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# Study the Effect of Biofertilization and Cobalt on Growth and Productivity of Guar Plant under New Valley Conditions

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TO INVESTIGATE the effect of biofertilization using *Pseudomonas fluorescens* and cobalt on growth and productivity of guar (*Cyamposis tetragonoloba* L.) under desert soil conditions. A field experiment was carried out for two successive seasons of 2010 and 2011 at the agriculture experimental station at El-Kharga Oasis, New Valley Governorate, Egypt. *Bradyrhizobium spp*. was used to inoculate seeds of all treatments and control as base application. *Pseudomonas fluorescens* was used as seed inoculant and cobalt at concentrations (5,10 and 20ppm) as foliar application in single and mixed treatment with *Pseudomonas*. Obtained results indicated that, interaction treatment between *P. fluorescens* inoculation and cobalt foliar application (20ppm) had the highest record for guar plant growth parameters, yield and its components as well as mineral contents of seeds (N,P,K as macronutrients) and (Zn, Mn, Fe and Cu as micronutrient). Cobalt content in plant and seed, nodulation and its efficiency and microbial activity in guar rhizosphere.

**Keywords:** Guar, Cyamposis tetragonolba, Cobalt , Biofertilization, *Pseudomonas fluorescens* .

Guar (*Cyamopsis tetragonoloba* L.), is a member of the family fabaeace important summer season crop, It has good drought tolerant mechanism (Garg and Burman, 2002 and Tran, 2013) and ability to fix atmospheric nitrogen (Ahmad, 2008).

Guar is a promising summer forage crop that could be used in Egypt to reduce the gap between the available and required summer forage crops for livestocks feeding especially in grass-legume mixtures to increase dry matter yield and give forage of better quality than pure crop (Farag and Abd El-lateef, 1997 and Khalid *et al*., 2010). Also, guar has been well grown in wide range of soils. The most excellent performance by the fertile medium to light sandy loam soil with pH values of 7.5 to 8 (Francois *et al.*, 1990).

Guar (clusterbean) is a rich source of high quality galactomannan gum which is in great demand in the world market because of it multi-purpose use in textiles, foods, cosmetics, mining, explosives and oil industries. Despite multipurpose use of clusterbean, no systematic work has been done to improve the nodulation, nitrogen-fixing ability and crop productivity using bioinoculants. Thus, it is

desired that efficient Rhizobium/*Bradyrhizobium* cultures should be isolated and introduced in clusterbean growing areas to improve its nodulation status, seed quality and crop productivity (El-Sheikh and Ibrahim, 1999).

In sustainable agriculture, different soil microorganisms with beneficial characteristics were used as bioinoculants to improve productivity of cereal and legume crops, while minimizing the application of chemical fertilizers (Welbaum *et al*., 2004 and Compant *et al*., 2010). Among bioinoculants, nitrogen-fixing microorganisms offer an ecofriendly alternative to nitrogenous fertilizers in farming practices and supply fixed N to crop plants resulting in improved crop production (Burris and Roberts,1993 and Sindhu *et al*., 2010). Some plant growth-growth promoting rhizobacteria (PGPR) promote growth of cereals and legumes by solubilizing bound phosphorus(Sindhu *et al*., 2009) and potassium (Basak and Biswas, 2008 and Parmar & Sindhu, 2013) and by release of vitamins, auxins and plant growth regulating substances(Lugtenberg and Kamilova, 2009 and Jangu & Sindhu, 2011). PGPR have also been found to suppress plant diseases caused by potential pathogens by production of antibiotics, siderophores, hydrocyanic acid and/or hydrolytic enzymes (Stockwell and Stack, 2007 and Dua & Sindhu, 2012).

*Bradyrhizobium* inoculation to guar significantly improved nodulation and dry matter production particularly by locally isolated bradyrhizobia. Nitrogen fertilization improved dry matter production but depressed nodulation. Phosphate mitigated the depressive effect of nitrogen on nodulation and further enhanced its stimulatory effect on dry matter production (Gadallah *et al*., 2010). Inoculation of guar with *Rhizobium* on sandy loam soil elevated seed yield, seed gum and protein content Brokwell and Bottmely (1995). Nodulation in some legumes under field conditions is very poor, it may be due to absence of Rhizobium in such soils. Alternatively other environmental factors such as salinity, high temperature and draught may affect the nodulation and nitrogen fixation of leguminous plants (El-Sayed, 1997).

Andrade *et al*. (1998) found that *Pseudomonas fluorescens* enhanced nodulation by Rhizobium fourfold, While nodule produced were much larger and strongly pigmented (pink) as compared to those in other treatments. Myer and Linderman (1986) showed that *Pseudomonas putida* a siderophore producing plant growth promoting rhizobacterium, enhanced nodulation and nitrogen fixation by Rhizobium. The mechanism postulated was that effective scavenging for iron by this strain reduced populations of microorganisms that were deletrious to rhizobium while simultaneously providing the plant with extra iron.Similarly, Staley *et al.* (1992) showed that this strain increased nodulation on alfalfa. Cobalt is an essential element for legumes because of its use by microorganisms in fixing atmospheric nitrogen **(**Evan and kliwer, 1964). Bacteria on root nodules of legumes (Beans, alfalfa and clover) required cobalt to synthesize vitamin B12 and fix nitrogen from air (Young, 1983). Meanwhile Nasef *et al*. (2008) found that cobalt showed significantly higher nodule number and weight, nodule N concentration, leghaemoglobin content, total biomass production and seeds yield

compared with untreated plants. Recently, Vijayarengan *et al.* (2009), Jayakumar *et al*. (2009) and Kaliyamoorthy *et al*. (2013) showed that cobalt application at 50 mg / kg soil had a beneficial effect on biochemical contents, *i.e.*, sugar, protein and amino acids of groundnut seeds compared with control plants.

The biological importance of cobalt was first recognized by the discovery that small amounts of the element would certain deficiency symptoms in plants **(**Jayakumar and Vijayarengan, 2006). In abiotic stress, metal response will result in the production of reactive oxygen species (ROS) which leads to the activation of defense mechanisms in terms of antioxidant enzymes.

This study was conducted to investigate the effect of Biofertilization and cobalt foliar application on growth and productivity of guar(*Cyamposis tetragonoloba* L.) cultivated in El-Kharga Oasis, New Valley Governorate, Egypt.

## Material and Methods

The present investigation were carried out during the two successive seasons of 2010 and 2011 in newly cultivated lands under sandy soil conditions at the Agriculture Experimental Station at El-Kharga Oasis (30.53 longitude, 24.45 latitude and elevation78.8), New Valley Governorate, Desert Research Center (DRC),to investigate the effect of Biofertilization and Cobalt on growth and productivity of Guar (*Cyamopsis tetragonoloba* . L).

Guar (*Cyamopsis tetragonoloba*. L) seeds obtained from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt were sown in May 2010 and May 2011 in plots (3-3.5m) in rows. The mineral fertilization was applied as a general application. Calcium super phosphate 31kg$P_2O_5$/fed was mixed with soil before sowing, N and K fertilizers were added at a rate of 60 kg/fed as $NH_4NO_3$ and 75 kg $K_2O$/fed as $K_2SO_4$ into three split equal doses applied after 30,60,90 days. Sheep manure were applied at the rate of 20 $m^3$/feddan as organic manure containing O.C %28, N% 2.41,C/N ratio11.62 and O.M% 48.16. The physical and chemical analysis of soil and irrigation water were presented in Tables 1 and 2.

**TABLE 1. Some physical and chemical properties of the experimental soil.**

| **Mechanical analysis** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sand** | | | **Clay** | | | **Silt** | | **Soil Texture** | | |
| 50.8% | | | 30.9% | | | 18.3% | | Sandy clay loam | | |
| **Chemical analysis** | | | | | | | | | | |
| **pH** | **EC ds/m** | **T.N** | **Cations (meq/L)** | | | | **Anions (meq/L)** | | | |
| | | | **$Ca^{+2}$** | **$Mg^{+2}$** | **$K^+$** | **$Na^+$** | **$CO_3^{-2}$** | **$HCO^{-3}$** | **$Cl^-$** | **$SO_4^{-2}$** |
| 8.8 | 4.56 | 92 ppm | 8.76 | 4.68 | 1.26 | 29.44 | 0.00 | 8.96 | 13.52 | 21.66 |
| **Trace elements (mg/l)** | | | | | | | | | | |
| **Zn** | | **Mn** | | **Cu** | | **Fe** | | **B** | **Co** | |
| 5.744 | | 4.69 | | 1.77 | | 33.48 | | 0.49 | 0.03 | |

**TABLE 2. Chemical analysis of irrigation water.**

| pH | E.C ds/m | Soluble ions (meq/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cations | | | | Anions | | | |
| | | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{-2}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{-2}$ |
| 7.2 | 0.814 | 3.92 | 1.96 | 3.3 | 0.184 | 0.8 | 5.36 | 2.28 | 1.23 |

*Isolation, purification and selection of pseuomonas isolates*

Different soil samples were collected from different sites of El-Kharga Oasis, New valley used for isolation,7 *Pseudomonas* isolates were obtained as shown in Table 3. Cultures of *Pseudomonas* isolates were purified by successive streaking on King's medium B is based on the formulation of Murray *et al*. (2003). Microscopical examination was carried out to check the purity of cultures. The purified 7 *Pseudomonas* isolates were tested for their phosphate dissolving efficiency quantitative and qualitative according to deFreitas *et al*. (1997), siderophore production (Reeves,1983) and salycilic acid production (Meyer *et al*., 1992).

**TABLE 3. Phosphate solubilization,Siderophore and Salicylic acid production by *Pseudomonas* isolates.**

| *Pseudomonas* isolates | Phosphate solubilization | | Siderophores (µg/ml, 410 nm) | SA (µg/ml, 527 nm) |
|---|---|---|---|---|
| | Mean diameter of lysis (cm) | Phosphorus concentration mg/l | | |
| 1 | 0.9 | 1.5 | 31.9 | 36 |
| 2 | 1.1 | 2.5 | 42 | 23.7 |
| 3 | 0.6 | 1.4 | 38.1 | 20.1 |
| 4 | 2.3 | 4.8 | 59.73 | 39.4 |
| 5 | 1 | 1.7 | 40.5 | 28 |
| 6 | 1.5 | 2.9 | 46 | 41.9 |
| 7 | 0.8 | 1.6 | 54 | 33 |

*Identification of Pseuomonas isolate*

The most active fluorescent *Pseudomonas* isolates with phosphate dissolving activity, siderophore production and Salycilic acid production were identified using Bergey's Manual of determinative bacteriology (1984 and 1994) (Table 4).

*Bradyrhizobium spp*.

Locally isolated *Bradyrhizobium* spp. were maintained at 4°C on yeast extract mannitol agar(YEMA).

**TABLE 4. Identification of *Pseudomonas* isolate no.4.**

| Biochemical reactions | Results | Biochemical reactions | Results |
|---|---|---|---|
| **Morphological character:** | | Catalase production | + |
| Shape | Short rod | Lipase (Tween 80 | + |
| Motility | Motile | hydrolysis) | |
| Gram reaction | Gram -ve | Utilization of : | |
| KOH solubility test | + | Glucose | + |
| **Physiological character :** | | Trehalose | - |
| Pyoverdin production | + | L-Rhamnose | - |
| Pyocyanin production | + | D-Mannose | + |
| Oxidase reaction | + | D-Galactose | + |
| Gelatin liquefaction | + | Mannitol | + |
| Starch hydrolysis | - | L-Tryptophan | + |
| Hydrogen sulphide production | + | D-Alanine | + |

*Biochemical activities of studied microbial isolate*

The ability of the tested microbial isolates to produce biochemical activities was evaluated under in vitro conditions, through determination of their efficiency for growth regulators production (Rizzolo *et al*., 1993), nitrogen fixation (Page *et al*., 1982), enzymes (Barrow and Velthan, 1993), antibiotics production (Jarlier *et al*., 1996) (Table 5).

**TABLE 5. Biochemical activities of microbial isolates.**

| | ***P. fluore-scens*** | ***Bradyrhiz-obium*** | | ***P. fluore-scens*** | ***Bradyrhiz-obium*** |
|---|---|---|---|---|---|
| Nitrogenase ($\mu lC_2H_4H^{-1}I^{-1}$) | - | 395 | Hormonal activity µg/ml | | |
| | | | IAA | 9.4 | 2.7 |
| | | | GA3 | 2.48 | 3.29 |
| | | | Cytokinine | 23.67 | 12.4 |
| Enzyme activity: | | | Antibiotic roduction(mm) | | |
| Amylase | - | + | *E.coli* | 28 | - |
| Cellulase | + | + | *Salmonella typhi* | 21 | - |
| Protease | + | + | *F.oxysporum* | 35 | - |
| Pectinase | + | + | *R.solani* | 29 | - |

*Purification and maintenance of Cultures*

*Pseudonomonas fluorescens* and *Bradyrhizobium* spp., were isolated and purified by streak plate method on king's B medium and Yeast Extract Mannitol Agar medium respectively. Individual colonies were streaked on respective slants and stored in a refrigerator at 4$^{o}$C for further studies.

The inoculum of each strain was prepared by growing them in 500 ml flasks containing selective media, flasks incubated at 30 °C for 48 hr under shaking, the suspension containing $10^8$ cfu/ml used for inoculation.

*Compatibility test of the inoculants*

*Bradyrhizobium* spp. and *Pseudomonas fluorescens* were tested for compatibility of growth by cross streak assay in nutrient agar medium. Nutrient agar medium was prepared and sterilized. The medium was poured into sterile petri plates and allowed for solidification. To test the compatibility of *P. fluorescens* with *Bradyrhizobium* spp. *P. fluorescens* was streaked as a strip at one end of the plate and incubated for 24 hr to form a thick growth. The test cultures of *Bradyrhizobium* spp. was streaked perpendicular to *P.fluorescens* growth. The plates were incubated for 48hr and observed for the growth of *Bradyrhizobium* spp. and *P. fluorescens*.

Seeds of all the experimental plots inoculated with *Bradyrhizobium* spp. as base application (Seeds of guar were washed and immersed for 30 min in liquid culture of *Bradyrhizobium* spp.to be tested. Carboxymethyl cellulose (CMC 0.5%) was used as an adhesive agent. Seeds were then dried at room temperature for two hours.

The concentration of cobalt were used as foliar spray (5,10 and 20 ppm) alone and in combination with *Pseudomonas fluorescens* inoculation.

The experiment included 8 treatments where *Bradyrhizobium* used as base treatments:

| | |
|---|---|
| 1- control(*Bradyrhizobium*). | 2- *Pseudomonas.* |
| 3- Co 5ppm. | 4-Co 10ppm. |
| 5- Co 2ppm. | 6- *Pseudomonas+ Co 5ppm.* |
| *7- Pseudomonas+Co 10ppm.* | *8- Pseudomonas+Co 20ppm.* |

After 45 days, the soil samples were taken for nodulation testing. At 60 days old, three replicates were left for determination of guar growth parameters, yield and its components as follow: Plant height (cm), fresh weight and dry matter %, the nitrogen % according to Page *et al*. (1982), protein content calculated by multiplying N% by 6.25., phosphorus, potassium and micronutrients were determined (colorimetric method) in seed according to Cottenie *et al*. (1982).

Rhizosphere soil samples were collected and analyzed for determination of total microbial counts on Bunt and Rovira medium (Nautiyal, 1999). $CO_2$ evolution according to Anderson (1982) and Estimates of number of *Pseudomonas* by MPN technique were calculated using Garthright (1993). Nodulation (nodule number and dry weight of nodule ), the leg-haemoglobin content of root nodules were analyzed by the method of Wilson and Reiesenauer (1963), Nitrogenase activity was determined according to Haahtela *et al*. (1981).

Plants and seed were analyzed for Co concentration by Atomic Absorption spectrophotometer (Miller, 1998 and Malik & Teuswal, 2000). The completely randomized block design was used for statistical analysis of the subjected obtained data. The combined analysis of data, for the two seasons was conducted and the difference between means of various treatments had done by using L.S.D. at 5% significant level according to Snedecor and Cochran (1990).

## Results and Discussion

*Isolation and selection of pseuomonas isolates*

A number of 7 *Pseudomonas* isolates were isolated (Table 3) , purified and examined for their activities to dissolve phosphate qualitative (deFreitas *et al*., 1997) and quantitative (Schinner *et al*., 1996), production of siderophore (Reeves,1983) and production of salicylic acid (Meyer *et al*., 1992).

*Identification of Pseudomonas isolate*

The most active isolate no. (4) was completely identified according to Bergey's Manual of determinative bacteriology (1984 and 1994). The morphological and physiological characters presented in Table 4. Selected Pseudomonas isolate was found to belong to *P. fluorescens.*

*Biochemical activities of Pseudomonas fluorescens and Bradyrhizobium spp.*

Microbes under study known to produce a number of secondary metabolites (Table 5) which may affect growth, health of plants, and the relationships between rhizosphere soil microorganisms. Plant growth regulators (quantitative (HPLC) / µg/ml),nitrogen fixation, enzyme and antibiotic production. Also, Table 5 showed the biochemical activities of the *Bradyrhizobium* spp. and *Pseudomonas fluorescens* used in the trial for production of plant hormones, antibiotics, enzymes and nitrogen fixation. As shown in Table 5 the microorganisms exhibited biochemical and hormonal activities in vitro that could result in beneficial action in field (Aml and Abd El-Hai, 2011).

*Compatibility test of the inoculants*

*Bradyrhizobium* spp. and *P. fluorescens* were found to be compatible with each other and were able to grow simultaneously without and inhibition in growth.

*Effect of Biofertilization and Cobalt on growth parameters of Guar*

The obtained results in Table 6 reported that, in the presence of *Bradyrhizobium* spp. as base treatment, inoculation with *P. fluorescens* and cobalt applications increased plant height, fresh weight and dry matter % higher than individual treatments with cobalt or *P. fluorescens* application through first, second cuts, harvesting stage and for the first cut during the two growing seasons.

Plant growth promoting *P. fluorescens* induced positive response on growth and physiological parameters through production of 2,4-diacetylphloroglucinol which increase root length, root weight, transiently enhanced lateral root

formation, thickening of leaf palisade layer, spreading of lateral roots and production of root hairs. The growth and yield parameters were significantly improved as compared with the control (Boruah *et al.*, 2003).

**TABLE 6. Effect of biofertilization and Cobalt on growth parameters of Guar.**

| Treatments | 1st season | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | | | F.W kg/f | | | D.M% | | |
| | 1st cut | 2nd cut | Harv. | 1st cut | 2nd cut | Harv. | 1st cut | 2nd cut | Harv. |
| **Control** | 100 | 98 | 93 | 1250 | 1244 | 1239 | 20.12 | 17.61 | 17.43 |
| *Ps* | 107 | 105 | 101 | 1280 | 1251 | 1247 | 22.2 | 18 | 16.52 |
| Co 5ppm | 109 | 106 | 97 | 1286 | 1258 | 1251 | 24.1 | 19.1 | 17.81 |
| Co 10ppm | 113 | 108 | 103 | 1290 | 1283 | 1269 | 27.2 | 23.6 | 20.4 |
| Co 20ppm | 120 | 117 | 115 | 1297 | 1289 | 1280 | 27.6 | 24.1 | 21.6 |
| Ps+Co5ppm | 127 | 120 | 118 | 1303 | 1294 | 1286 | 28.2 | 24.8 | 22 |
| Ps+Co10ppm | 132 | 126 | 122 | 1308 | 1302 | 1291 | 29 | 26.3 | 22.5 |
| Ps+Co20ppm | 135 | 129 | 124 | 1321 | 1317 | 1310 | 29.6 | 27.1 | 24.3 |
| L.S.D at 5%: Bio | 1.8763 | | | 1.125 | | | 0.35362 | | |
| Cut | 1.68 | | | 1.00531 | | | 0.216549 | | |
| Interaction | 1.018 | | | 0.61563 | | | 1.90795 | | |
| 2nd season | | | | | | | | | |
| **Control** | 104 | 97 | 93 | 1252 | 1247 | 1243 | 20.46 | 18.1 | 17.93 |
| *Ps* | 108 | 105 | 98 | 1287 | 1273 | 1256 | 24.39 | 22.8 | 19.25 |
| Co 5ppm | 111 | 106 | 102 | 1298 | 1292 | 1281 | 25.81 | 23.72 | 20.11 |
| Co 10ppm | 117 | 112 | 105 | 1309 | 1298 | 1288 | 26.92 | 24.18 | 21.93 |
| Co 20ppm | 122 | 119 | 110 | 1316 | 1311 | 1297 | 27.55 | 24.66 | 22.5 |
| Ps+Co5ppm | 129 | 123 | 116 | 1321 | 1314 | 1308 | 28.4 | 25.81 | 24.26 |
| Ps+Co10ppm | 133 | 128 | 121 | 1329 | 1319 | 1316 | 29.98 | 26.1 | 24.7 |
| Ps+Co20ppm | 141 | 132 | 127 | 1328 | 1322 | 1318 | 30.1 | 27.9 | 26.5 |
| L.S.D at 5%:Bio | 0.9738 | | | 1.3996 | | | 0.06777 | | |
| Cut | 0.59633 | | | 0.8571 | | | 0.041501 | | |
| Interaction | 1.0556 | | | 2.1801 | | | 1.55001 | | |

F.W: fresh weight. Co: Cobalt. Ps: *Pseudomonas fluorescens.* DM: Dry matter of plant.

The enhancement effect due to cobalt application on development process including growth parameters as stem cleoptile elongation, leaf expansion, bud development and shoot dry weight (Nadia Gad, 2006).

The highest % of increase than control in plant height, fresh weight (kg/fed) and Dry matter % recorded with interaction treatment between *P. fluorescens* inoculation and cobalt 20ppm foliar application during two cuts and harvesting stage being 35%, 32% and 34% cm in first growth season and 36%, 36.1% and 37% in second growth season for plant height respectively. 57%, 59% and 57% in first growth season and 61%, 60% and 60% in second growth season for fresh weight, respectively. Also, highest dry matter % were 47,54 and 39% in first growth season and 47, 54 and 48% in second growth season, respectively. Data at second growing season were higher than that of first growth season. These results are in line with (Ayub *et al.*, 2012 and Gomaa & Magda, 2007), interaction application of *P. fluorescens* inoculation and cobalt 20ppm foliar application resulted in synergistic effect.

*Effect of biofertilization and Cobalt on Guar yield and its components*

The impact of various tested treatments on number of pods/plant, No.seeds/pod, No. seed/plant, 100 seed weight and seed yield as compared with control (*Bradyrhizobium* spp. as application treatment) during two growth season was demonstrated in Table 7.

**TABLE 7. Effect of biofertilization and Cobalt on yield of Guar.**

| Treatments | 1st season | | | | | |
|---|---|---|---|---|---|---|
| | **No. Pod/Plant** | **No.seed/ pod** | **No.seed/ plant** | **100 seed wt gm** | **Seed yield kg/f** | **Protein % in seeds** |
| **Control** | 15.3 | 5.4 | 86 | 3.11 | 450 | 23.75 |
| *Ps* | 17.6 | 6.3 | 109 | 3.42 | 472 | 24.5 |
| Co 5ppm | 18.1 | 6.8 | 116 | 3.45 | 479 | 25.38 |
| Co 10ppm | 18.2 | 7.4 | 139 | 3.59 | 516 | 25.94 |
| Co 20ppm | 18.9 | 7.9 | 154 | 3.84 | 548 | 26.25 |
| Ps+Co5ppm | 21.62 | 8 | 183 | 4.35 | 580 | 27.3 |
| Ps+Co10ppm | 22.3 | 8.6 | 192 | 4.42 | 594 | 28.1 |
| Ps+Co20ppm | 24 | 10.2 | 196 | 4.94 | 619 | 29.4 |
| L.S.D at 5% | 0.63403 | 0.1731 | 1.731 | 0.018696 | 1.7311 | 0.37499 |
| **2nd season** | | | | | | |
| **Control** | 15.7 | 5.5 | 91 | 3.19 | 463 | 24.5 |
| *Ps* | 17.85 | 7.2 | 123 | 3.61 | 481 | 27.25 |
| Co 5ppm | 18.3 | 8 | 138 | 3.84 | 490 | 26.4 |
| Co 10ppm | 19 | 8.4 | 154 | 4.13 | 534 | 27.3 |
| Co 20ppm | 22 | 9 | 167 | 4.29 | 556 | 27.44 |
| Ps+Co5ppm | 23.8 | 9.3 | 189 | 4.37 | 583 | 28 |
| Ps+Co10ppm | 24.6 | 9.8 | 194 | 4.72 | 608 | 29.1 |
| Ps+Co20ppm | 24.9 | 11.3 | 201 | 5.26 | 630 | 30.2 |
| L.S.D at 5% | 0.63894 | 0.65053 | 1.730895 | 0.01731 | 2.44786 | 0.65996 |

Co: Cobalt. Ps: *Pseudomonas fluorescens* .

Obviously inoculation with *P. fluorescens* and cobalt 20ppm foliar application gave the highest record for tested yield components. Biofertilization proved to be effective in increasing the yield of guar for No.pods/plant, No.seeds/pod and No.of seeds/plant, the highest values being 24,10.2 and 196 in first growing season and 24.9, 11.3 and 201 in the second growing season respectively. 100 seed weight (gm) and seed yield (kg/fed) being 4.94and 619kg/fed in the first growing season and 5.26gm and 630 kg/fed in second growing season, respectively. Obtained trend for protein% which was 29.4% and 30.2% in first and second growing season, respectively. The obtained results were found to be on the same line with those obtained by Gaballah and Gomaa (2005) and Mohamed & Gomaa (2005) they reported that, plant growth promoting rhizobacteria (PGPR) proved to be effective in increasing the yield of guar through production of amino acids like niacin and pantothenic acid, it also produced different other vitamins such as biotin, thiamine, cobalamine and pyridoxine. Cobalt application strongly influenced protein %, production of four new polypeptides and induction of antioxidant enzyme ( Peroxidase, catalase, polyphenoloxidase, Ascorbic acid, glutathione , α-tocopherol and cartenoid) in cluster bean (Gurusaravanan *et al*., 2012 and Kaliyamoorthy *et al*., 2013).

*Effect of biofertilization and Cobalt application on some minerals contents of seeds.*

As demonstrated in Table 8 in the presence of *Bradyrhizobium* spp. as a base application, inoculation with *P. fluorescens* increased nitrogen contents in seeds also, application of cobalt increased nitrogen content of seed and as cobalt concentration increased nitrogen content increased. Cobalt enhance total N accumulation (Vanek and Knob, 1997 and Nadia Gad, 2006), Seed inoculation with *Pseudomonas* strains of two chickpea cultivar significantly increased plant N contents (Goel *et al.,* 2002). *Pseudomonas* could stimulate growth directly through production of growth hormones like indole acetic acetic, phosphate-solubilization and uptake of iron, whereas indirect mechanisms include check on phytopathogens by the release of HCN, antibiotics and siderophores (O'Sullivan and O'Gara, 1992). Both of *P. fluorescens* and cobalt foliar application enhanced nitrogen fixation process by *Bradyrhizobium* where cobalt application played a greater role higher than *Pseudomonas* inoculation. *P.flouresence* and cobalt foliar application (20ppm) as interaction treatment gave the maximum nitrogen, phosphorus and potassium contents in seed being (4.7% and 4.83%) and(0.63% and 0.69%) and (1.23% and 1.35%) for nitrogen, phosphorus and potassium in first and second growing seasons respectively. Both of *P.flouresence* inoculation and cobalt application gave synergistic effect and enhanced mineral uptake and their metabolism in plant. The second growing season showed higher enhancement effect than the first growth season, these results are in agreement with those reported by ( Khalid *et al*., 2010).

**TABLE 8. Effect of biofertilization and Cobalt application on some minerals contents of seeds.**

| Treatments | 1st season | | | | | | |
|---|---|---|---|---|---|---|---|
| | Macronutrients % | | | Micronutrients (ppm) | | | |
| | N | P | K | Zn | Mn | Fe | Cu |
| Control | 3.8 | 0.53 | 1.08 | 12.6 | 14.9 | 26.53 | 10.84 |
| *Ps* | 3.92 | 0.55 | 1.1 | 16.2 | 16 | 31.3 | 11.6 |
| Co 5ppm | 4.06 | 0.54 | 1.12 | 12.73 | 15.5 | 25.1 | 11 |
| Co 10ppm | 4.15 | 0.57 | 1.5 | 14.5 | 15.9 | 24.3 | 11.28 |
| Co 20ppm | 4.2 | 0.58 | 1.18 | 16 | 16.7 | 22 | 11.9 |
| Ps+Co5ppm | 4.36 | 0.6 | 1.19 | 16.8 | 17.3 | 34.6 | 13.8 |
| Ps+Co10ppm | 4.5 | 0.61 | 1.2 | 18.6 | 17.8 | 36.1 | 14.7 |
| Ps+Co20ppm | 4.7 | 0.63 | 1.23 | 20.2 | 18 | 37 | 16.2 |
| L.S.D at 5% | 0.12303 | 0.06418 | 0.17814 | 0.52187 | 0.6428 | 0.87622 | 0.6274 |
| **2nd season** | | | | | | | |
| **Control** | 3.92 | 0.55 | 1.11 | 13.8 | 15.6 | 27 | 11.2 |
| ***Ps*** | 4.16 | 0.58 | 1.13 | 16.8 | 16.8 | 33 | 12.1 |
| Co 5ppm | 4.22 | 0.56 | 1.16 | 14.2 | 16.1 | 24.5 | 11.8 |
| Co 10ppm | 4.36 | 0.59 | 1.17 | 16.4 | 16.4 | 23.6 | 12.5 |
| Co 20ppm | 4.39 | 0.61 | 1.2 | 17.1 | 18 | 21.5 | 13.4 |
| Ps+Co5ppm | 4.48 | 0.63 | 1.24 | 18 | 17.6 | 35 | 15.1 |
| Ps+Co10ppm | 4.65 | 0.67 | 1.31 | 20.2 | 18 | 36.7 | 16.9 |
| Ps+Co20ppm | 4.83 | 0.69 | 1.35 | 22.6 | 18.6 | 37.2 | 17.8 |
| L.S.D at 5% | 0.030802 | 0.02523 | 0.07853 | 0.2609 | 0.876911 | 1.06875 | 0.20296 |

Concerning micronutrients (Fe,Zn, Mn and Cu) data recorded in Table 8 showed the same trend as macronutrients (N,P,K), where combined application of cobalt 20ppm with *P.flouresence* inoculation in mixed treatment gave the highest response and superiority of micronutrients contents in seeds being (10.2, 18.1,37and 16.2) for Zn,Mn,Fe and Cu in first season and (11.3,18.6,37.2 and 17.8) for second season. Cobalt significantly increased the content of N,P,K,Mn and Zn as well as chemical contents ( Nadia Gad, 2012).

*Effect of biofertilization and Cobalt application on cobalt content in plant and seed*

Cobalt is essential for symbiotic nitrogen fixation by legumes, it is part of cobalmin (vitamine B12and its derivatives) (Nadia Gad, 2006, Mohamed *et al*., 2011 and Weisany *et al*., 2013), thus it is important to evaluate cobalt contents in plant and seeds. Also, data showed in Fig. 1 (A and B) confirmed the previous findings where inoculation with *P. fluorescens* increased the uptake of cobalt and related directly with increasing the concentration of applied cobalt. The interaction treatment between *P. fluorescens* and cobalt foliar application (20ppm) exhibited the maximum cobalt contents in plant and seed being 0.49 and 3.89 for first season and 0.54 and 4.17 for second season, respectively.

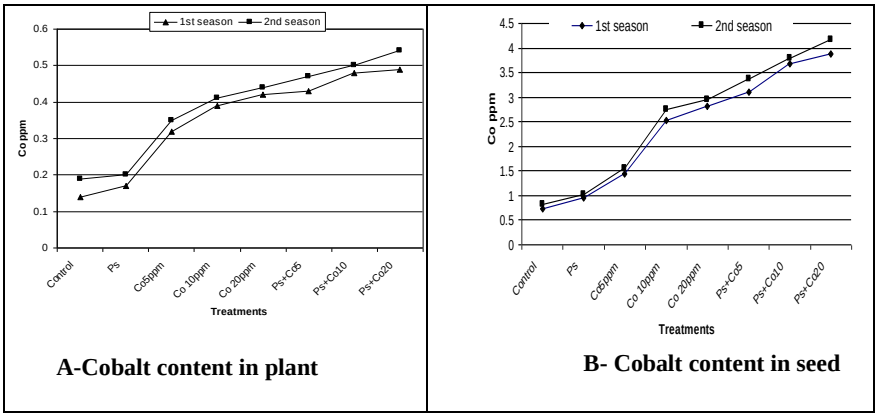


**Fig. 1. Cobalt content in seed and plant.**

It has been noticed that, there were a gradually increasing in cobalt by adding each of *P. fluorescens* and cobalt (5,10,20ppm) and the interaction between the abovementioned treatments respectively. Cobalt played an important role in increasing macro and micronutrient contents and promoted many developmental process like stem coleoptiles elongation , leaf expansion and bud development as reported by Abd El Moez and Nadia Gad (2002) and Weisany *et al*. (2013).

Although the competition between cobalt and iron contents in plant and seeds, the inoculation with *P.flouresence* may increase the iron content in plant and seeds due to siderophore formation. This is agrees with (Nadia Gad, 2006, Nadia Gad, 2012, Mohamed *et al*., 2011 and Gomaa & Magda, 2007).

*Effect of biofertilization and Cobalt application on nodulation and its efficiency*

Data presented in Table 9 clearly showed that, biofertilization with *P.flouresence* in the presence of *Bradyrhizobium* spp. as base treatment and cobalt foliar application with different concentrations(5,10 and 20 ppm) had a significant promoting effect on nodule number/plant, fresh and dry weights of nodules, nitrogenase activity and leghaemoglobin content compared with control. The interaction treatment with *P.flouresence* and Cobalt 20ppm foliar application gave greatest increase in the aforementioned parameters for nodulation and its efficiency (Table 9). The positive response increase as cobalt concentration increased from 5,10 and 20 ppm in the presence of *P.flouresence* inoculation as mixed treatment higher than application of cobalt concentration or *P.flouresence* inoculation alone as single treatment that confirmed the presence of synergistic effect in their action on the studied parameters. These results are in harmony with those obtained by Abd El moez and Nadia Gad (2002) who reported that the addition of cobalt improved nodule formation process, number of root nodule, leghaemoglobin content, essential constituent of vit B12 which is important for root nodule production and nitrogense activity. Also, these results are in a good agreement with those found by Tenywa ( 2003 ) and Vanek & knop (1997) and Nadia Gad (2006). The inoculation of groundnut with PGPR like *Pseudomonas sp*. enhanced growth, nodule and yield (Anandaraj and Leema, 2010). Also, Andrade *et al.* (1998) reported that *P.flouresence* enhanced nodulation by Rhizobium fourfold while the nodule produced were much large and strongly pigmented (Pink) as compared with other treatments.

**TABLE 9. Effect of biofertilization and Cobalt application on nodulation and its efficiency.**

| Treatments | No.nodule/ plant | Fw.nodule (gm) | DW.nodule (gm) | Nitrogenase µmole $C_2H_2$ | Leg.haemo mg/g fresh nodule |
|---|---|---|---|---|---|
| | **1st season** | | | | |
| **Control** | 4 | 30.4 | 11.2 | 16.93 | 5.2 |
| *Ps* | 5 | 37.1 | 13.4 | 17.2 | 5.7 |
| Co 5ppm | 5 | 34.8 | 12.1 | 18.53 | 5.3 |
| Co 10ppm | 6 | 42.3 | 14.8 | 19.1 | 6 |
| Co 20ppm | 7 | 48 | 16.5 | 19.7 | 6.2 |
| Ps+Co5ppm | 7 | 51 | 17.81 | 20.4 | 6.6 |
| Ps+Co10ppm | 8 | 56 | 20.2 | 20.9 | 7 |
| Ps+Co20ppm | 9 | 63 | 21.7 | 21.3 | 7.3 |
| L.S.D at 5% | 0.14989 | 1.2381 | 0.16203 | 0.15015 | 0.90975 |
| **2nd season** | | | | | |
| **Control** | 6 | 39.61 | 14.3 | 18.81 | 5.6 |
| *Ps* | 6 | 44.2 | 15.6 | 19.2 | 6.1 |
| Co 5ppm | 6 | 48.1 | 15.8 | 19.27 | 5.9 |
| Co 10ppm | 8 | 55 | 19.3 | 20.3 | 6.3 |
| Co 20ppm | 8 | 56.4 | 19.82 | 20.51 | 6.4 |
| Ps+Co5ppm | 9 | 61 | 20.5 | 21.94 | 6.8 |
| Ps+Co10ppm | 9 | 64 | 22.3 | 22.35 | 7.2 |
| Ps+Co20ppm | 11 | 71 | 24.6 | 24.8 | 7.5 |
| L.S.D at 5% | 1.0635 | 1.3892 | 0.215001 | 0.69901 | 0.27368 |

*Effect of biofertilization and Cobalt application on microbial activity in Guar rhizosphere*

*A- Total microbial counts*

Initial total microbial counts in New valley soil was $34\times10^5$ cfu/gm dry soil. Data in Table 10 showed that the counts tended to increase with all treatments refer to control. Total microbial counts proved an increase in second cut higher than first cut followed by harvesting in both growing seasons. Also, interaction treatment between *P.flouresence* inoculation and cobalt foliar application 20ppm produced the highest total microbial counts as compared with other treatments and control being ($91\times10^5$ and $98\times10^5$cfu/gm dry soil) in $2^{nd}$ cut of first and second seasons respectively. these results agreed with Subba Rao (1988) and Abd El-Ghany *et al.* (1997).

*B- $CO_2$ evolution*

The generation of carbon dioxide ($CO_2$) was determined as an indication of the biological activity in plant rhizosphere. Results in Table 10 clearly showed that mixed treatment with *P.fluorescens* inoculation and cobalt foliar application (20ppm) gave higher rate of $CO_2$ evolution being 45 and 98 mg$CO_2$/100g dry soil/24 hr than all other treatments in $2^{nd}$ cut of first and second season respectively. Data of $CO_2$ evolution were almost in harmony with those of total microbial counts discussed before.These results agreed with (Visser and Dennis, 1992).

C- *Pseudomonas* counts

The initial *Pseudomonas* counts in new valley were $8.3\times10^2$ cfu/gm dry soil.Data recorded in Table10 proved a marked increase in *Pseudomonas* counts in first and second season. The increase in *Pseudomonas* counts was in second season higher than first one and the increase was in second cut higher than first cut but decreased at harvest. The counts under interaction treatment of *P.flouresence* with cobalt foliar application (20ppm) recorded the highest counts being $31\times10^2$ and $34\times10^2$ cfu/gm dry soil in second ($2^{nd}$) cut of first and second season respectively.The highest increase in *Pseudomonas* counts were obtained by using the interaction treatment with cobalt (20ppm) and *P.flouresence* inoculation followed by *P.flouresence* inoculation treatment and cobalt foliar application treatments (5,10,20 ppm) respectively. The promoting effect due to application of *P.flouresence* not only due to production of organic acid and siderophore which increase the availability of iron uptake but also to the production of plant growth promoting substances and antimicrobial substances as well which increase soil fertility, microbial communities and plant growth (Yadav *et al*., 2007).

Anandaraj and Leema (2010) confirmed that, combined inoculation increased the population of total bacteria, *Rhizobium sp.*and *Pseudomonas fluorescens* over uninoculated control indicating the ability of the introduced microorganisms to establish themselves in the rhizosphere. Cobalt is an essential element for growth of *Bradyrhizobium* spp. on root nodules and to synthesize vitamin B12 which required for the microorganisms fixing nitrogen in nodules (Nadia Gad, 2012).

**TABLE 10. Effect of biofertilization and Cobalt on microbial activity in Guar rhizosphere.**

| Treatments | 1st season | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TC×10$^5$ cfu/gm dry soil | | | $CO_2$ mg$CO_2$/100g dry soil/24hr | | | Ps×10$^2$ cfu/gm dry soil | | |
| | 1st cut | 2nd cut | Harv. | 1st cut | 2nd cut | Harv. | 1st cut | 2nd cut | Harv. |
| **Control** | 42 | 61 | 56 | 18.6 | 23.1 | 21 | 11.6 | 13.2 | 12.9 |
| *Ps* | 53 | 75 | 69 | 26.2 | 39 | 33 | 17 | 29 | 21.26 |
| Co 5ppm | 45 | 67 | 61 | 19.8 | 27 | 24 | 12 | 14.8 | 14.1 |
| Co 10ppm | 51 | 74 | 63 | 22.4 | 31 | 25 | 13.1 | 16.5 | 15.3 |
| Co 20ppm | 54 | 79 | 68 | 24.9 | 35 | 28 | 14.5 | 17.3 | 15.8 |
| Ps+Co5 | 60 | 83 | 74 | 27.6 | 41 | 34 | 19.3 | 26.1 | 23.6 |
| Ps+Co10 | 63 | 89 | 77 | 29 | 43 | 38 | 22 | 29 | 27.59 |
| Ps+Co20 | 64 | 91 | 81 | 30 | 45 | 41.3 | 24 | 31 | 12.9 |
| L.S.D at :Bio | 1.1608 | | | 0.778796 | | | 0.5281 | | |
| Cut | 0.71087 | | | 0.47693 | | | 0.3234 | | |
| Interaction | 1.5 | | | 0.6754 | | | 0.31044 | | |
| 2nd season | | | | | | | | | |
| **Control** | 47 | 64 | 59 | 19.4 | 24 | 21.5 | 11.9 | 13.8 | 13.1 |
| *Ps* | 55 | 83 | 76 | 28 | 40 | 34.3 | 19.4 | 31 | 23 |
| Co 5ppm | 48 | 69 | 64 | 21 | 29 | 25 | 13.8 | 16 | 15.2 |
| Co 10ppm | 54 | 77 | 68 | 24 | 34 | 26.8 | 14.2 | 17.4 | 15.9 |
| Co 20ppm | 57 | 80 | 71 | 27.4 | 38 | 29 | 15.3 | 18.6 | 16.4 |
| Ps+Co5 | 62 | 88 | 78 | 31 | 42 | 37 | 21.8 | 28 | 25.71 |
| Ps+Co10 | 66 | 94 | 79 | 35 | 47 | 42.8 | 25 | 30 | 28 |
| Ps+Co20 | 73 | 98 | 84 | 37 | 49 | 44.7 | 27 | 34 | 30.2 |
| L.S.D at 5%: Bio | 1.133651 | | | 1.044750 | | | 1.1849 | | |
| Cut | 0.69422 | | | 0.63978 | | | 1.8493 | | |
| Interaction | 1.43056 | | | 1.215 | | | 0.2991 | | |

Tc: total microbial count. Co: Cobalt. Ps: *Pseudomonas fluorescens.*

## Conclusion

It could reasonably concluded from the abovementioned results that biofertilization with *P.flouresence* showed a powerful effects on the growth, yield of guar and microbial community in rhizosphere of inoculated plant. Cobalt foliar application resulted in enhancement effect toward aforementioned parameters within range used in this study. interaction treatment between *P.flouresence* inoculation and cobalt foliar application (20ppm) resulted in a maximum benefits toward growth parameters, yield and its components, nutritional status, nodulation characters and microbial activity in rhizosphere of guar plant as compared with control (*Brady-rhizobium* spp.only).

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## دراسة تأثير التسميد الحيوى والكوبلت على النمو وانتاجية نبات الجوار تحت ظروف الوادى الجديد

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لبيان تأثير التسميد الحيوى باستخدام السيدوموناس فلوريسنس والكوبلت على انتاجيه الجوار تحت ظروف ارض صحراوية. اجريت تجربة حقلية لموسمين متتابعين 2010و 2011 بمحطة التجارب الزراعية بالخارجة –الوادى الجديد– مصر. تم التلقيح بالبرادى ريزوبيم لبذور الجوار لكل المعاملات واستخدمت بكتريا السيدوموناس فلوريسنس لتلقيح البذور والرش بالكوبلت بتركيزات(5، 10، 20 جزء فى المليون) معامله رش بالكوبلت ومع استخدام السيدوموناس وقد أظهرت النتائج ما يلى :

1- ادى الرش بالكوبلت بتركيزات(5و10و20 جزء فى المليون) الى زيادة معنويه للصفات المدروسه للمحصول ومكوناته ، المحتوى المعدنى للبذور(النيتروجين، الفوسفور والبوتاسيوم) والعناصر الصغرى (الزنك ، المنجنيز، الحديد ، النحاس) محتوى الكوبلت فى النبات والبذور، العقد الجذرية وكفاءتها والنشاط الميكروبى فى ريزوسفير نبات الجوارخلال الحشتين الاولى والثانية وموسمى النمو.

2- كما أظهرت النتائج زيادة معنويه باستخدام السيدوموناس فلوريسنس منفردة ومع الرش بالكوبلت بتركيزاته المختلفة مقارنة بالكنترول (برادى ريزوبيا فقط).

3- وقد سجلت المعامله المشتركة بالسيدوموناس والكوبلت (20 جزء فى المليون) اعلى قيم لمقاييس نمونبات الجوار و المحصول ومكوناته، المحتوى المعدنى للبذوروالعناصر الصغرى ومحتوى الكوبلت فى النبات والبذور، العقد الجذرية وكفاءتها والنشاط الميكروبى فى ريزوسفير نبات الجوارخلال الحشتين الاولى والثانية و موسمى النمو.