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# Evaluation of Effect of Zinc Oxide and Zinc Solubilizing Bacteria on Morphological and Enzymatic Activities on Radish (*Raphanus Sativus* L.)

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## ABSTRACT

Zinc is an essential micronutrient required by all plants in variable concentrations but certain inherent properties of soil make the accessibility of Zinc a problem for crop plants. Different approaches can be use to increase Zn uptake by plants, one of which can be the application of Zinc solubilizing bacteria (ZSB). Although highly useful but only a handful of studies document the effect of concentration of Zinc Oxide with different inoculants of bacteria. Various PGPR have found to be effective zinc solubilizers. These bacteria improve the plant growth and development by colonizing the rhizosphere and by solubilizing complex zinc compounds into simpler ones, thus making zinc available to the plants. Various PGPR have shown enhanced growth and Zinc content when inoculated in plants. These include *Pseudomonas, Bacillus* sp., and *Azospirillum*. To investigate the physiological and biochemical basis with Zinc oxide (ZnO) and inoculation of plant growth promoting rhizobacteria in Radish plants (Japanese White Radish). Four different strains of bacteria were used i.e. MTCC 102 – *Pseudomonas putida*, MTCC 103 – *Pseudomonas fluorescens*, MTCC 121 – *Azospirillum lipoferum*, MTCC 2694 – *Bacillus subtilis*, and untreated Control in field of Radishes. Field and Laboratory experiments were conducted in Department of Botany and Botanical garden at DAV University Jalandhar. Results of our study indicate that morphological data (Fresh Weight, Dry Weight of Radish Plants), and Chlorophyll Content, Catalase activity and Protein Estimation Content showed the best performance with application of ZnO and PGPR as compared to the plants with no external treatment on Radish plants.

***KEYWORDS:*** *Fresh Weight, Dry Weight of Radish Plants, Chlorophyll Content, Catalase activity and Protein Estimation Content, Radishes, PGPR, Zinc solubilizing bacteria (ZSB), Zinc oxide (ZnO)*

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## INTRODUCTION

Zinc solubilizing bacteria are living fertilizers found on a select strain of naturally occurring useful autotropic, acidophiclic bacteria. They are gram negative, rod shaped, nonsporing bacteria. Zinc solubilizing bacteria are used as an effective soil inoculants. Zinc solubilization can be influence by numerous factors with activity of the Zinc solubilizing bacteria. The bacteria solubilize Zn through some mechanisms, which comprises of excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents **(Eshaghi et al., 2019)**. This solubilization property is main in nutrient cycling chiefly during planting. Zinc solubilizing bacteria are prospective alternatives for zinc supplementation and transform applied inorganic Zinc to available forms **(Kamran et al., 2017)**.

ZSB proficiently solubilizes both the unsolvable Zinc compounds i.e. ZnO and $ZnCO_3$, though ZnO was efficiently solubilized in comparison to $ZnCO_3$. The application of Zn solubilizing bacteria upsurges the obtainability of native zinc for plant assimilation and ultimately plant growth promotion **(Gontia-Mishra**

**et al., 2017**). Rice plant under ZSB inoculation had been reported, that the inoculation of ZSB with Rice seedlings, recorded increase in shoot and root lengths as well as higher dry weights root and shoot in contrast to un-inoculated seedlings. **(Sharma et al., 2012; Goteti et al., 2013; Vaid et al., 2014)** founded similar increase in root mass, shoot length and total dry weight of Soybean, Maize. Application of ZSB in agriculture can rise the availability of Zn in soil which can circumvent the yield loss and therefore the nutritional quality of rice. Results from the recent study demonstrate that ZSB can surely modify Zn-regulated transporters and iron -regulated transporter-like protein (ZIP) family, which play vital role in Zn uptake from soil, translocation within root and subsequently into the grains **(Krithika and Balachandar, 2016**).

## MATERIALS AND METHODS

Experiments were conducted in the Field and Laboratory during the year 2017-2018. One commercially available Radish variety was used in the experiment. Seeds were sown in a randomized block design, with four treatments and untreated Control. First plot plants of Radish were treated with ZnO and *Pseudomonas putida*, second plot plants were treated with ZnO and *Pseudomonas fluorescens*, third plot plants were treated with ZnO and *Azospirillum lipoferum*, fourth plot plants were treated with ZnO and *Bacillus subtilis*, and fifth plot plants were not treated. At 10, 20 days morphological data were recorded and at 7, 14, 21 days different enzymatic activities were recorded. Radishes crop were harvested and Fresh and Dry weight of plants were recorded.

### Fresh Weight of Radish Plants:-

Fresh Weight of Radish Plants (Units Gram) were measured after Zinc Oxide fertilizer application and different treatments of bacterial inoculants (PGPR), in treated plants and untreated Control.

### Dry Weight of Radish Plants:-

Dry Weight of Radish Plants (Units Gram) were measured after Zinc Oxide fertilizer application and different treatments of bacterial inoculants (PGPR), in treated plants and untreated Control.

**Catalase (CAT) activity:-** Catalase activity in the leaves and fruits were estimated by the method given by Kato and Schimizu (1987).

### Chlorophyll quantification:-

Chlorophyll was quantified in leaves using the protocol given by Hiscox and Israelstam (1979).

### Protein Estimation Content:-

Protein was estimated from leaves and fruits of Radish plant material given by Bradford (1976).

### Statistical analysis of morphological data and enzymatic activities

Significance of the observed values were determined by SPSS.

## RESULTS AND DISCUSSION

Significant differences between different bacteria were showed by one-way ANOVA. The results of this study revealed that application of Zinc Oxide and PGPR application in plants had a significant effect on all measured traits of Radish seedlings.

## FRESH WEIGHT OF PLANTS

Fresh Weight of Plants were higher in the plants that received Zinc Oxide and PGPR as compared to Control plants. Fresh weight is important parameter in case of Radish because the tap root of Radish is the important part of the plant and is widely used as a vegetable, salad preparations worldwide. In the context Fresh Weight of plants were measured in order to estimate the effect of PGPR and its interaction with ZnO by plants.

The effect of different PGPR application were founded highly significant during the year. During the crop season, at all PGPR application the highest values were founded in MTCC 103 and the lowest values were founded in MTCC 102. The % increase in the fresh weight of plants in comparison to Control were 66.72. The Graph in Figure-1 showed data of Dry weight of plants after different applications of PGPR and ZnO, and of untreated Control at harvesting stage.

**(Singh et al., 2018)** founded Similar results envisaged that $T_3$ (60% RDF + *Azotobacter* + VAM) treatment was found significantly superior to improve the growth, yield and quality of Radish root. The maximum fresh weight of root per plant (358.25 g), and leaves (7.57%) were recorded with $T_3$ treatment (60% RDF + Azotobacter + VAM) followed by $T_7$ (RDF200:80:80 NPK).

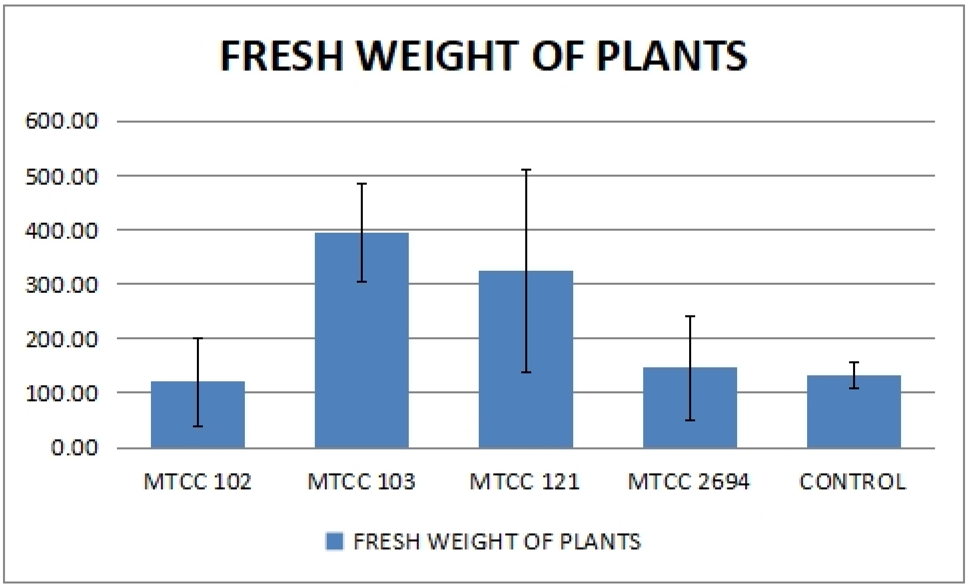


**Figure 1: Fresh weight of radishes in treated and control plants. The treatments were given at 3 leaf stage and the observations were recorded at harvesting stage of Plants. The values presented are mean of 5 rows of 6 replicates each. Series 1 represents data of Fresh weight of Plants.**

## DRY WEIGHT OF PLANTS

Dry weight is an important parameter. Dry weight was affected by the application of the Zinc along with bacterial treatments suggesting that Zinc Oxide fertilization and bacterial inoculation both can promote plant growth of the Radish plants along with improving the micronutrient content of the Roots.

In the context Dry Weight of plants were measured in order to estimate the effect of PGPR and its interaction with ZnO by plants. The effect of different PGPR application were founded highly significant during the year. At all PGPR and Zinc Oxide application the highest values were founded in MTCC 103 and the lowest values were founded in Control. The % increase in the dry weight of plants were 84.07. The Graph in Figure-2 showed data of Dry weight of plants after different applications of PGPR and ZnO, and of untreated Control at harvesting stage.

**(Singh et al., 2018)** founded similar results in Radishes, the maximum (287.00 q $ha^{-1}$) root yield was observed from $T_3$ (60% RDF + *Azotobacter* + VAM) followed by $T_6$, $T_4$ and $T_5$ which were found at par to each other. The maximum dry matter content of root (8.59%) were recorded with $T_3$ treatment (60% RDF + *Azotobacter* + VAM) followed by $T_7$ (RDF200:80:80 NPK).

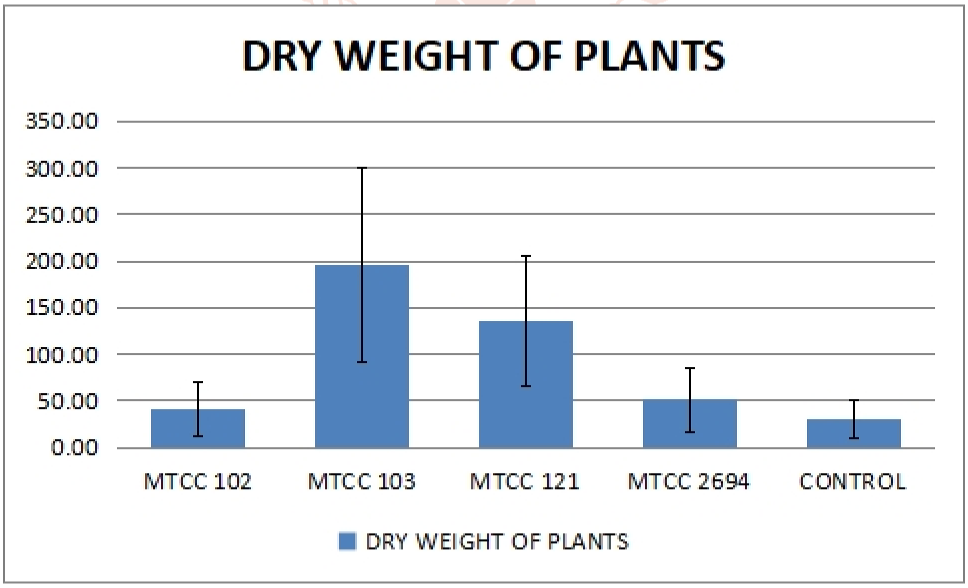


**Figure 2: Dry weight of Radishes in treated and Control plants. The treatments were given at 3 leaf stage and the observations were recorded at harvesting stage of Plants. The values presented are mean of 5 rows of 6 replicates each. Series 1 represents data of Dry weight of Plants.**

## CHLOROPHYLL CONTENT

Chlorophyll is the pigment that gives plants their characteristic green color. The amount of Chlorophyll in plants is affected by many factors one of them being the amount of Zinc taken up by the plants. As Zinc is utilized as an important cofactor in the conversion of protochlorophyllide to chlorophyll, increase in the amount of Zinc results in an increase in Chlorophyll content of plants.

Chlorophyll A was measured in leaves, At all PGPR and ZnO application the highest values were founded for MTCC 2694 and the lowest values were founded for MTCC 121. The % increase in the Chlorophyll A content in comparison to Control were 34.78.

Chlorophyll B is the subsidiary Chlorophyll and its measurement is important to establish the ability of the plants to photosynthesize as it forms an important component of antenna molecules of photosynthetic light harvesting complex 1 and 2. At all PGPR and ZnO application the highest values were founded for MTCC 103 and the lowest values were founded for Control. The % increase in the chlorophyll B content in comparison to control were 40.47.

Total Chlorophyll content involves the primary Chlorophylls i.e. Chlorophyll A and B as well as the subsidiary pigments that help in photosynthesis of the green plants. At all PGPR and ZnO application the highest values of Total Chlorophyll were founded for MTCC 2694 and the lowest values were founded for MTCC 121. The % increase in the total chlorophyll content in comparison to Control were 24.77. The Graph in Figure-3 showed data of Chlorophyll content in leaves after different applications of PGPR and ZnO, and of untreated Control at different days.

Similar results were obtained in Wheat Chlorophyll a, chlorophyll b and total chlorophyll content was correlated with higher iron acquisition **(Sadaghiani et al., 2009)**. The concentration of chlorophyll a in leaves of sid $^{+}$ and sid $^{-}$ treatments were 1.27 and 0.41 g mg $^{-1}$ of fresh weight, respectively, and the concentration of chlorophyll b were measured to be 1.09 and 0.35 g mg $^{-1}$ of fresh weight, respectively, indicating significantly more chlorophyll formation due to inoculation with sid $^{+}$ as compared with sid $^{-}$. These results may also indicate that siderophores of strain 7NSK2 involved in Fe uptake.

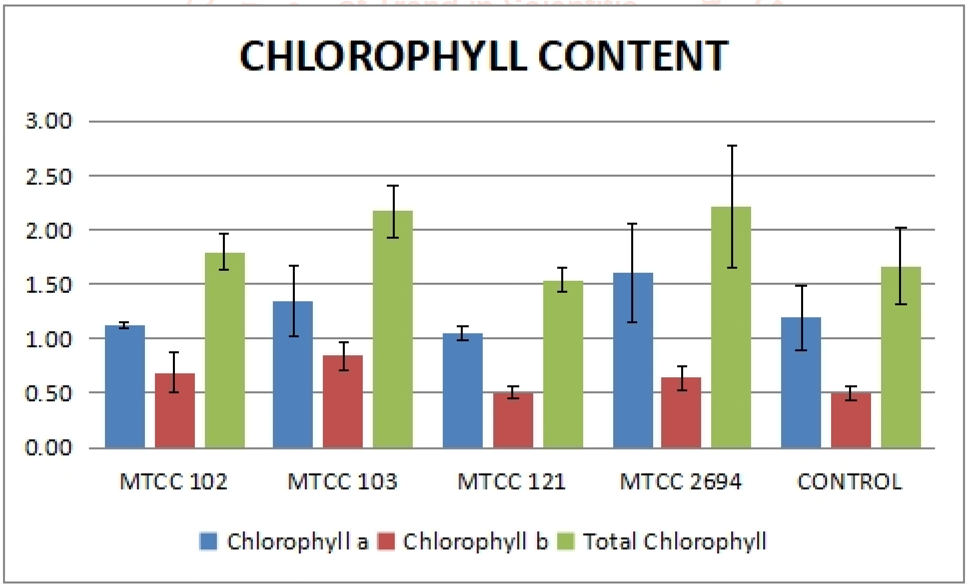


**Figure 3: Chlorophyll Content in Leaves of Radish in treated and Control plants. The treatments were given at 3 leaf stage and the observations were recorded 7 days later. The values presented are mean of 3 replicates each.**

## CATALASE ACTIVITY

CATALASE enzyme catayses the decomposition of the hydrogen peroxide into water and oxygen. It protects the cell from oxidative damage by reactive oxygen species (ROS) and ultimately increases the plant productivity. The activity of Catalase was measured in the context to check the effect of ZnO and different bacterial application on enhancement of Iron content. The Catalase Activity was enhanced by the application of Zinc oxide and bacterial treatment. Zinc and Iron also are synergistic to each other in terms of absorption i.e. each promotes the absorption of other therefore, application of ZnO fertilizer enhances the content of Iron in plants hence the enhancement of Catalase Activity was observed.

Analysis of the data showed increase in the CAT Activity for 7 days, 14 days, 21 days and in Fruits. At 7 days the highest values were founded in MTCC 102 and the lowest values were founded in MTCC 121. The % increase in the Catalase Activity in comparison to Control were 26.67. At 14 days the highest values were founded in MTCC 102, MTCC 2694 and the lowest values were founded in MTCC 121. The % increase in the Catalase Activity in comparison to Control were 65.32. At 21 days the highest values were founded in MTCC 2694 and the lowest values were founded in MTCC 121 and Control. The % increase in the Catalase Activity in comparison to Control were 34.49. In Fruits the highest values were founded in MTCC 2694 and the minimum for MTCC 102. The % increase in the Catalase Activity were 7.89. The Graph in Figure-4 showed data of Catalase Activity in leaves and fruits after different applications of PGPR and ZnO, and of untreated Control at different days. At fruiting stage of Radish, the Catalase Activity were recorded in siliques.

**(Rasouli Sadaghiani et al., 2007)** showed that Tabasi genotype as bread Wheat released high amount of root exudates mainly phytosiderophores in Fe and Zn deficiency conditions. Therefore, the uptake of Fe by roots and its rate of translocation to the shoots were greater for the sid $^+$ treated plants as compared with the sid $^-$ treated ones, indicating that siderophores increased the rate of Fe uptake by wheat.

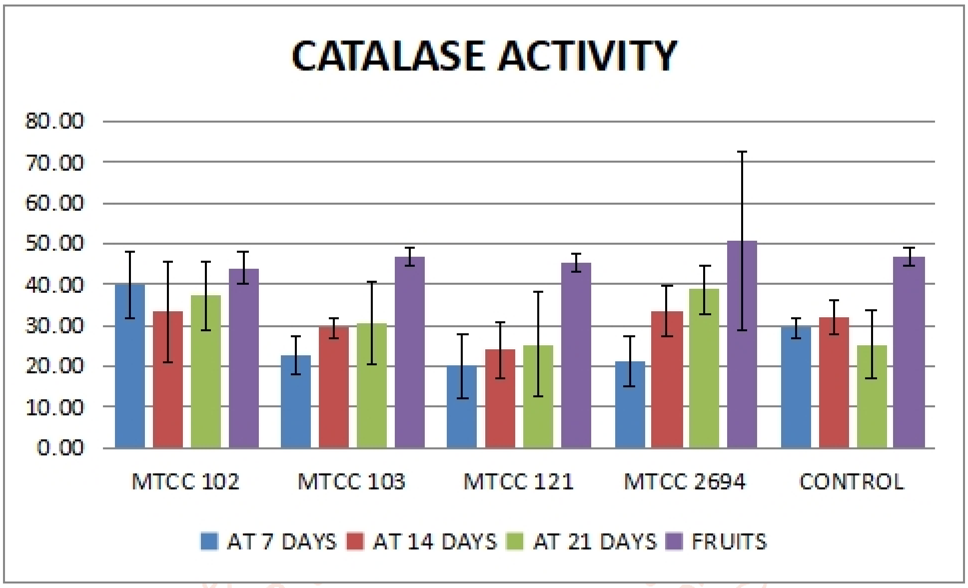


**Figure 4: Catalase Activity in Leaves and Fruits of Radish in treated and control plants. The treatments were given at 3 leaf stage and the observations were recorded 7 days later. The values presented are mean of 3 replicates each.**

## PROTEIN ESTIMATION CONTENT

Protein Estimation Content is an important parameter of plants. In the context Protein Estimation Content was measured in order to estimate the effect of PGPR and its interaction with ZnO on amount of Protein Estimation Content synthesized by plants. During the crop season, at all PGPR and ZnO application at 7 days the highest value were founded in MTCC 103 and the minimum for MTCC 2694. The % increase in the Protein Estimation Content in comparison to Control were 9.38%. At 14 days the highest values were founded in MTCC 102 and the minimum for MTCC 121. The % increase in the Protein Estimation Content in comparison to Control were 0.57%. At 21 days the highest values were founded in MTCC 2694 and the minimum for MTCC 102. The % increase in the Protein Estimation Content in comparison to Control were 1.71%. In Fruits the highest values were founded in MTCC 2694 and the lowest values were founded in MTCC 102. The % increase in comparison to Control were 16.99. The Graph in Figure-5 showed data of Protein Estimation Content in leaves and fruits after different applications of PGPR and ZnO, and of untreated Control at different days. At fruiting stage of Radish, the Protein Estimation Content were recorded in siliques.

(**Khan et al., 2019**) studied the similar results that the leaf Protein Content of the sensitive variety (S) decreased by 18% under drought stress as compared to irrigated control; however, the tolerant variety (T) showed greater protein content over the control. The consortium of PGPR + PGRs significantly enhanced the leaf protein content and the increase was even greater (20% and 33%) than control for both the sensitive (20%) and tolerant varieties (33%), respectively.

Graph 5 showed data for different treatments at harvesting stage after the application of PGPR and ZnO and of untreated Control.

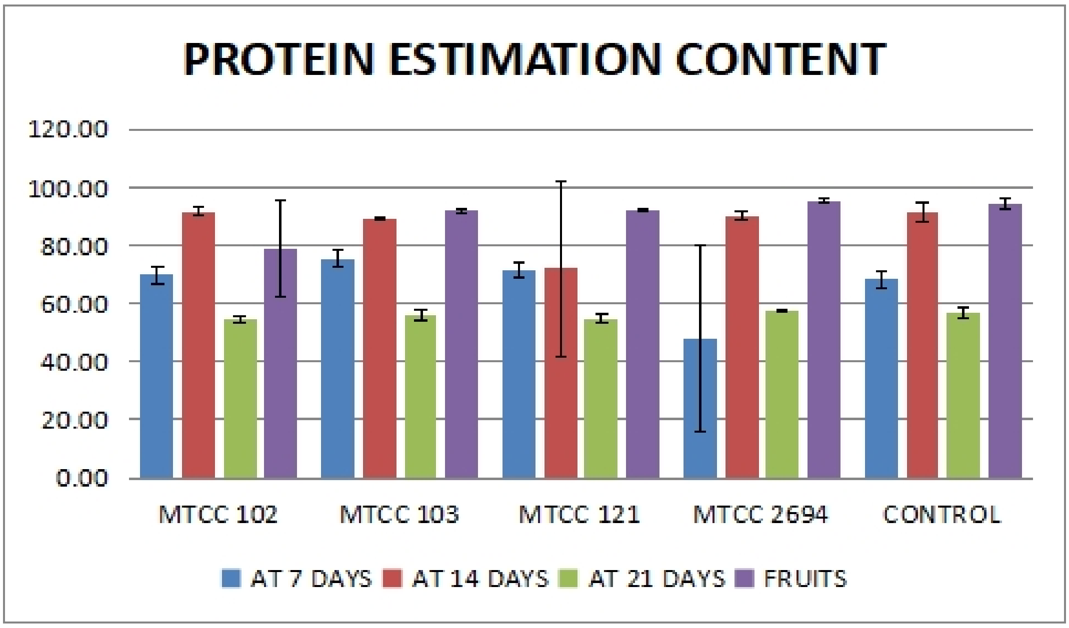


**Figure 5: Protein Activity in Leaves and Fruits of Radish in treated and control plants. The treatments were given at 3 leaf stage and the observations were recorded 7 days later. The values presented are mean of 3 replicates each.**

## CONCLUSIONS

Zinc solubilizing bacteria had well-known to promote plant growth under abiotic stress conditions. Zinc solubilizing microorganisms solubilize Zinc by diverse mechanisms, one of which is acidification. The microbes produce organic acids in soil which sequester the Zinc cations and decrease the pH of the nearby soil. The anions can also chelate Zinc and enhance Zinc solubility. Other mechanisms perhaps involved in Zinc solubilization include production of siderophores and proton, oxido-reductive systems on cell membranes and chelated ligands. Various PGPR have shown enhanced growth and Zinc content when inoculated in plants. Our study confirms the relationship among the Fresh Weight, Dry Weight of Radish Plants, Chlorophyll Content, Catalase activity (Enzyme), Protein Estimation Content by the application of ZnO and PGPR and in untreated Control. Fresh Weight, Dry Weight of Radish Plants, and Chlorophyll Content, Catalase activity (Enzyme), Protein Estimation Content increased by *Pseudomonas* strains, *Azospirillum lipoferum*, *Bacillus subtilis*. The data of Radish plants recorded showed that Zinc Oxide and ZSB increased yield of plants. Increase in Fresh weight and Dry weight of Roots, Petiole and Leaves can be correlated to the increase in the Chlorophyll Content in the plants as well as enhancement of Protein Estimation Content and CAT Activity. Zinc O

xide, Iron and ZSB increases uptake also improves the translocation and storage of photosynthetic product from Leaves to Roots hence Weight and Length of Roots increases.

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