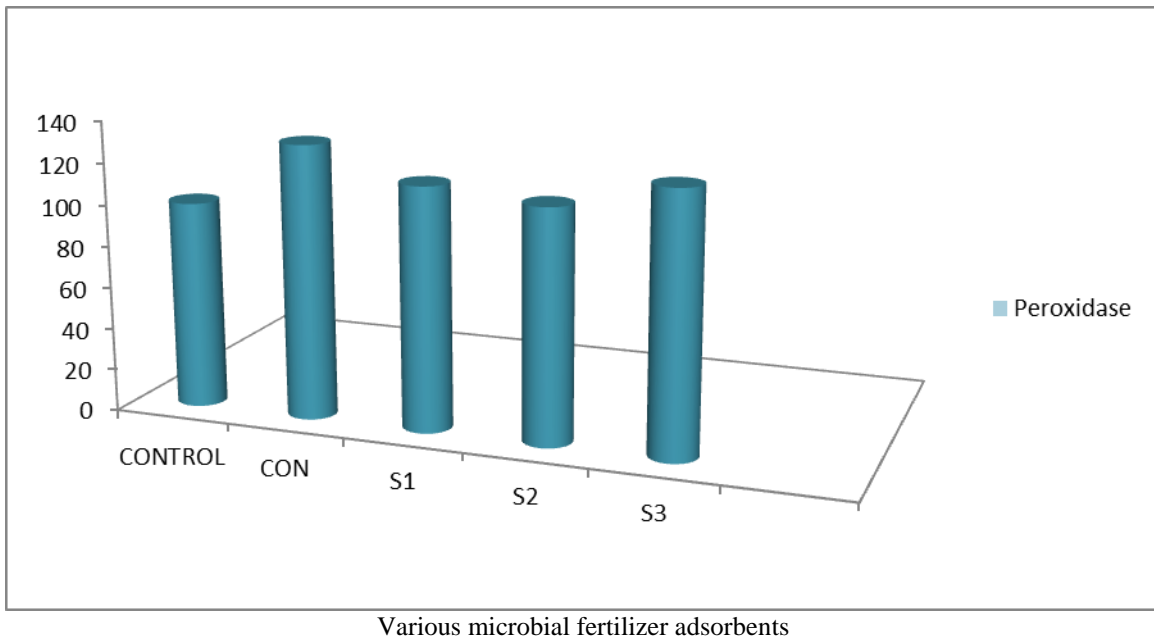


Fig.7 Microbial fertilizers treated Nickel chloride on Nitrate Reductase Activity in *P. americanum*

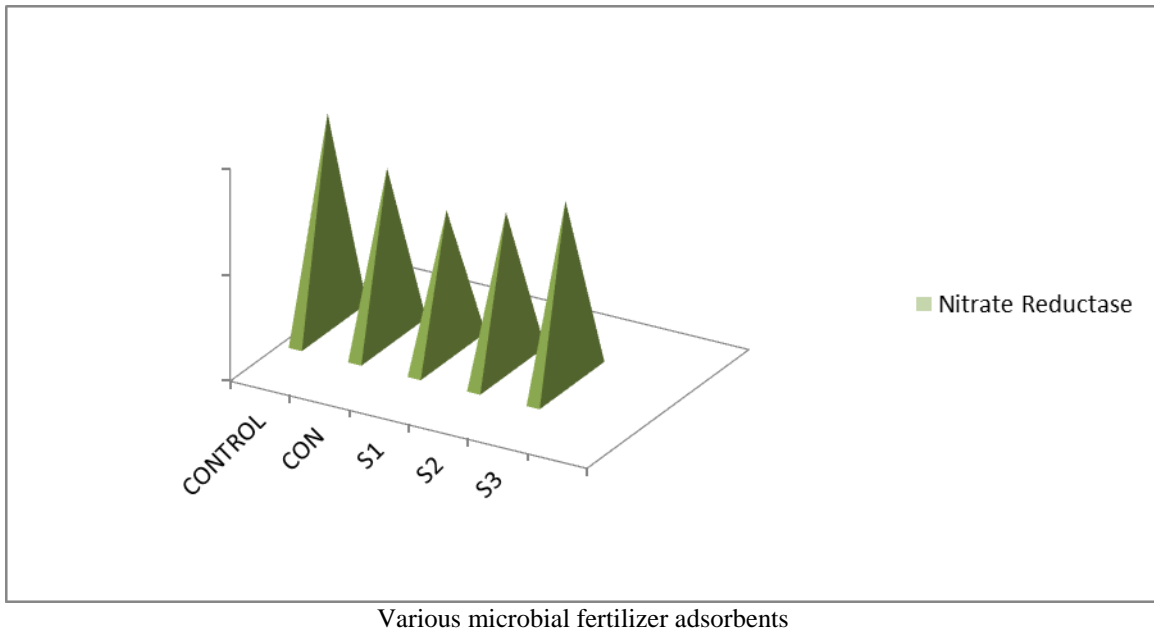


Fig.8 Microbial fertilizers treated Nickel chloride on Superoxide Dismutase Activity in *P. americanum*

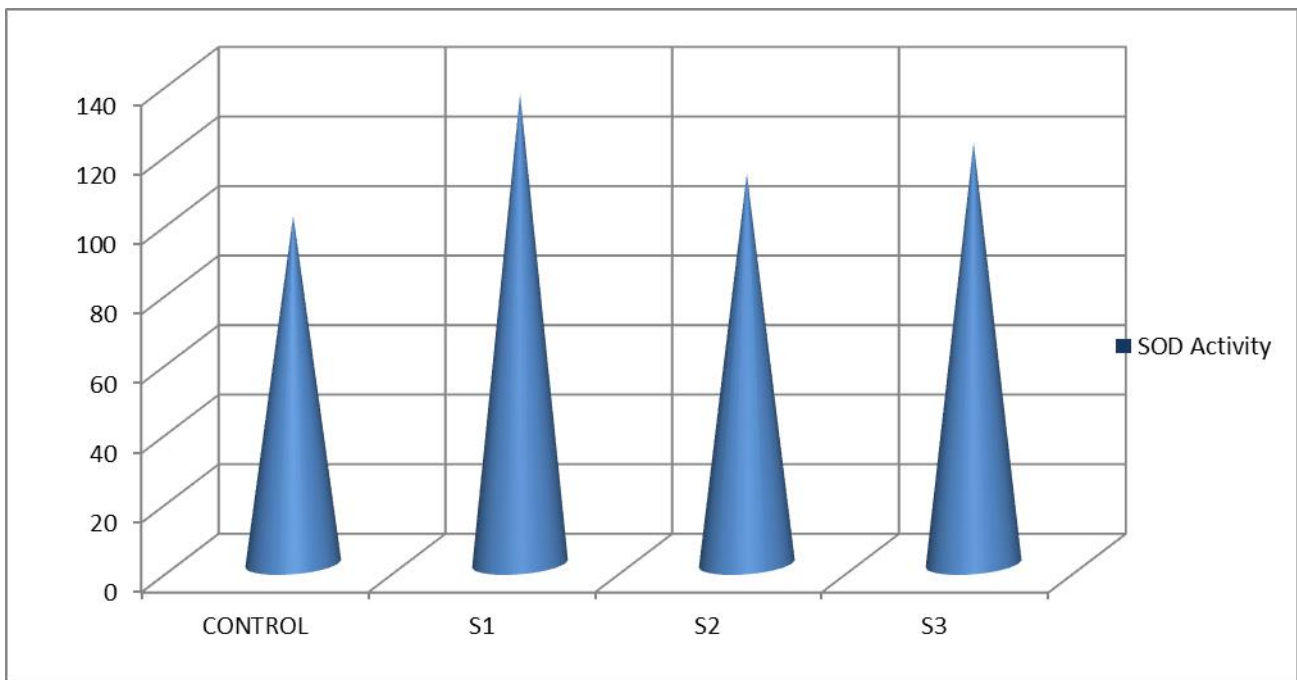


Table.1 Microbial fertilizers treated Nickel chloride application in Growth of *Pennisetum americanum*, L seedlings

S. No	Parameters	Control	Control NiCl ₂ (6mM)	S1	S2	S3
1.	Shoot length (cm)	13.5±0.12 (100)	10.12 ±0.01 (77)	12.1±0.13 (92)	12.8±0.034 (97)	11.2±0.021 (85)
2.	Root length (cm)	2.9±0.14 (100)	2.3±0.12 (82)	2.7±0.121 (96)	2.8±0.07 (99)	2.5±0.14 (89)
3.	Fresh weight (mg)	0.21±0.21 (100)	0.17±0.132 (81)	0.19±0.12 (90)	0.20±0.04 (99)	0.18±0.13 (86)
4.	Dry weight (mg)	0.013±0.13 (100)	0.010±0.01 (83)	0.013±0.07 (108)	0.015±0.31 (125)	0.011±0.14 (92)
5.	Leaf area (cm ²)	3.14±0.07 (100)	2.85±0.11 (91)	3.10±0.01 (99)	3.21±0.02 (103)	2.93±0.12 (94)
6.	Plant growth rate	1.27±0.04 (100)	1.02±0.10 (81)	1.18±0.03 (94)	1.23±0.05 (98)	1.09±0.11 (87)

Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE

Table.2 Microbial fertilizers treated Nickel chloride application in Biochemical composition of *Pennisetum americanum* L

S. No	Parameters	Control	Control NiCl ₂ (6mM)	S1	S2	S3
1.	Glucose (ug/gl fw)	0.27±0.14 (100)	0.15± 0.12 (81)	0.24±0.11 (91)	0.26±0.10 (95)	0.20±0.14 (88)
2.	Protein (ug/gl fw)	1.48±0.12 (100)	1.23± 0.01 (78)	1.38±0.13 (94)	1.44±0.11 (98)	1.33±0.14 (85)
3.	Amino acid (ug/gl fw)	2.41±0.13 (100)	2.72± 0.03 (121)	2.17±0.04 (91)	1.93±0.12. (96)	1.93±0.10 (85)
4.	Leaf Nitrate (ug/gl fw)	4.17±0.12 (100)	3.78± 0.02 (93)	4.05±0.01 (98)	4.18±0.11 (101)	3.93±0.11 (95)

Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE

Table.3 Microbial fertilizers treated Nickel chloride application in enzymatic activities of *Pennisetum americanum*, L

S. No	Parameters	Control	Control NiCl ₂ (6mM)	S1	S2	S3
1.	Catalase (ug/gl fw)	0.66±0.21 (100)	0.85±0.15 (130)	0.72±0.12 (110)	0.69±0.13 (106)	0.79 ±0.04 (121)
2.	Peroxidase (ug/gl fw)	0.019±0.13 (100)	0.029±0.14 (150)	0.023±0.07 (121)	0.021±0.14 (110)	0.025±0.31 (131)
3.	Nitrate Reductase (ug/gl fw)	3.33±0.07 (100)	4.63±0.01 (148)	3.82±0.01 (122)	3.63±0.12 (116)	4.21±0.02 (134)
4.	Superoxide Dismutase (ug/gl fw)	1.20±0.04 (100)	1.62±0.31 (129)	1.48±0.03 (118)	1.37±0.11 (109)	1.52±0.05 (121)

Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE

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