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# AGROCHEMICALS TOLERANT EFFICACY BIOINOCULANTS *Vigna mungo* L. HEPPER GROWTH IN NURSERY TRIALS

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## ABSTRACT

The efficient bioinoculants are gaining importance in sustaining agriculture. In the present study, to analyses the effects caused by agrochemicals and to find the best alternative agrochemicals tolerance bioinoculants to where the crop production. Chemical fertilizers were treated with bioinoculants at different concentrations. Decrease in growth rate of both *A. brasilensis* and *B. megaterium* strains was observed at higher concentrations of urea and superphosphate. *Azospirillum brasilensis* did not inhibit the growth of *B. megaterium* at co-culture conditions in well diffusion test as no inhibition zone was observed around the culture well of *A. brasilensis* on the lawn of *B. megaterium*. Similarly, *B. megaterium* did not suppress the growth of *A. brasilensis.* Applications of urea and superphosphate along with adaptive bacterial Inoculants *A. brasilensis* and *B. megaterium* resulted in maximum increase in number plant growth. After 30 and 60 days different treatments of plant growth parameters was obtained. Chlorophyll content was increased with the application of half dose of fertilizer. The experimental soil physicochemical properties were analyzed for 30 and 60 days,the pH, EC, calcium carbonate level, micronutrients (Fe, Mn, Z, and Cu) and macronutrients (N, P,K) levels were increased in all treatments compared to control. Overall results suggest application of 0.5% chemical fertilizers with bioinoculants increase the plant growth and soil chemical profiles such as enzymes, macro and micro nutrients.

**Keywords: Agrochemicals, Bioinoculants, *Vigna mungo* L., *Azospirillum brasilensis, Bacillus megaterium***

## INTRODUCTION

In Indian subcontinent black gram in one of the staple food crop, on which the lives of more than 1.1 billion people rely. Although this crop can serve as constituents of a nutritious diet, malnutrition is a widespread phenomenon in the rural Indian population, and currently 231 million people are undernourished. The main problems are energy deficiency, lack of proteins, and the shortage of micronutrient supply. Black gram (*Vigna mungo* L. Hepper) belonging to family Fabaceae is an important nitrogen fixing, short-duration, tropical pulse crop grown in many parts of the Asian countries and is also in other countries including Africa and Australia for its protein-rich edible dry seeds. Among the Asian countries India is the largest producer accounting for more than two-thirds of the world's total production of black gram **[1]** and it is thought to have originated in the Indian subcontinent with maximum diversity in the Western Ghats **[2]**.

Nutrient efficiency is an important goal in crop production. Access to this nutrient may be hampered by soil characteristics and water availability which renders the application of readily available chemical fertilizers is one key factor to boost the grain yields particularly when grown in poor or degraded soils. In high input agronomic practices, fertilizers are essential components of modern agriculture because they provide essential plant nutrients. Moreover, after application, a large portion of fertilizers accumulate in the soil layer and exert deleterious effects on ecosystem including microbial diversity associated with rhizosphere soil **[3]** thereby leading to a loss in soil fertility. Microbial communities of soils play an important role in cycling of elements in ecological systems and provide essential nutrients to plants. In addition over uses of some chemical fertilizers leads to softening the plant tissue resulting in plants that are more sensitive to diseases and pests. Hence, the application of suitable fertilizers in appropriate doses is considered as one of the most important factors for increasing crop yield per unit area. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth promoting rhizobacteria (PGPR) for improving crop productivity and improvement of the soil fertility for sustainable crop production has gained significant importance.

Pertaining to these facts, in present study, two rhizospheric isolates *Azospirillum brasilensis* and *Bacillus megaterium* were studied for their potential for nutrient management studies to improve growth and yield of black gram and reduce use of chemicals. The strains were

previously reported to elicit significant effects on root development, plant growth, biocontrol and/or induced systemic resistance. Further, to substantiate applicability of *A. brasilensis* and *B. megaterium* in rhizosphere, their tolerant towards two chemical fertilizers such as urea and super phosphate was evaluated. Also, they were applied in nursery along with reduced dose of fertilizers, as individual trials or in co-inoculated conditions.

## MATERIALS AND METHODS

### Bioinoculants

The plant growth promoting bacterial strains such as *Azospirillum brasilensis* and *Bacillus megaterium* were obtained from commercial Sun Agro Biosystem, Chennai, Tamil Nadu. *Azospirillum brasilensis* and *B. megaterium* were maintained on nitrogen free agar medium and pikovskaya's agar medium. Both strains were stored at 4° C until use.

### Isolation of Urea and Super phosphate Adaptive Variants of *Azospirillum brasilensis*and *Bacillus megaterium*

The lethal concentrations of urea and superphosphate on bacterial strains were determined by growing them in medium having different concentrations of urea (0.4–1.6m) and superphosphate (0.5–1.5m). For this, nitrogen free medium broth was used for *A. brasilensis* and pikovskaya's medium broth was used for *B. megaterium*. The log phase cultures ($10^8$ cells $ml^{-1}$) were transferred under aseptic conditions to the respective supplemented medium and incubated at 28°C; 150 rpm for 48 hrs. Optical density was measured at 610 nm after every 6 h intervals. Growth stimulatory, sub-lethal ($LC_{50}$) and lethal ($LC_{100}$) concentrations of urea and superphosphate were determined by calculating specific growth rate and, growth rate of control/specific growth rate of treatment (Vo/V) **[3]**. The adaptive variants of both *A. brasilensis* and *B. megaterium* were raised against the sub-lethal concentrations ($LC_{50}$) of urea and superphosphate by transferring the surviving colonies on growth medium and medium supplemented with sub-lethal ($LC_{50}$) concentrations of urea and superphosphate, respectively.

### Interaction Study between *Azospirillum brasilensis* and *Bacillus megaterium* Strains

Effect of interaction between the strains of *brasilensis megaterium* on their growth rates was determined **[4]**. Supernatants of log phase cultures ($10^8$ cells $ml^{-1}$) of *A. brasilensis* and *B. megaterium* were prepared by centrifuging the late-log phase cultures at 12,000 g for 20 min. The supernatants were passed through Whatman No.1 filter paper. The culture supernatant of *B. megaterium* was filled in the well of nitrogen free agar medium plates

preinoculated with *A.brasilensis* and well filled with supernatant of *A. brasilensis* was placed on pikovskaya's medium solidified plates pre-inoculated with *B. megaterium*. The inhibition of growth around the well was assessed and recorded after 24 h of incubation at 28°C, in form of zone of inhibition.

**Experimental Design**

Soil samples were collected from Field Research Facility of Department of Microbiology, Bharathidasan University. Black gram seeds were treated with agrochemicals and bioinoculants with the ratio of 1%, 0.5% and 2%. Seeds were sown in each pot of the treatment. Nursery experiment was conducted at the Field Research Facility Department of Microbiology, Bharathidasan University, Tiruchirappalli. The experiment was set up in a completely randomized block design with 11 treaments and 66 replicates. All the plants were kept under nursery condition upto 60 days **[5, 6]**.

Experimental Set up (Treatments)

$RS_1$ - Control

$RS_2$ - *Aspergillus brasiliensis*

$RS_3$ - Urea

$RS_4$ - *Aspergillus brasiliensis* + Urea 1%

$RS_5$ -*Aspergillus brasiliensis* + Urea0.5%

$RS_6$ -*Aspergillus brasiliensis* + Urea 2%

$RS_7$ - *Bacillus megaterium*

$RS_8$ - Super Phosphate

$RS_9$ - Super Phosphate +*Bacillus megaterium*1%

$RS_{10}$ - Super Phosphate +*Bacillus megaterium*0.5%

$RS_{11}$ - Super Phosphate +*Bacillus megaterium*2%

**Soil Physico- Chemical Analysis**

The experimental soils were collected and analyses to physicochemical parameters such as pH, EC, soil texture, macro (total nitrogen (N), available phosphorous (P) and exchangeable potassium (K) and micro nutrients iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu)) in Soil Testing Laboratory, Tamil Nadu Agricultural Department, Tiruchirappalli, Tamil Nadu India **[7, 8]**.

**Soil Protein Estimation by Lowry's Method**

500 mg of the sample was mixed well and the solution was centrifuged at 6000 rpm for 20 mins. The supernatant solution was collected, from that 0.1ml of the supernatant was used for the protein estimation and the final volume for protein estimation was made up to 1ml in all the test tubes using the following reagents A, B, C and D. 5ml of reagent C was added to all the test tubes including the blank, mixed well and allowed to stand for 10min. 0.5ml of reagent D was then added, mixed well and was incubated at room temperature in the dark for 30min. The blue

colour developed was read in a spectrophotometer at 660nm. A tube with 1ml of water served as a blank **[9]**.

Where, Reagent A is 2% sodium carbonate in 0.1N sodium hydroxide.

Reagent B is 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate.

Reagent C is alkaline copper sulphate solution: 50ml of reagent A was mixed with 1ml of reagent B, prior to use.

Reagent D is Folin-Ciocalteau Reagent.

**Soil Enzymes**

**Estimate of Urease Enzyme**

One gm of rhizosphere soil was treated with 0.25 ml of toluene. Then to this suspension 0.75 ml of citrate buffer (pH 6.7) and 1 ml of urea solution was added and it was incubated at 37°C for 24 h. Optical density of the soil urease enzyme was measured using UV-Vis-absorption spectroscopy at 578 nm **[10, 11]**.

**Protease**

One gm of rhizosphere soil was incubated at 37° C for 1 h with 1 ml of 1% casein in 50mM Tris HCL buffer (pH 10.5), to this 3 ml of 5 % Tri-chloro acetic acid (TCA) was added. Then the suspension was centrifuged at 8000 rpm for 10 min. The supernatant solution was collected and used for protease measurement using UV-Vis-absorption spectroscopy at 470 nm **[12, 13]**.

**Phosphatase**

Two gm of soil sample was weighed and poured into screw-cap tubes (Soil blank, Reagent blank, and test). Then 5 ml of 0.5 M CaCl2 solution was added into each of the three tubes. One ml of PNPP solution transferred into the tubes labeled test and reagent blank. 1ml of phosphate buffer was pippeted out into the soil blank tube to serve as a control. All three tubes were incubated at 37°C for 1 hour. Then 4 ml of the liquid from each tube transferred into other test tubes. The test tubes were centrifuged for 5 min at 2500 rpm. The supernatant solution was collected for phosphatase enzyme estimation by using UV-Vis-absorption spectroscopy at 440 nm **[14, 15]**.

**Estimation of Soil carbohydrate**

Five mg of sample was mixed with 1 ml of 5% solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 sec and it was placed in a water bath at room temperature for 20 min for color development. Then, light absorption at 485 nm is recorded using UV-Vis-absorption spectroscopy. Reference solutions were prepared in identical manner as above, except that the 5 mg sample is replaced by DDI water **[16, 17]**.

**Isolation and Enumeration of Soil Microorganisms from Soil Sample**

Soil samples were collected from each treatment and serially diluted up to $10^{-6}$ as per the serial dilution technique, diluted soil suspensions were platted on various microbiological medium such as nutrient agar, potato dextrose agar, NFB medium and Pikovskaya's agar for enumeration of bacteria and fungi **[18, 19]**.

**Analysis of Black gram Morphometric variable**

The following measurements were recorded at the period of 30 and 60 days of harvested plant for each treatment per replication:plant shoot and root fresh weight and Dry weight. Shoot, root length and numbers of leaves and root nodules, root fresh weight and dry weight were measured **[20]**.

**Biochemical Analysis**

**Total Protein**

The 500 mg of leaf sample was grounded using mortar and pestle in 5-10 ml of the phosphate buffer and the solution was centrifuged at 6000 rpm for 20 min. The supernatant solution was collected, from that 0.1ml of the supernatant was used for the protein estimation and the final volume for protein estimation was made up to 1ml in all the test tubes using the following reagents A, B, C and D. 5ml of reagent C was added to all the test tubes including the blank, mixed well and allowed to stand for 10 min. 0.5ml of reagent D was then added, mixed well and was incubated at room temperature in the dark for 30min. The blue color developed was read in a spectrophotometer at 660nm. A tube with 1ml of water served as a blank **[21, 22]**.

Where,

Reagent A is 2% sodium carbonate in 0.1N sodium hydroxide.

Reagent B is 0.5% copper sulphate ($CuSo_4.5H_2O$) in 1% potassium sodium tartarate.

Reagent C is alkaline copper sulphate solution: 50ml of reagent A was mixed with 1ml of reagent B, prior to use.

Reagent D is Folin-Ciocalteau Reagent.

**Total Carbohydrate**

100 mg of leaf sample mixed with 1 ml of 5% solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 S and it was placed in a water bath at room temperature for 20 min for color development. Then, light absorption at 485 nm is recorded using UV-Vis-absorption spectroscopy at 440 nm. Reference solutions were prepared in identical manner as above, except that the 5 mg sample is replaced by DDI water **[23]**.

**Total Chlorophyll**

The 100 mg of black gram leaves was placed in a test tube containing 10 ml of 80% buffered acetone (80 ml of acetone made up to100 ml with 20 ml of 2.5 Mm sodium phosphate buffer pH 7.8) and the tubes were placed under refrigeration. At the desired period of incubation the extract liquid was filtered into another tube. Optical density was measured at 645 nm and 663nm using UV-Vis-absorption spectroscopy **[24, 25]**.

**Statistical Analysis**

Means of soil enzymes and growth parameters were compared using analysis of variance and significant differences were identified with using Graph pad prism software.

**RESULTS**

**Chemical Fertilizers (Urea and superphosphate) Adaptive A*zospirillum brasilensis* and *Bacillus megaterium*.**

Chemical fertilizers were treated with bioinoculants at various concentrations, While decreased in growth rate of both *A. brasilensis* and *B. megaterium* strains was observed at higher concentrations of urea and superphosphate respectively. The growth of *A. brasilensis* was completely inhibited at 1.6 gm of urea while in case of *B. megaterium*, 2 gm concentrations of superphosphate proved to be toxic as evidenced by absence of growth on respective medium, hence considered as lethal concentrations. However, the growth rate of *A. brasilensis* was found to increase at lower concentrations of urea (0.4 gm) and *B. megaterium* was found to increase at lower concentrations of superphosphate (0.5 gm) respectively **(Figure 1 and 2)**. These tolerant variants showed growth pattern identical to non-adaptive variants and also gave PGPR activities similar to non-adaptive variants.

**Interaction between *Azospirillum brasilensis* and *Bacillus megaterium***

*Azospirillum brasilensis* does not inhibit the growth of *B. megaterium* under co-culture conditions in well diffusion test as no inhibition zone was observed around the culture well of *A. brasilensis* on the lawn of *B. megaterium*. Similarly, *B. megaterium* does not suppress the growth of *A. brasilensis* **(Figure 3)**.

**Nursery Trial**

Seeds of *Vignamungo* were inoculated with chemical fertilizers and bioinoculants and sowed into the experimental soil. Seeds without inoculation act as control. After 30 and 60 days plant has harvested and necessary plant indexes were observed. Dry root and shoot weight, root and shoot length were increased significantly in all the treatments as compared to control. Increase in fresh shoot weight of black gram plants. The synergistic effect of *A. brasilensis* and *B. megaterium* was apparent in co-inoculated trial, for growth

enhancement of black gram. Increase in shoot length and fresh shoot weight was recorded in co-inoculated trial as compared to control. Further, plant growth was significantly improved with application of half dose of fertilizers. Interestingly, application of urea and superphosphate along with adaptive bacterial inoculants **Table 1** of *A. brasilensis* and *B. megaterium* resulted in maximum increase in number plant growth.

Total Chlorophyll content was estimated from fresh leaves of plants the level content was increased with the application of half dose of fertilizer **(Figure 4 and 5)**. Plant leaves total carbohydrate and protein was analyzed, co-inoculation of half dose of agrochemicals with bioinoculants improved the levels for 30 and 60 days **(Figure 6 and 7)**.

**Physico-chemical Properties of Soil**

The experimental soil physicochemical properties were analyzed for 30 and 60 days in the present study. Physicochemical characters pH, EC, calcium carbonate level, micronutrients (Fe, Mn, Z, and Cu) and macronutrients N, P and K levels were increased in all treatments compared to control **(Figure 8, 9, 10 and11)**.

**Analysis of Soil Enzymes, Carbohydrate and Protein**

Soil enzymes urease, phosphatase and protease were estimated from the experimental soils. Urease enzyme level was improved with the application of half dose of fertilizers (*A. brasilensis* 0.5% + urea 0.5%) and phosphatase concentration was increased in the half dose fertilizers (*B. megaterium* 0.5% + phosphate 0.5%), **(Figure 12, 13, 14 and 15)**. Total carbohydrate and protein was analyzed from experimental soils for 30 and 60 days.

**Isolation of Soil Microorganisms**

Soil microorganisms are experimental blackgram rhizosphere soils microbial profiles were analyses such as bacteria, fungi, free living nitrogen fixing bacteria, phosphate solubilizing bacteria were isolated from the rhizosphere regions of treated black gram plants. Different types of colonies were isolated from soil. Microbial profiles were increased with application of half dose of fertilizers and colonies decreased in the higher concentrations of fertilizers **(Figure 16 and 17)**.

**DISCUSSION**

The experimental results were presented to support the hypothesis that *Azospirillum brasilensis* and *Bacillus megaterium* can improve the nutrient use efficiency of fertilizers. In the present study, the population of *A. brasilensis* and *B.megaterium* was subjected to increased concentrations of urea and super phosphate. The low doses were found to stimulate the bacterial population, but increasing the

concentration of urea and super phosphate resulted in decreased the population rate, and also lead to cell lysis and death, which was in accordanceto earlier observations **[26]**. Recently, **[27]** found that higher concentrations of urea are inhibitory to rhizobial growth, because of alteration in cell membrane permeability and/or effect on cellular DNA synthesis, but extremely low doses urea proved stimulatory for growth rate of rhizobia, which is similar to our findings. Both *A. brasilensis* and *B.megaterium* were good colonizers of *V. mungo* rhizosphere. The population of both isolates increased ten times after 60 Days, as compared to population of 30 Days.

Super phosphate when applied to the field release ammonia and provide HPO4- a soluble and available form of inorganic phosphate but soon after it become unavailable due to low solubility and high sorption capacity in soil. Acidification was responsible for inorganic phosphate solubilization by phosphate solubilizing bacteria, which had been reported to form acids, resulting drop in pH and cause phosphate solubilization. Phosphate solubilizing bacteria (*Bacillus polymyxa*) release organic and inorganic acids, which is reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants. One of the main disadvantages of chemical fertilizers is that, in contrast to organic fertilizers, several chemical fertilizers have high acid content like sulfuric acid and hydrochloric acid. This high acid content results in the destruction of the nitrogen-fixing bacteria, which is helpful in supplying the nitrogen to a growing plant. In contrast, organic fertilizers support the growth of nitrogen-fixing bacteria.

When chemical fertilizers are used, just a certain amount of the chemicals within the fertilizers are utilized by the plant. The balance unused chemicals enter the groundwater and get carried from there to various water bodies. Hence, these fertilizers cause pollution of groundwater and other water bodies. Danger with the incorrect application of chemical fertilizer and over-concentration leads to dehydration of plant tissues. The excessive use of nitrogenous fertilizers concentrates nitrates in the soil and water. Nitrate rich water is unfit for drinking, and is rather difficult to treat. Excessive use of fertilizers over a long period may affect the alkalinity or acidity of the soil and may adversely affect the crop production. When chemical fertilizers are used correctly, plants will be healthy. Chemical fertilizers make plants healthier and work well in poor soil conditions. These fertilizers aid in resisting bugs and disease. Distinct microbial populations in rhizosphere frequently interact with each other. Therefore mixed inoculants (combination of micro-

organisms) that interact synergistically are currently being devised, which yield better and quick results **[28]**. The secondary metabolites produced by *A. brasilensis* and *B. megaterium* were non-reactive against each other; hence both were able to co-exist. During present study, enhanced plant growth and developments parameters revealed the significance of integrated use of co-inoculant of variants with reduced dose of urea and super phosphate.

Co-inoculation of bioinoculants with 0.5% chemical fertilizer resulted in plant uptake of nutrient that was significantly higher than with full fertilizer rates. However, 0.5% fertilizer plus bioinoculants resulted in more nutrient uptake than the corresponding treatment with chemical fertilizer alone.

The co-inoculation of *A. brasilensis* and *B.megaterium* proved very effective for growth promotion of *V. mungo,* when applied with half dose of fertilizers. When the percentage of recommended fertilizer was reduced and inoculants were used, plant height, plant fresh and dry weight and nutrient uptake were comparable to those with the full rate of fertilizer without inoculants. The increase in plant growth with co-inoculation of both strains and half dose of fertilizers was almost similar to that obtained after application of recommended doses of urea and super phosphate. When, 0.5% chemical fertilizer rate or lower was supplemented with bioinoculants, higher growth of black gram was observed growth compared to the 1% chemical fertilizer control. The results were clearly suggested that the possibility of reduction in chemical use by application of *A. brasilensis* and *B. megaterium.*

**[29, 30]**, suggested that the possible exploitation of pesticide resistant mutant rhizobial strains for integrated use with chemical fertilizers. **[31, 32]** observed that 48% increases in black gram yield by using rhizobacteria with half dose of chemical fertilizers. Similarly, **[33, 34]** have reported 5–30% enhancement in growth and yield of cereals by inoculation with *Azospirillum* sp. With reduced dose of fertilizers. **[35, 36, 37]** have suggested that integrated use of biofertilizers with reduced dose of chemical fertilizers in wheat. Overall, the results were suggested that inoculants could be used to allow reductions in the current high rates of fertilizer and the resulting environmental problems without compromising plant productivity. However, here we report that the reduction of chemical fertilizer and growth enhancement of *V. mungo* by integrated use of two PGPR, chemotactically active for these fertilizers, with immense potential for these chemical fertilizer adaptive variants for commercial and environmental benefits.

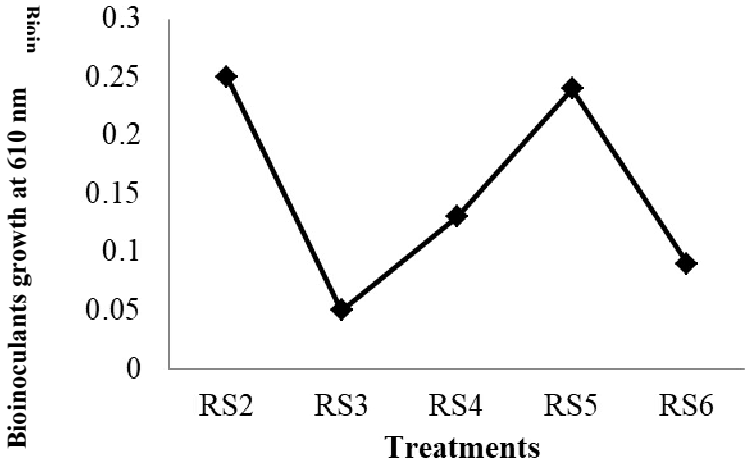


**Figure 1: *In vitro* studies of agrochemical tolerating bioinoculants (*Azospirillum* + Urea)**

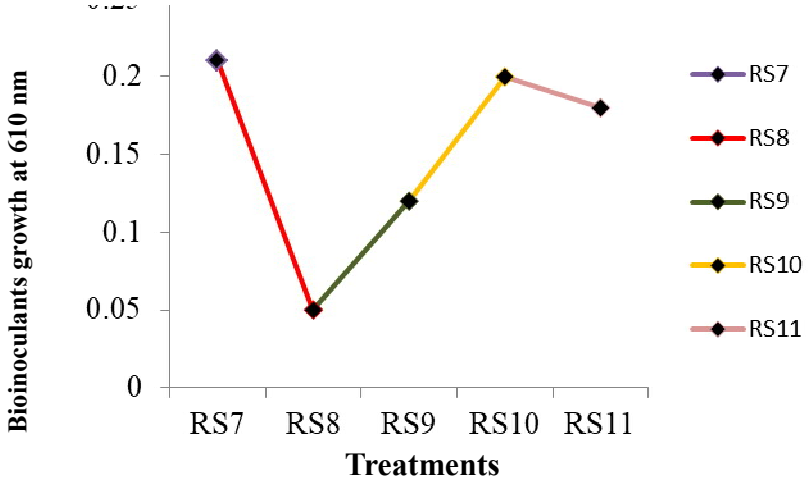


**Figure 2: *In vitro* studies of agrochemical tolerating bioinoculants (Phosphobacteria + superphosphate)**


**Figure 3: Interaction between *Azospirillum brasilensis* and *Bacillus megaterium***

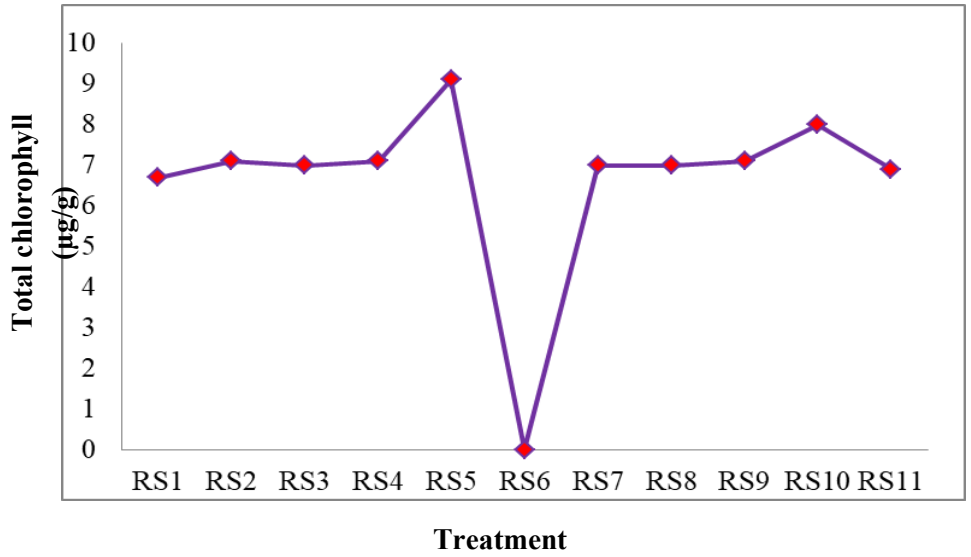


**Figure 4: Plant leaves total chlorophyll for 30 days**

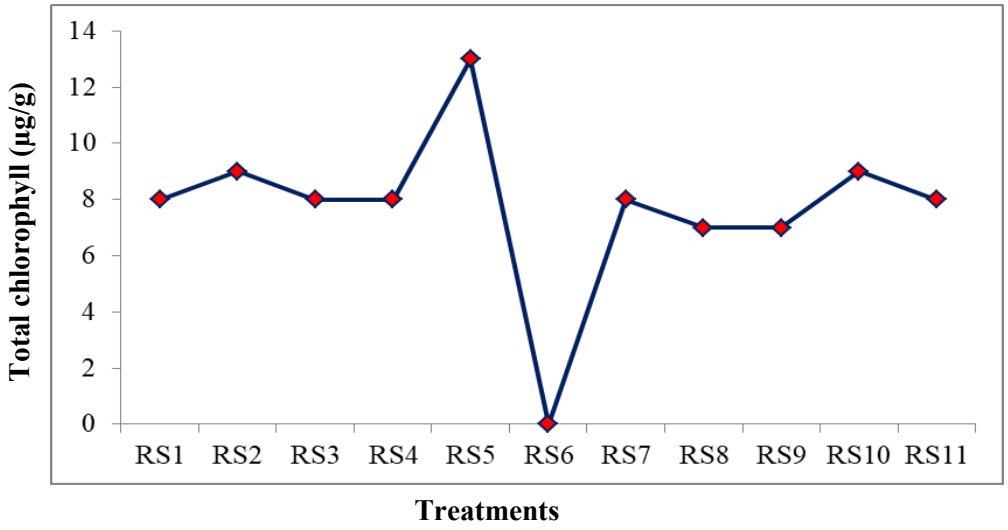


**Figure 5: Plant leaves total chlorophyll for 60 days**

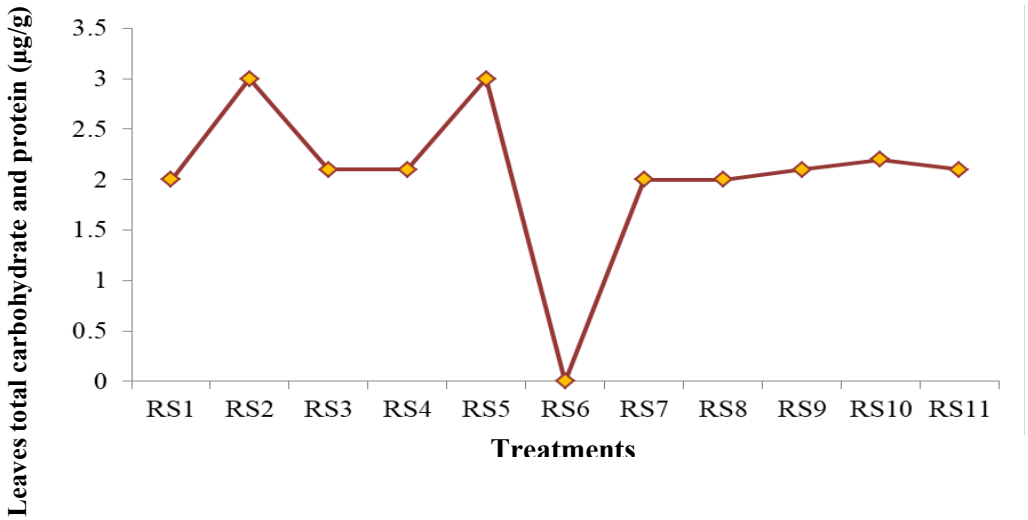

**Figure 6: Plant leaves total carbohydrate and protein for 30 days**

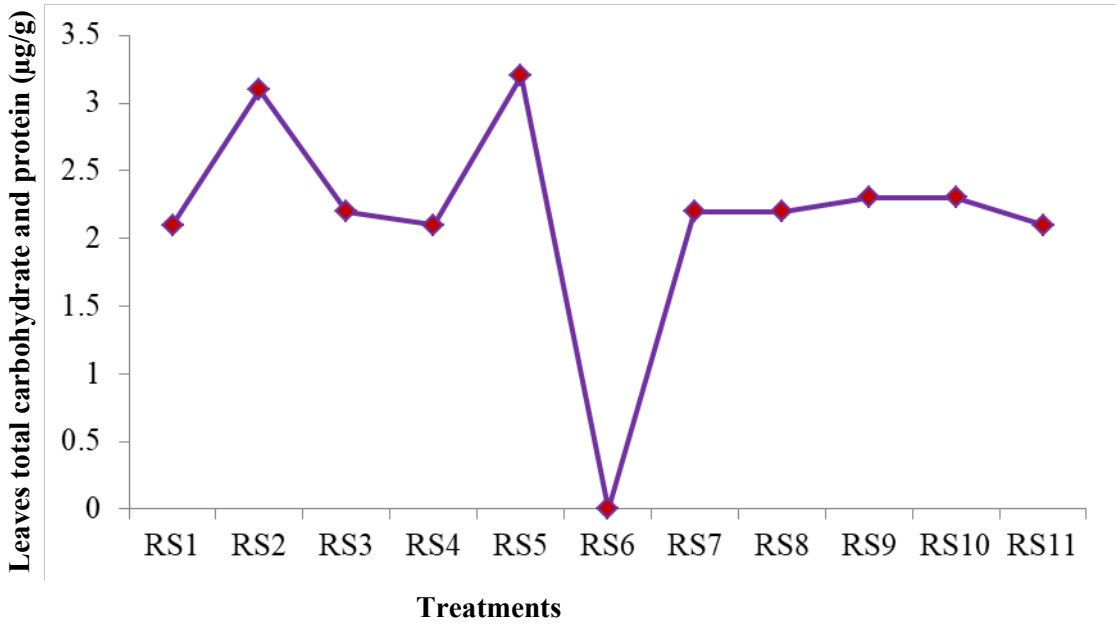


**Figure 7: Plant leaves total carbohydrate and protein for 60 days**

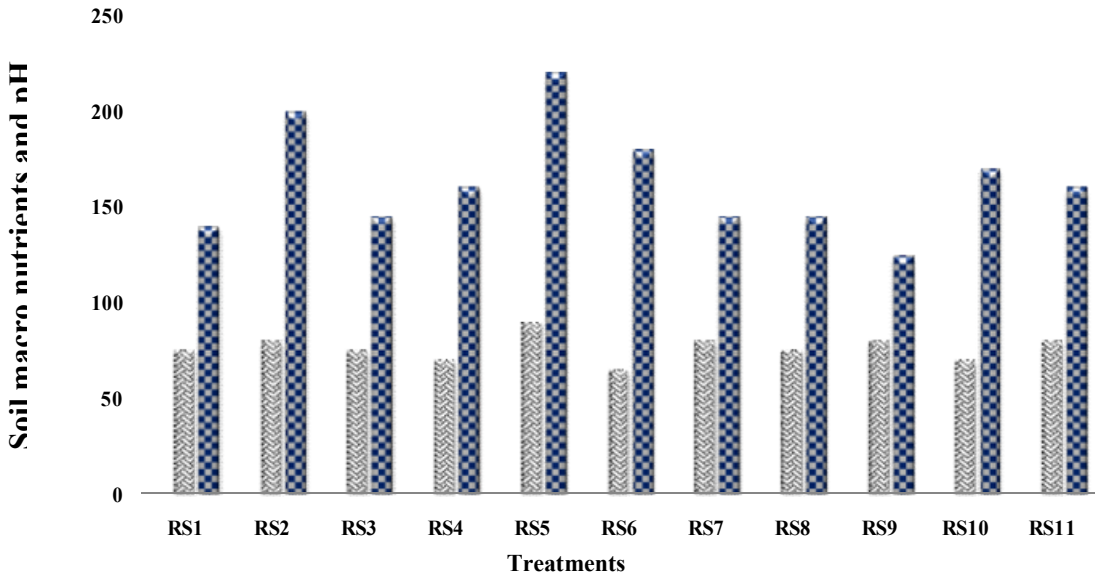


**Figure 8: Soil pH and macro nutrients level at 30 days**

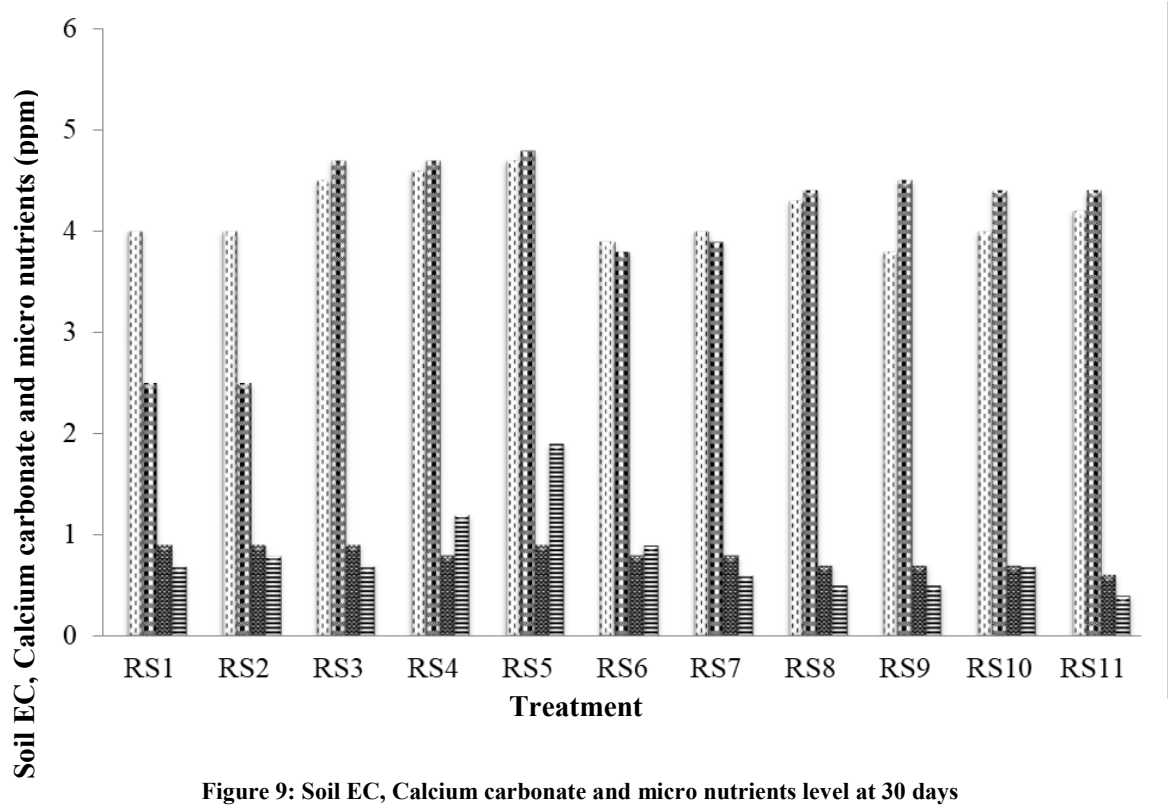


**Figure 9: Soil EC, Calcium carbonate and micro nutrients level at 30 days**

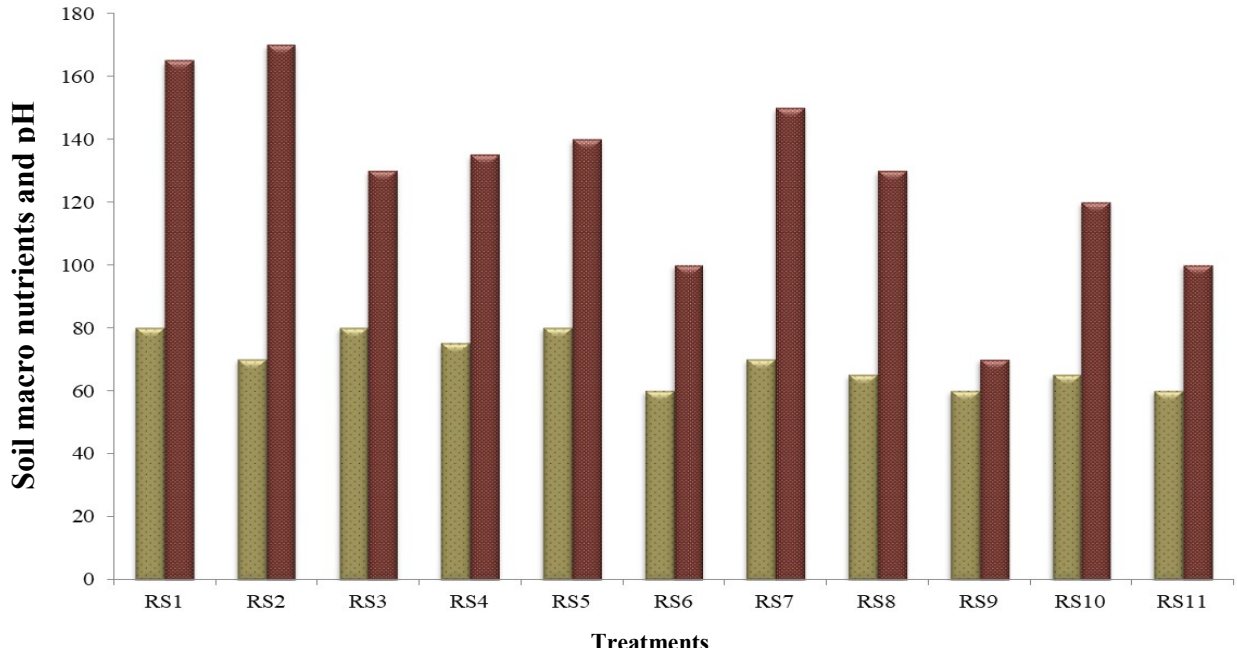


**Figure 10: Soil pH and macro nutrients level at 60 days**

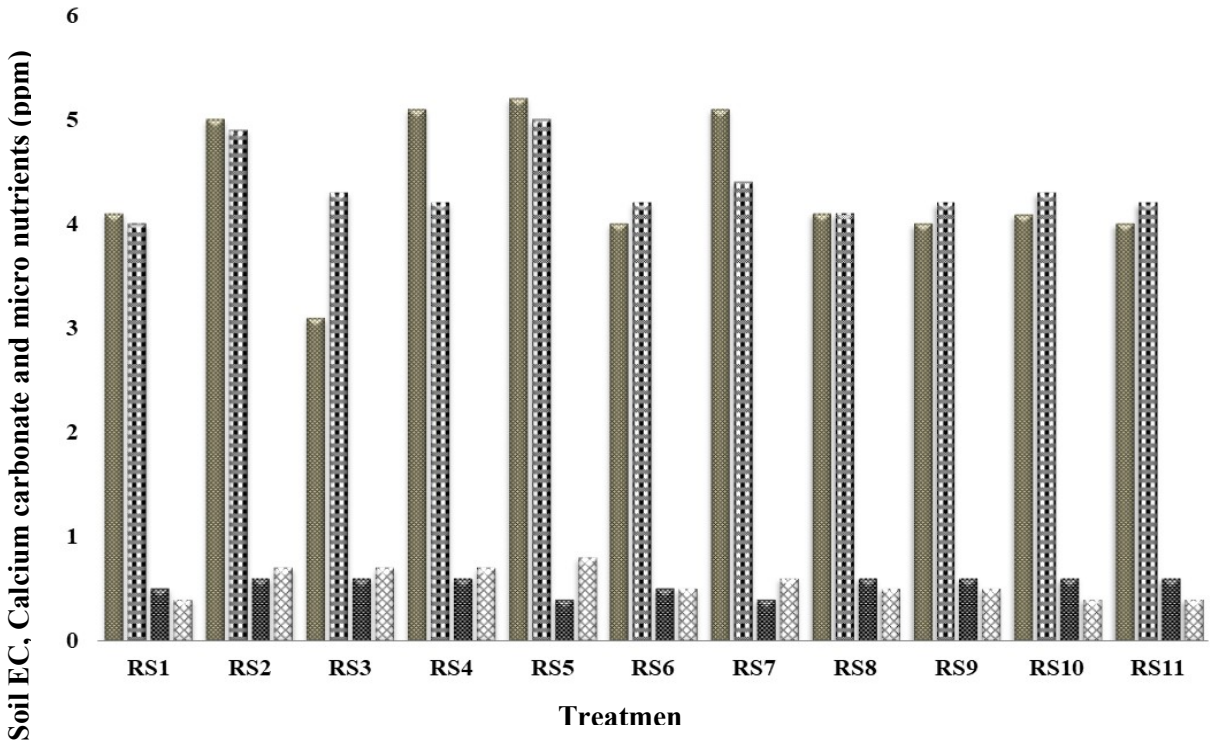


**Figure 11: Soil EC, Calcium carbonate and micro nutrients level at 60 days**

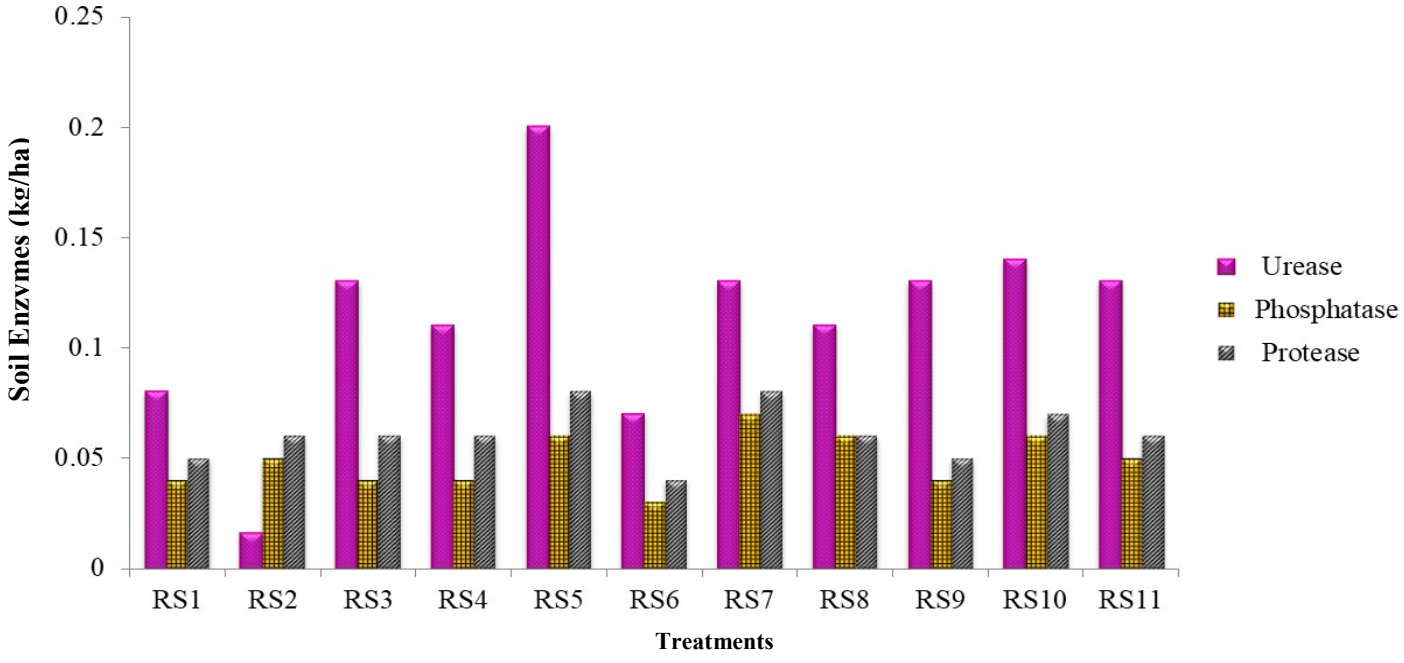


**Figure 12: Soil enzymes enzymes level at 30 days**

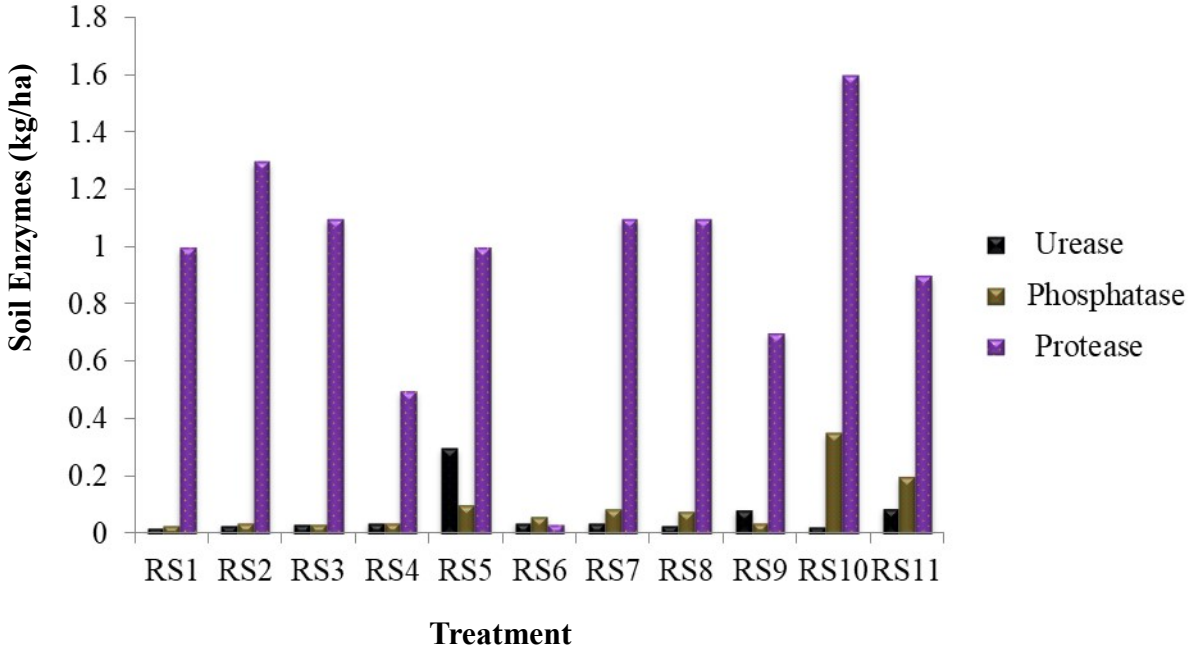


**Figure 13: Soil enzymes level at 60 days**

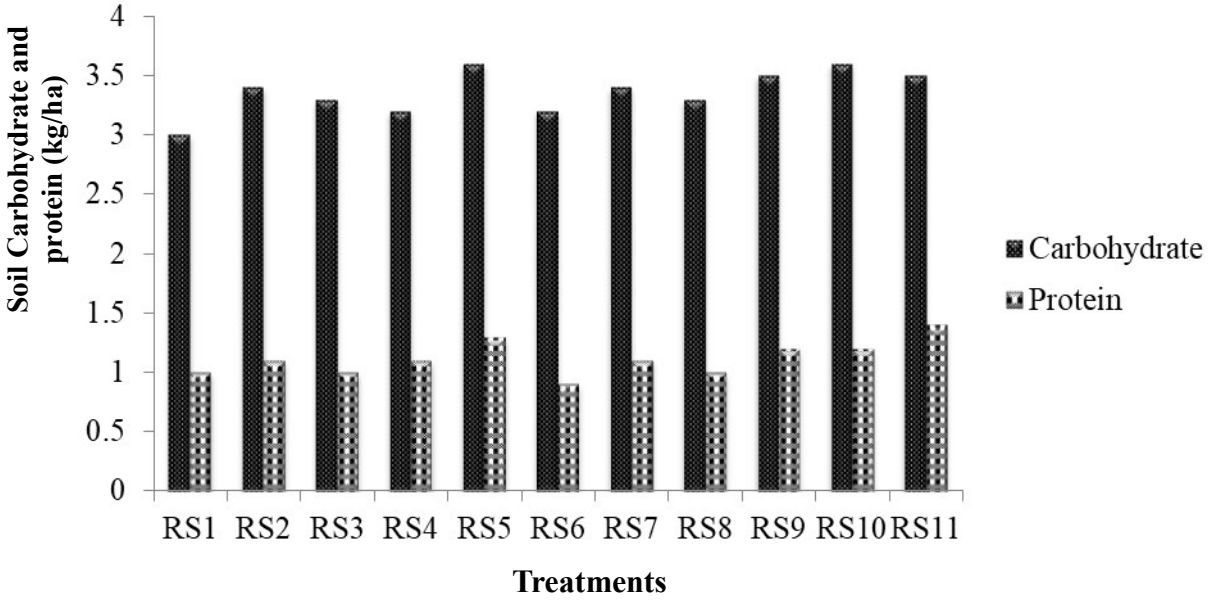


**Figure 14: Soil carbohydrate and protein level at 30 days**

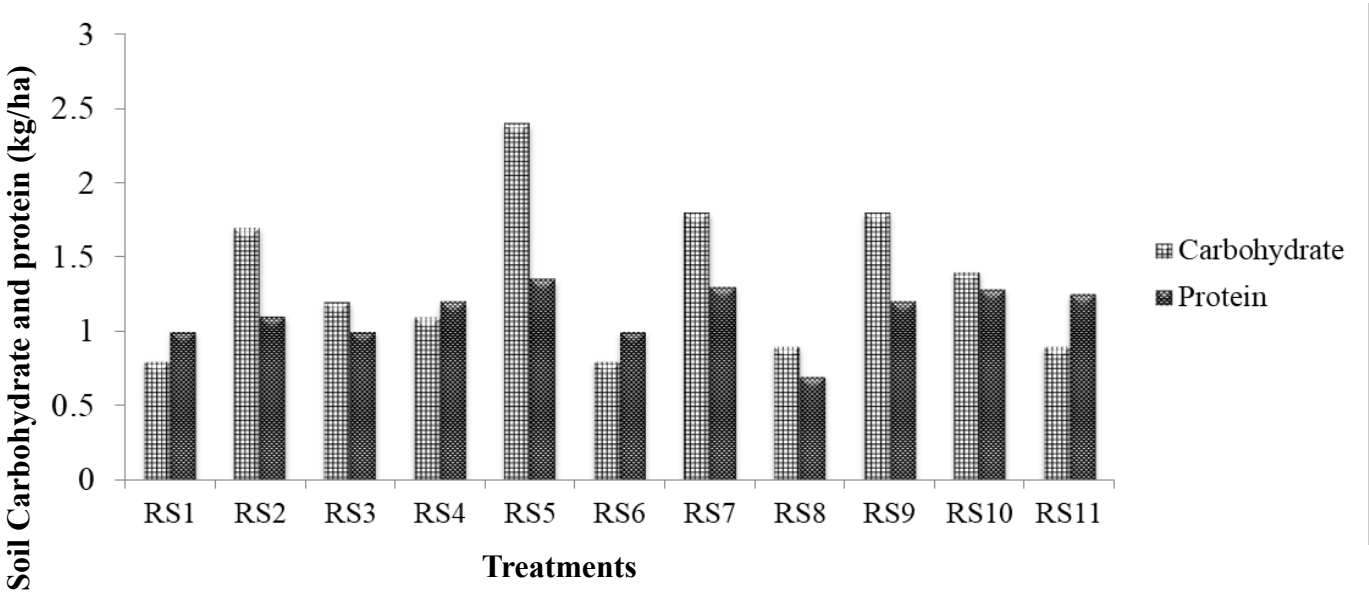


**Figure 15: Soil carbohydrate and protein level at 60 days**

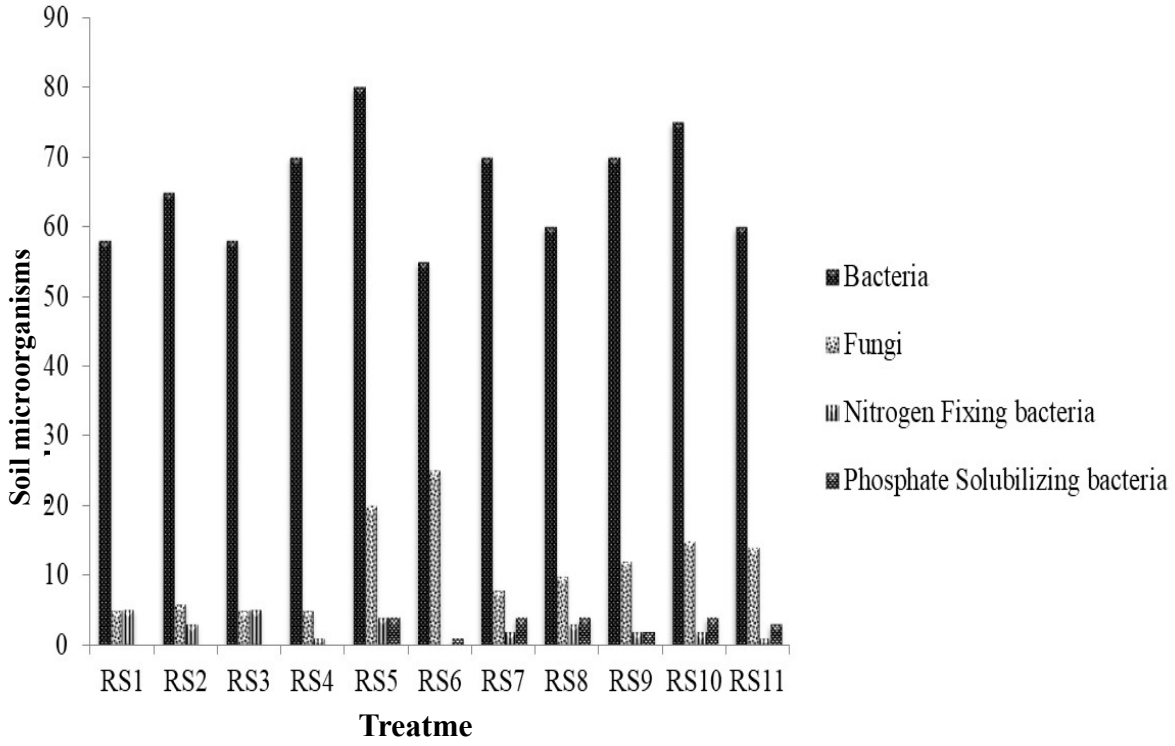


**Figure 16: Microorganisms level in soil at 30 days**

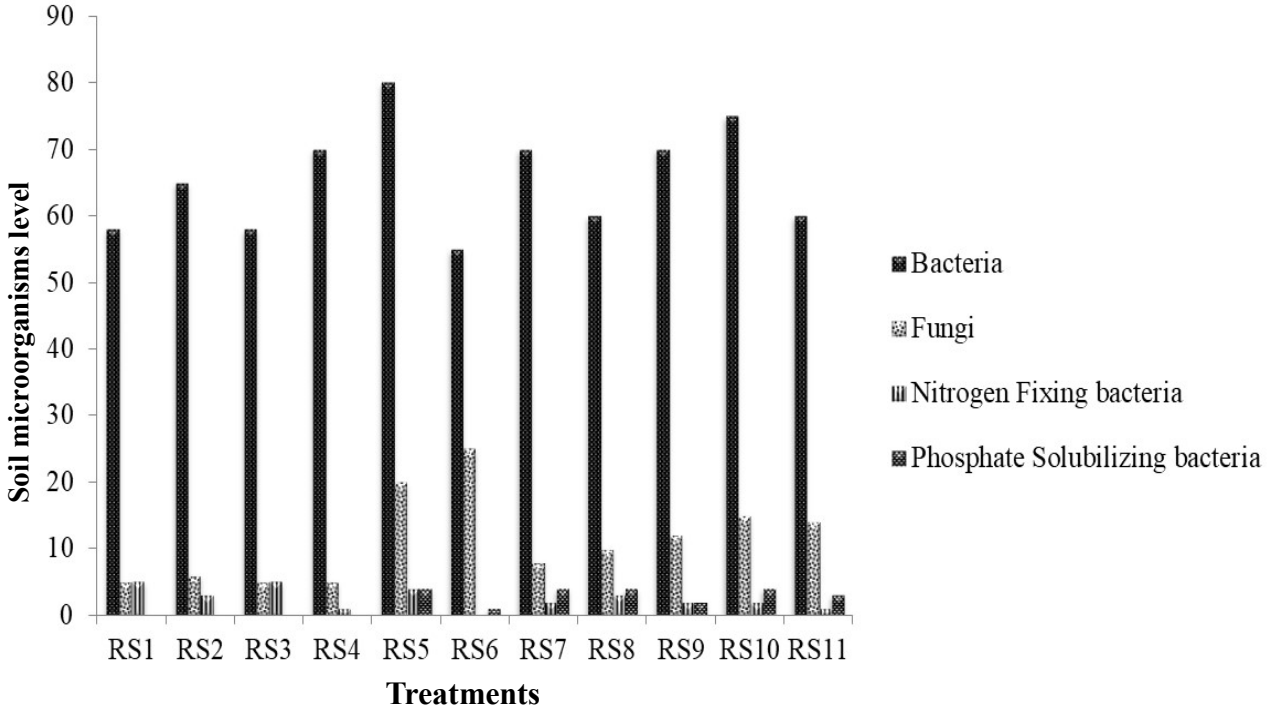


**Figure 17: Microorganisms level in soil at 30 days**

**Table 1: Morphometric analysis of *Vignamungo* L. Hepper for 30 and 60 days after incubation**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant dry weight (gm)** | **60 days** | **0.09** | **0.26** | **0.20** | **0.15** | **0.33** | **0** | **0.14** | **0.29** | **0.17** | **0.31** | **0.17** |
| | **30 days** | **0.08** | **0.18** | **0.15** | **0.11** | **0.22** | **0** | **0.12** | **0.12** | **0.14** | **0.22** | **0.14** |
| **Plant fresh weight (gm)** | **60 days** | **0.89** | **1.21** | **1.09** | **1.20** | **1.48** | **0** | **1.26** | **1.37** | **1.27** | **1.45** | **1.27** |
| | **30 days** | **0.87** | **1.15** | **0.96** | **1.12** | **1.34** | **0** | **1.18** | **1.18** | **1.24** | **1.33** | **1.27** |
| **No. of root nodules** | **60 days** | **6** | **10** | **10** | **7** | **13** | **0** | **10** | **6** | **11** | **13** | **11** |
| | **30 days** | **4** | **6** | **8** | **5** | **9** | **0** | **7** | **6** | **8** | **8** | **7** |
| **Shoot length (cm)** | **60 days** | **25.2** | **28.3** | **25.3** | **34.4** | **36.5** | **0** | **32.8** | **30.7** | **32.5** | **34.6** | **33.06** |
| | **30 days** | **23.9** | **25.6** | **24.1** | **31.4** | **32.6** | **0** | **29.9** | **28.4** | **31.6** | **33.6** | **31.9** |
| **Root length (cm)** | **60 days** | **5.2** | **9.8** | **10.6** | **6.8** | **11.7** | **0** | **9.26** | **10.4** | **7.63** | **9.93** | **8.96** |
| | **30 days** | **5.2** | **9.8** | **10.6** | **6.8** | **11.7** | **0** | **9.26** | **10.4** | **7.6** | **9.9** | **8.9** |
| **No. of leaves** | **60 days** | **7** | **7** | **6** | **7** | **9** | **0** | **8** | **7** | **5** | **7** | **6** |
| | **30 days** | **6** | **7** | **6** | **6** | **8** | **0** | **6** | **6** | **5** | **7** | **6** |
| **Morphometric Analysis** | | **RS 1** | **RS 2** | **RS 3** | **RS 4** | **RS 5** | **RS 6** | **RS 7** | **RS 8** | **RS 9** | **RS 10** | **RS 11** |

## CONCLUSION

Bioinoculants (*A. brasilensis* and *B. megaterium*) were treated with chemical fertilizers (Urea and super phosphate) at different concentrations such as 1%, 0.5% and 2 %. Fertilizers applied in the soil and black gram used as model plant. Plant growth was observed and harvested at the intervals of 30 and 60 days. Experimental Soil physico-chemical parameters, enzymes, carbohydrate and protein levels were estimated. Plant growth parameters, total chlorophyll content, leaf protein and carbohydrate was analyzed.

Soil microorganisms were enumerated by serial dilution technique. Application of half dose of fertilizers in soil showed the highest growth of black gram plants. Overall results suggested these are application of 0.5% chemical fertilizers with bio-inoculants increase the plant growth and soil parameters such as enzymes, macro and micro nutrients.

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