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# Application of biofertilization and biological control for cowpea production

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**Abstract**

This study was carried out during two successive seasons 2014 and 2015 for evaluating the effect of inoculation with *Bradyrhizobium* sp, *Bacillus megaterium*, *Bacillus circulans*, *Glomus macrocarpum* instead a part of chemical fertilization in the presence of biocontrol agent i.e., *Pseudomonas fluorescens* on some microbial enzymes activity, growth characteristics, some biological constituent as well as yield and its components for optimal nutrition and some attributes of cowpeas. Obtained data showed significant increases of dehydrogenase (DH), nitrogenase and phosphatase activity in both seasons using of the combination of biofertilization and chemical nitrogen fertilization. Moreover, the values of $NH_4$-N, $NO_3$-N, available-P and soluble-K were the highest records with dual application of biofertilizers and half dose of chemical fertilization. Concerning, vegetative growth parameters and total yield, NPK-microbial inoculants combined with NPK-mineral fertilizers had the highest values. The results of this study suggest that, it may be replaced the mineral fertilizers by biofertilizers, even partially, to produce a better food and such integrated nutrient management program should be followed to produce the highest yield of cowpea.

**Key word:** biofertilization, biological, cowpea

## Introduction

Cowpea (*Vigna unguiculata* L.Walp) is considered the furthermost vital yields in Egypt, also as an inexpensive source of protein **(Mohamed *et al*., 2012)**. Cowpea is mainly cultured as a source of their maintenances **(Fang *et al*., 2007)**. Cowpea is globally cultivated as a vegetable, cover and cash crops, it is an abundant protein quality and has vigor content almost corresponding to that of cereal grains. Cowpea protein is rich in lysine and tryptophan compared to cereal seeds **(Rabia *et al*., 2015)**.

Chemical nitrogen fertilization performs a role of enhancing crop yield **(Abyomi *et al*., 2008)**, application nitrogen to cowpea had a positive effect on yield and its components of cowpea **(Gohari *et al*., 2010)**. Chemical nitrogen fertilization may apply as a substantial quantity to increase crop productivity by farmers. The continuous application of mineral fertilizers may adversely affect soil degradation, soil chemical composition, nutrient imbalance and vegetable crop yield (**Mousa and Mohamed 2009**). To avoid the environmental pollution resulting from the over-use of mineral fertilizers, biofertilizers could be considered important candidates from sustainable agriculture point of view.

Biofertilizers can be defined as a substance contains living microorganisms, which colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant when applied to seed, plant surfaces or soil (Vessey 2003). Furthermore, biofertilizers are essential components of organic farming and play a vital role in maintaining long term soil fertility and sustainability. Moreover, biofertilizers as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers helping in achieving sustainability of farms). **(Abou-Aly *et al*, 2006, Zaghloul *et al*., 2009 and Kumar *et al.* 2013; 2015**).

*Bradyrhizobium* is the valuable root bacteria, due to their ability to alter atmospheric nitrogen into a useful form in association with legume plants **(Weir, 2012)**.

Most plants inoculation with arbuscular mycorrhizal fungi has noticing consequence due to pronounced capability to increase plant growth and yield (**Zayed *et al*., 2013** and **Eissa *et al*., 2015)**. Phosphate solubilizing bacteria (*Bacillus megaterium*) emits several organic acids including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, fumaric and ketogluconic, which solubilize phosphate and micronutrients and subsequent reduction in soil pH **(Ahmed, 2010)**.

Biological control with introduced microorganisms is still opening in its stages. The use of *Ps. fluorescens* is gaining importance for plant growth elevation and biological control **(Nandi *et al*., 2013)**.

Therefore, the main target of this study was to evaluate the possibility of using biofertilizers in combination with chemical fertilizers and biocontrol agent in improving microbial enzymes activity, maintaining higher growth, productivity and yield quality of cowpea.

## Materials and Methods

The experiments were executed during two successive seasons, 2014 and 2015, at Faculty of Agriculture, South Valley University, Qena Governorate, Egypt to study the effect of biofertilizers and mineral fertilizers as well as their interactions on the growth and yield components of

**Cowpea seeds**

Seeds of cowpea (*Vigna unguiculata* ( L.) Walp ) Creeam 7 were obtained from Vegetable Crops Research Dept., Horticultural Institute, Agriculture Research Centre, Giza, Egypt.

**Chemical fertilizers**

Chemical fertilizers were got from regional market in Qena, Egypt.

**Biofertilizers**

Biofertilizers contain four microbial strains namely *Bradyrhizobium* sp, *Bacillus megaterium, Bacillus circulans,* and *Glomus macrocarpum* were kindly obtained from Microbiological Resources center, Cairo MIRCEN, Ain Shams University, Egypt.

**Biocontrol agent strain**

Biocontrol agent strain *Pseudomonas fluorescens* was kindly obtained from Microbiological Resources center, Cairo MIRCEN, Ain Shams University, Egypt.

**Experimental soil**

Experimental soil was subjected to chemical and mechanical examinations as stated by the method explained by **Page *et al*. (1982)**. Mechanical and chemical soil features are tabulated in **Table (1)**.

**Table 1.** Mechanical and chemical analyses of the experimental soil.

| Parameter | | Mechanical analysis | | | | Chemical analysis | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Total and available macronutrients (ppm) | | | | | | Soluble cations meq/1 | | | | Soluble anions meq/1 | | | | |
| | | | | | | | | | | N | | P | | K | | | | | | | | | | |
| | | Coarse sand (%) | Fine sand(%) | Silt(%) | Clay(%) | Soil texture | pH (1:1) in water | E.C. (dS/m) | Organic matter | Calcium carbonate | Total | available | Total | Availabl | Total | available | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $HCO_3^{-}$ | $+CO_3^{--}$ | $Cl^{-}$ | $SO_4^{--}$ |
| Value | 1st | 8.12 | 69.8 | 14.7 | 7.38 | Sandy loam | 7.88 | 3.62 | 0.12 | 9.32 | 200 | 17.2 | 220 | 6.4 | 250 | 130 | 3.20 | 2.80 | 10.75 | 1.38 | 1.32 | - | 10.31 | 6.50 |
| | 2nd | 7.82 | 70.2 | 14.9 | 7.08 | | 8.01 | 3.42 | 0.13 | 9.29 | 200 | 18.3 | 220 | 9.5 | 250 | 133 | 3.18 | 2.83 | 10.7 | 1.36 | 1.30 | - | 10.3 | 6.51 |

**Experimental design**

Field experiment was carried out during two successive seasons of 2014 and 2015 at the Experimental Research Farm, Faculty of Agriculture, South Valley University, Qena Governorate, Egypt to study the effect of biofertilizers and mineral fertilizers as well as their interactions on the growth and yield components of cowpea (*Vigna unguiculata* ( L.) Walp ). Cowpea seeds (cv. Creem 7) were sown in the soil in 15th of March in both two seasons. Experimental treatments were randomly arranged in a randomized complete blocks design with three replicates. The plot area was 10.5 $m^2$ (3 x 3.5 m). All treatments were tabulated in Table 2. Before cultivating cowpea seeds were inoculated by seed-dressing technique with biofertilizers cell suspension consists of $8\times10^9$ cfu per ml of each one and 40 % sucrose solution and 10% Arabic gum as an adhesive for inocula, then spread in plates and endorsed to air drying before sowing. The rest of this solution was added to plants with irrigation. The inocula of biocontrol agent and mycorrhiza were add to the soil immediately before cultivation with rate $8\times10^9$ cfu and 160 spores $ml^{-1}$ respectively. The boost inocula were added three times (every month) throughout the emergent season.

**Table 2.** Experimental design

| Treatments | Description |
|---|---|
| T1: | Control (non-fertilized and non-inoculated). |
| T2: | Nitrogen fertilization (full dose) . |
| T3: | Full dose N + fungicide |
| T4: | Full dose N+ Bio- control |
| T5: | Half dose N+ Biofertilizers |
| T6: | Half dose N + Biofertilizers + Bio-control |

Biofertilizers cell suspension was containing (*Bradyrhizobium* sp, as nitrogen fixing bacteria, *Bacillus megaterium* as phosphate dissolving bacteria, *Bacillus circulans* as potassium dissolving bacteria as well as *Glomus macrocarpum*. The biofertilizer cultures were prepared by strains obtained from Microbiological Resources center, Cairo MIRCEN, Ain Shams University, Egypt.

In fungicide treatment, cowpea seeds were dressed with Rizolex-T 50% at recommended dose (3g/kg of seeds).

Ammonium sulphate, calcium super phosphate and potassium sulphate fertilizers were added at a rate of 106, 160 and 53 kg $fedd^{-1}$, respectively as a recommended dose. Phosphate fertilizer was added for experimental plots during soil preparation, whereas both $NH_4NO_3$ and $K_2SO_4$ fertilizers were divided into two doses, where the first and second doses added before the first and second irrigation, respectively.

**Determinations**

Dehydrogenase activity (DHA) was assayed in soil rhizosphere of cowpea plants according to **Glathe' and Thalmann (1970)**. $N_2$-ase activity was assessed in nodules, the measurement built on the diminution of acetylene to ethylene as quantities by gas chromatography. Acetylene reduction was achieved by a procedure altered from **(Silvester, 1983)**. Alkaline Phosphatase activity was estimated in soil rhizosphere of cowpea plants according to **Drobrikova (1961)**.

Ammoniacal and nitrate nitrogen ($NH_4$-N and $NO_3$- N) were determined according to the method described by **Bremner and Keeny (1965)**. Soluble-phosphorus was determined according to the method described by **Watanabe and Oleson (1965)**. Available-P was evaluated by **Jackson (1973)**.

Vegetative growth characteristics were estimated by taken five plants randomly from each replicate at flowering stage (60 days after sowing) to measure plant height (cm), number of branches and leaves, fresh and dry weights/plant (g). As well as, yield and its components were assessed by taken five plants from each plot to evaluate number of pods/plants, average weight of 100 seeds (g) and total green pods yield per feddan (kg/fed).

**Statistical analysis**

Statistical analysis was carried out according to **Snedecor and Cochran (1989)**. The differences between the means value of various treatments were compared by Duncan's multiple range test **(Duncan's, 1955)**.

**Results and Discussions**

**Effectiveness of biofertilization, biological control and inorganic nitrogen fertilizer on cowpea production.**

Two field experiment were carried out during two successive seasons of 2014 and 2015 aiming to study the efficiency of biofertilization and biological control in open field on soil enzymes activity, macronutrients content, total phenol, peroxidase and polyphenol oxidase activity, plant growth characteristics, yield and active substances of cowpea.

**Periodical changes of dehydrogenase activity.**

Dehydrogenase (DH) activity was estimated as a guide of respiration rate and total microbial activity populations in soil under different investigated treatments.

Except the control treatment, data presented in **Table (2)** showed that the lowest DH activity was observed in the treatment of full dose from chemical nitrogen fertilization combined with fungicide. This trend of results was observed at all determination periods and during the two growing seasons. In addition to, obtained data revealed that the rhizosphere of cowpea plants treated with fungicide gave lower records of DH activity compared to untreated one.

Inoculation with biofertilizers and bio-control agent gave higher records of DH activity than non-inoculated ones. Moreover, inoculation plants with biofertilizers led to increase DH activity value in soil rhizosphere than inoculation with bio-control agent.

**Table 2.** Periodical changes of dehydrogenase activity (DHA) under biofertilization and biological control in soil cultivated with cowpea.

| Treatments | Dehydrogenase activity (µg TPF / g dry soil) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First season | | | | Second season | | | |
| | 30 | 45 | 60 | 90 days | 30 | 45 | 60 | 90 days |
| Control | $8.36^{g}$ | $10^{g}$ | $11.13^{i}$ | $9.15^{i}$ | $8.52^{g}$ | $7.59^{h}$ | $12^{h}$ | $9.15^{i}$ |
| Full dose of N fertilizer | $17.51^{d}$ | $18.41^{d}$ | $21.1^{de}$ | $12.51^{g}$ | $18.31^{c}$ | $18.64^{cd}$ | $19.44^{f}$ | $18.54^{d}$ |
| Full dose N + fungicide | $9.75^{f}$ | $12.04^{f}$ | $12.75^{g}$ | $11.54^{h}$ | $10.62^{e}$ | $14.65^{e}$ | $22.13^{c}$ | $11.56^{h}$ |
| Full dose N + Bio- control | $17.52^{d}$ | $19.41^{b}$ | $21.48^{d}$ | $12.58^{g}$ | $18.54^{c}$ | $19.3^{bc}$ | $20.85^{e}$ | $19.29^{c}$ |
| Half dose N + Biofertilizers | $18.46^{c}$ | $21.43^{a}$ | $22.5^{c}$ | $13.22^{f}$ | $19.54^{b}$ | $19.2^{bc}$ | $21.43^{d}$ | $20.01^{b}$ |
| Half dose N+ Biofertilizers + Bio-control | $19.21^{b}$ | $21.69^{a}$ | $24.02^{b}$ | $16.25^{e}$ | $20.94^{a}$ | $19.43^{b}$ | $25.15^{a}$ | $22.04^{a}$ |

It is clear from the obtained results that plant treated with half dose of N fertilizer combined with biofertilizers and bio-control agent gave the highest records of DHA values. This result could be attributed to the synergistic effect between strains in inoculated soil with mixture of biofertilizer strains in both growing seasons (*Bradyrhizobium* sp., *Bacillus megaterium* var. *phosphaticum and Bacillus circulans*). The highest records of DHA in dual inoculation of cowpea with biofertilizers and biological control agent was observed in all determination periods and during two growing seasons. This result is in accordance with **Nour and Hager (2015)** who found that higher records of DHA with biofertilization are likely be due to the effective role of inoculation for enhancing colonization of introduced biofertilizers for plant roots. Moreover, the inoculation might lead to accumulation of available nutrients and stimulate the microorganisms in rhizosphere. The proliferation and activation of microorganisms in rhizosphere of the inoculated plants may explain the observed increase in the dehydrogenase activity. In this respect, a good correlation between microbial biomass and soil dehydrogenase activity has been demonstrated by **Carlile *et al.* (2004)**.

DHA activity increased in various treatments with the increasing of growing time to reach their maximum records at 60 days (flowering stage) and decreased thereafter. This results may due to the difference in multiplication rate of different soil microorganisms which usually be maximum during flowering stage. Moreover, could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages These results are in harmony with **Abdel-Jawad (1998) and Zaghloul *et al*. (2007)**. In the entire crop period, the enzyme activity increased initially at 60 days and then declined with the age of the crop **(Singaram and Kamalakumari, 1995)**. These observations are in accordance with the findings of the present investigation. More than the microbial population and the enzyme activities are regulated (**Nagaraja *et al*., 1998).**

The difference between the two growing seasons may be due to changes in the climatic conditions. Data presented in **Table (2**) showed fluctuation in DH activity during growth period. This fluctuation is likely be due to the temperature changes and drying & remoistening during the experimental period which occurs in the field.

Results indicated that DHA widely varied among the studied treatments compared to control. Such results indicated that the rhizolex-T when reach to the soil change in quantitative aspects of several microorganisms and disturb the microbial equilibrium. This result is in agreement with **Zaghloul *et al*. (2007).**

**Periodical changes of nitrogenase activity**

Nitrogenase activity ($N_2$-ase) was periodically determined in root nodules as an indicator to $N_2$-fixation activity. Data in **Table (3)** showed that non-fertilized and non-inoculated treatment (control) gave lower records of $N_2$-ase activity than treated ones.

Except the control treatment, root nodules of cowpea plants which treated with full dose of chemical fertilizers amendment gave the lowest $N_2$-ase activity.

In addition to, obtained data in **Table (3)** revealed that the rhizosphere of cowpea plants treated with full dose of chemical nitrogen fertilization in combination with fungicide emphasized lower records of $N_2$-ase activity as compared to chemical nitrogen fertilization amendment individually.

Moreover, all treatment that were inoculated with biofertilizers gave higher records of $N_2$-ase activity in comparison with un-inoculated one.

Higher records of $N_2$-ase activity that observed in soil inoculated with biofertilizer strains (*Bradyrhizobium sp.*, *Bacillus megaterium* var. *phosphaticum*, and *Bacillus circulans)* externalized the importance of inoculation on proliferation and enhancement of $N_2$-fixers in rhizosphere. Dual inoculation of cowpea transplants showed higher values of $N_2$-ase activity. This result could be attributed to the synergistic effect in case of dual inoculation.

**Table 3.** Periodical changes of nitrogenase activity under biofertilization and biological control in soil cultivated with cowpea.

| Treatments | Nitrogenase activity (µL $C_2H_4$ / g dry soil / h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First season | | | | Second season | | | |
| | 30 | 45 | 60 | 90 days | 30 | 45 | 60 | 90 days |
| Control | $18.42^{j}$ | $21.57^{j}$ | $23.86^{l}$ | $19.86^{k}$ | $19.72^{i}$ | $22.23^{j}$ | $25.26^{k}$ | $21.84^{j}$ |
| Full dose of N fertilizer | $25.59^{f}$ | $41.36^{g}$ | $44.9^{g}$ | $27.13^{g}$ | $28.86^{d}$ | $43.92^{f}$ | $47.5^{d}$ | $28.86^{f}$ |
| Full dose N + fungicide | $22.44^{h}$ | $23.93^{i}$ | $27.5^{i}$ | $23.17^{i}$ | $23.87^{g}$ | $25.97^{h}$ | $28.78^{h}$ | $24.17^{h}$ |
| Full dose N + Bio- control | $26.14^{ef}$ | $48.63^{e}$ | $49.91^{f}$ | $36.82^{e}$ | $36.9^{b}$ | $50.54^{d}$ | $52.44^{e}$ | $38.57^{d}$ |
| Half dose N + Biofertilizers | $26.21^{e}$ | $53.00^{c}$ | $55.6^{d}$ | $37.99^{d}$ | $50.57^{d}$ | $56.9^{b}$ | $59.84^{c}$ | $39.85^{c}$ |
| Half dose N+ Biofertilizers + Bio-control | $31.6^{c}$ | $65.46^{a}$ | $67.46^{b}$ | $42.04^{b}$ | $38.8^{a}$ | $65.95^{a}$ | $69.6^{a}$ | $45.78^{a}$ |

Worthy, the higher values of $N_2$-ase activity in soil treated with half dose of chemical fertilization than ones treated with full dose may be due to the inhibition effect of nitrogen fertilizer on $N_2$-ase activity. These results are logic and in harmony with that obtained by **Anne-Sophie *et al.* (2002)** who demonstrated that the addition of chemical fertilizers such as ammonium nitrate decreased the nitrogenase activity. This trend of results was observed at all determination periods and during the two growing seasons.

Rhizobia require a plant host; therefore, they cannot independently fix nitrogen. These bacteria are located around root hair and fix atmospheric nitrogen using particular enzyme called nitrogenase. When this mutualistic symbiosis established, rhizobia use plant resources for their own reproduction whereas fixed atmospheric nitrogen is used to meet nitrogen requirement of both itself and the host plants. Supply of nitrogen through biological nitrogen fixation has ecological and economic benefits **(Ndakidemi *et al*., 2006)**.

As a result of continuous addition of biofertilizers during growth season, the values of $N_2$-ase activity in inoculated soil were higher than un-inoculated one. This result explains the synergistic effect of inocula addition on survival and activities of beneficial microorganisms. The highest records of $N_2$-ase activity were observed in dual inoculation of cowpea with biofertilizers and biological control agent. This result was observed in all determination periods and during two growing seasons.

From the obtained data in **Table (3)** showed that $N_2$-ase activity increased of the growing time to reach their maximum records at 60 days (flowering stage) and decreased thereafter. Higher records of $N_2$-ase activity at flowering stage could be attributed to the beneficial effect of root exudates which increase during this stage of cultivated plants. This result is in harmony with those obtained by **Neweigy *et al.* (1997)** and **Hanafy *et al.* (1998)** who found that the densities of $N_2$-fixer bacteria in rhizosphere were higher at heading (flowering) stage of plants than other plant growth stages. Also, **Aulakh *et al.* (2001)** reported that the exudation rates were in general lowest at seedling stage, increased until flowering but decreased at maturity.

Regarding the biological control with *Pseudomonas fluorescenes* effect, data showed that the biological control treatment gave slightly higher records of $N_2$-ase activity than chemical nitrogen with fungicide (Rhizolex-T), this result was observed at all determination periods as well as during the two growing seasons.

**Periodical changes of phosphatase activity.**

Data in **Table (4)** indicated that rhizosphere of cowpea plant treated with chemical nitrogen fertilizers gave lower alkaline phosphatase activity at the two seasons than ones inoculated with bio-inoculants. Significant increase in phosphatase activity in inoculated treatments externalize the beneficial effect of biofertilizer strains in phosphatase production.

**Table 4.** Periodical changes of phosphatase activity under biofertilization and biological control in soil cultivated with cowpea.

| Treatments | Phosphatase activity (µg inorganic phosphorus / g dry soil) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First season | | | | Second season | | | |
| | 30 | 45 | 60 | 90 days | 30 | 45 | 60 | 90 days |
| Control | 10.86$^{g}$ | 14.66$^{g}$ | 18.4$^{i}$ | 12.39$^{h}$ | 16.57$^{f}$ | 19.86$^{h}$ | 16.53$^{i}$ | 13.67$^{h}$ |
| Full dose of N fertilizer | 18.18$^{e}$ | 22.02$^{de}$ | 24.83$^{e}$ | 19.2$^{ef}$ | 20.21$^{c}$ | 23.81$^{b}$ | 29.04$^{d}$ | 20.38$^{de}$ |
| Full dose N + fungicide | 17.13$^{f}$ | 21.2$^{e}$ | 22.11$^{g}$ | 17.2$^{g}$ | 18.66$^{de}$ | 22.23$^{cd}$ | 23.92$^{f}$ | 17.96$^{fg}$ |
| Full dose N + Bio- control | 20.15$^{c}$ | 22.53$^{cd}$ | 32.53$^{b}$ | 21.51$^{cd}$ | 21.56$^{b}$ | 24.82$^{a}$ | 32.82$^{b}$ | 23.26$^{ab}$ |
| Half dose N + Biofertilizers | 19.22$^{d}$ | 23.14$^{bc}$ | 32.23$^{c}$ | 20.88$^{d}$ | 20.54$^{c}$ | 24.9$^{a}$ | 32.8$^{b}$ | 21.81$^{bcd}$ |
| Half dose N+ Biofertilizers + Bio-control | 22.47$^{b}$ | 24.81$^{a}$ | 33.02$^{b}$ | 23.01$^{abc}$ | 23.86$^{a}$ | 25.63$^{a}$ | 34$^{a}$ | 24$^{a}$ |

The highest records of phosphatase activity were observed in dual inoculation of cowpea with biofertilizers and biological control agent. This result was observed in all determination periods and during two growing seasons. Similar results were obtained by **Kumar (1998)** and **Subhita *et al.* (2005)** who reported that seed inoculation with biofertilization significantly increased the total and available P in soil after harvest and helped in releasing native P as well as in protecting fixation of added phosphorus. Increased microbial and root activity in the rhizosphere generally accounts for higher activity including phoshpatase as reported by **Singh *et al.* (2012)** and **Nath *et al.*, (2012)**. Maximum soil microbial biomass, total and available phosphorus in soil was recorded under treatment receiving dual inoculation and minimum in soil under no inoculation.

The highest records of phosphatase activity were observed in dual inoculation of cowpea with biofertilizers and biological control agent. This result was observed in all determination periods and during two growing seasons.

From the obtained data in **Table (4)** showed that phosphatase activity increased of the growing time to reach their maximum records at 60 days (flowering stage) and decreased thereafter. Higher records of phosphatase activity at flowering stage could be attributed to the beneficial effect of root exudates which increased during this stage of cultivated plants. Generally, phosphatase activity was fluctuated during the determination period. This was true under the investigated treatments and all periods in the two seasons. This fluctuation is likely be due to the temperature changes, soil drying & remoistening and synergistic doses of biofertilizers during the experimental period which occurs in field.

**Effect of biofertilization and biological control on ammoniacal nitrogen.**

Data in **Fig. (1)** clearly, showed that the ammoniacal nitrogen ($NH_4$-N) records in soil treated with full dose of chemical fertilizers gave lower values compared with soil treated with half dose and inoculated with biofertilizer strains. This result is in accordance with **Abdel-Rahman (2009)**. Also, data revealed that rhizosphere of cowpea were significantly increased under the investigated treatments compared with the control. Similar trend of results was observed in the two growing seasons.

The higher records of $NH_4$-N level were observed in dual inoculation of cowpea with biofertilizers and biological control agent at 60 days and decreased thereafter. This result was observed during the two growing seasons. Furthermore, from the obtained data we can notice that $NH_4$-N level was higher in the second season than the first one. This difference between the two growing seasons may be due to the changes in climatic conditions.

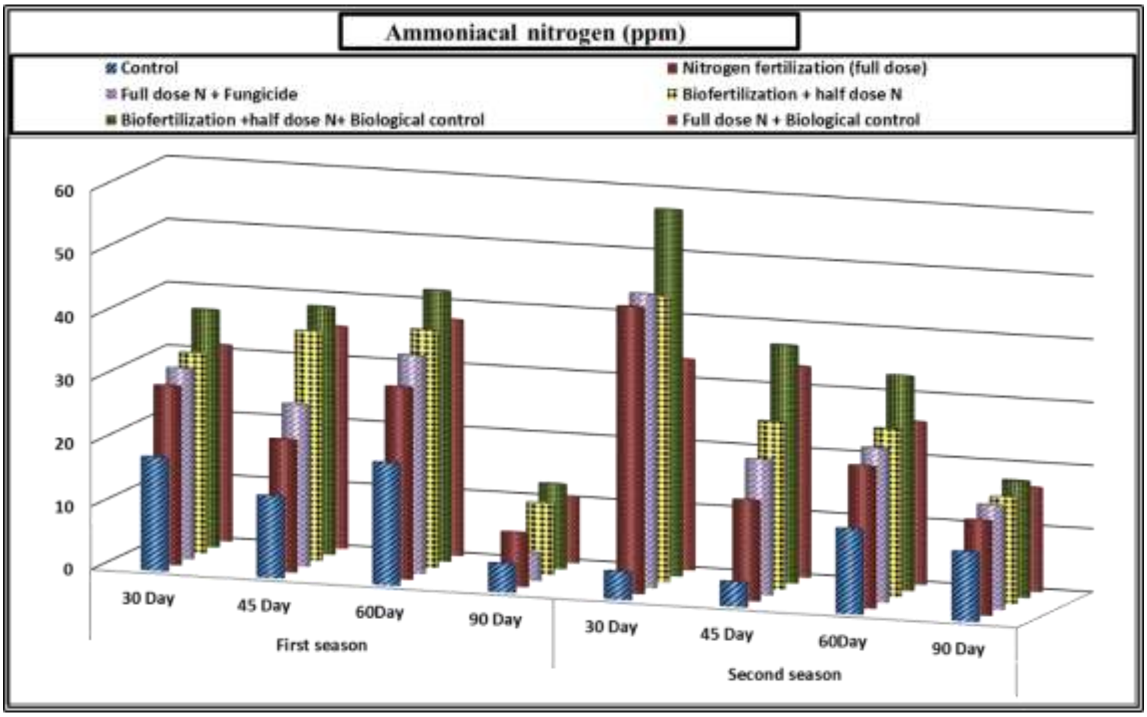



**Fig. (1).** Effect of biofertilization and biological control on ammoniacal nitrogen in soil cultivated with cowpea.

**Effect of biofertilization and biological control on nitrate nitrogen.**

Data in **Fig. (2)** showed that fertilized soil with different fertilization sources gave higher records of nitrate ($NO_3$-N) records compared with the control.

Also, data obtained emphasized that using of biofertilization led to significant increase of nitrate ($NO_3$-N) compared to use chemical fertilization alone. This result is in harmony with **Abd El-Satar (2014)** who found that using the biofertilization led to significant increase of $NO_3$-N levels compared with the chemical fertilization. The highest records of $NO_3$-N were observed in dual inoculation of cowpea with biofertilizers and biological control agent at 60 days and decreased thereafter. This result was observed in all determination periods and during two growing seasons.

The higher levels recorded at flowering stage can be attributed to the high multiplication of nitrifiers as a result of qualitative and quantitative changes in nature of the root exudates of cultivated plants during different growth stages. This result is in harmony with those obtained by **Zaghloul and Abou Aly (2002)**

From obtained data, it can notice that $NO_3$-N level was higher in the 2$^{nd}$ season than the 1$^{st}$ one. This difference between the two growing seasons may be due to the changes in the climatic conditions. Such increase could be interpreted as a result of nitrification process of ammoniacal nitrogen to $NO_3^-$ **(Abd El-Satar , 2014** and **Selim *et al*., 2012)**.

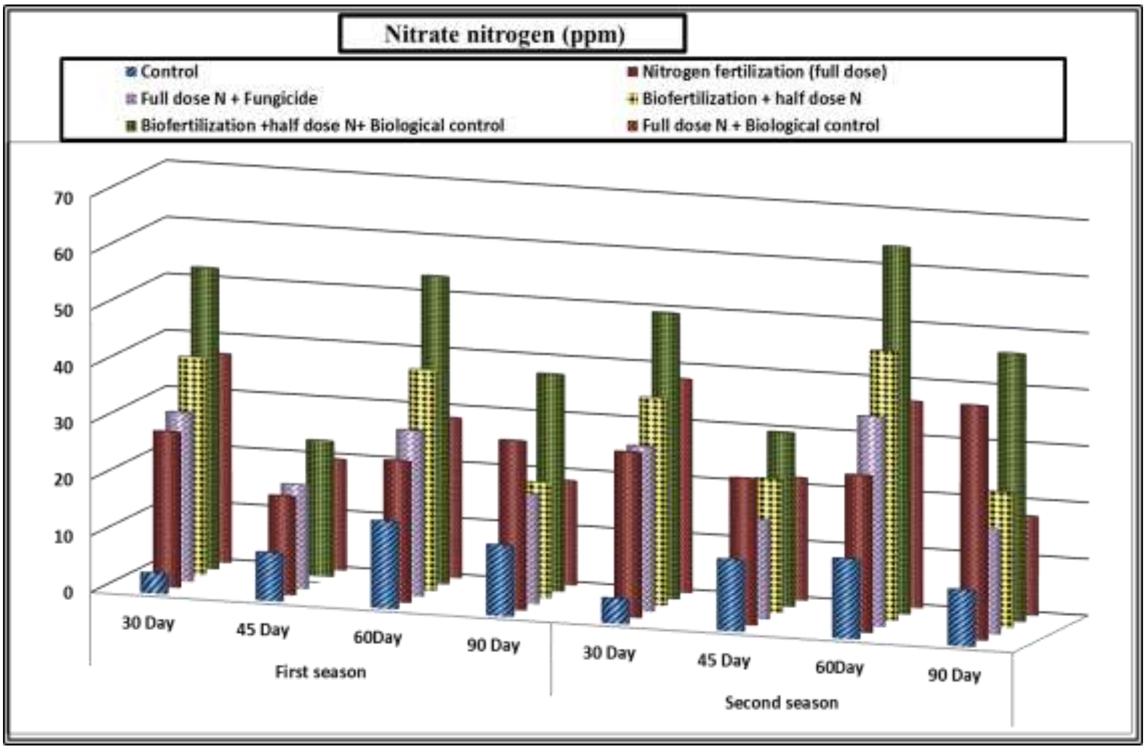



**Fig. (2).** Effect of biofertilization and biological control on nitrate nitrogen in soil cultivated with cowpea

**Effect of biofertilization and biological control on available phosphorus.**

Data in **Fig. (3)** showed that concentration of available phosphorus in rhizosphere of cowpea was significantly increased with biofertilization treatments compared with the chemical fertilization individual. This result is in harmony with **Talaat and Abdallah (2008)** who found that soil amended with the chemical fertilizers gave lower records of available phosphorus compared with the soil inoculated with biofertilizer strains. These results were happened in all growth periods and the two growing seasons.

the higher concentration of available phosphorus in rhizosphere of cowpea that inoculated with biofertilizer strains may be due to the effect of bio-inoculants on number of phosphate solubilizing bacteria where **Jyoti *et al*. (2013)** have reported that treatment soil inoculated with *Bacillus* sp. also showed the highest number of phosphate solubilizing bacteria (PSB) in the rhizosphere of plants and percent N content in shoots.

It is worthily to mention that amended soil with biofertilization + ½ chemical+ biological control showed the highest records of available phosphorus than soil amended with chemical fertilization only during the two successive growing seasons. This result distinguished the role of biofertilizer strains in phosphorus solubilization **(Bhat *et al*., 2013**).

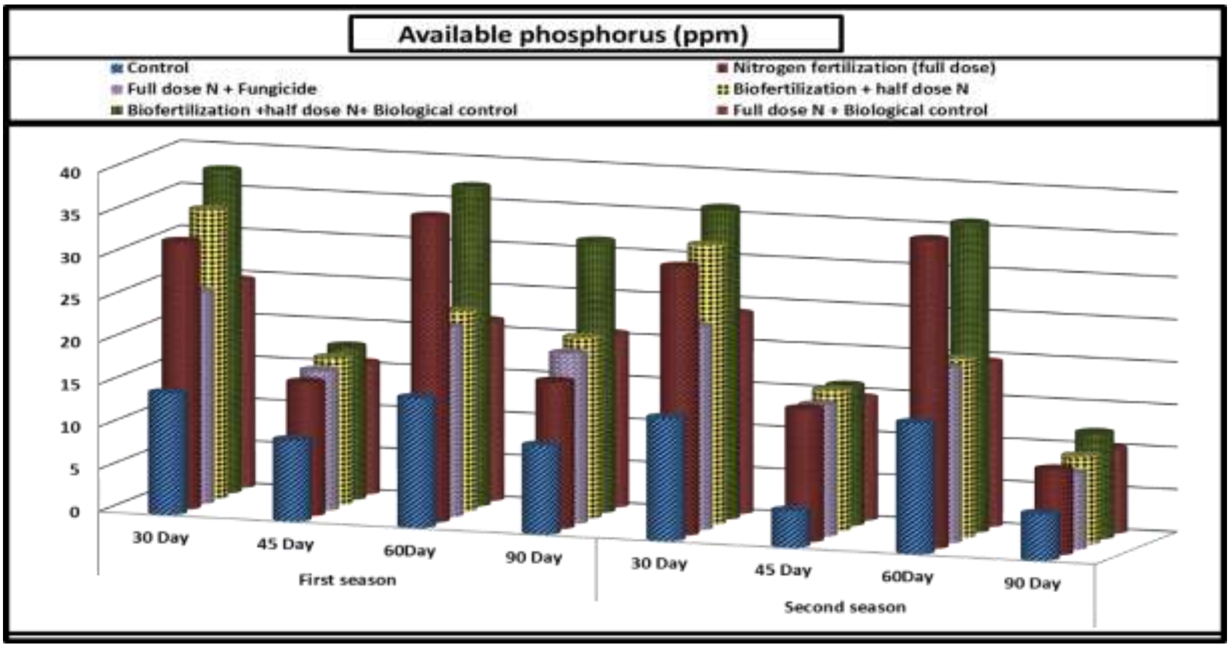



**Fig. (3). Effect of biofertilization and biological control on available phosphorus in soil cultivated with cowpea**.

**Effect of inoculation with BIOFERTILIZERS and biological control on soluble potassium.**

Soluble potassium was periodically determined as an indicator for silicate solubilizing bacteria activity. In this respect, data in **Fig. (4)** Showed that concentration of soluble-K in rhizosphere of cowpea was significantly increased under all investigated treatments compared with the control.

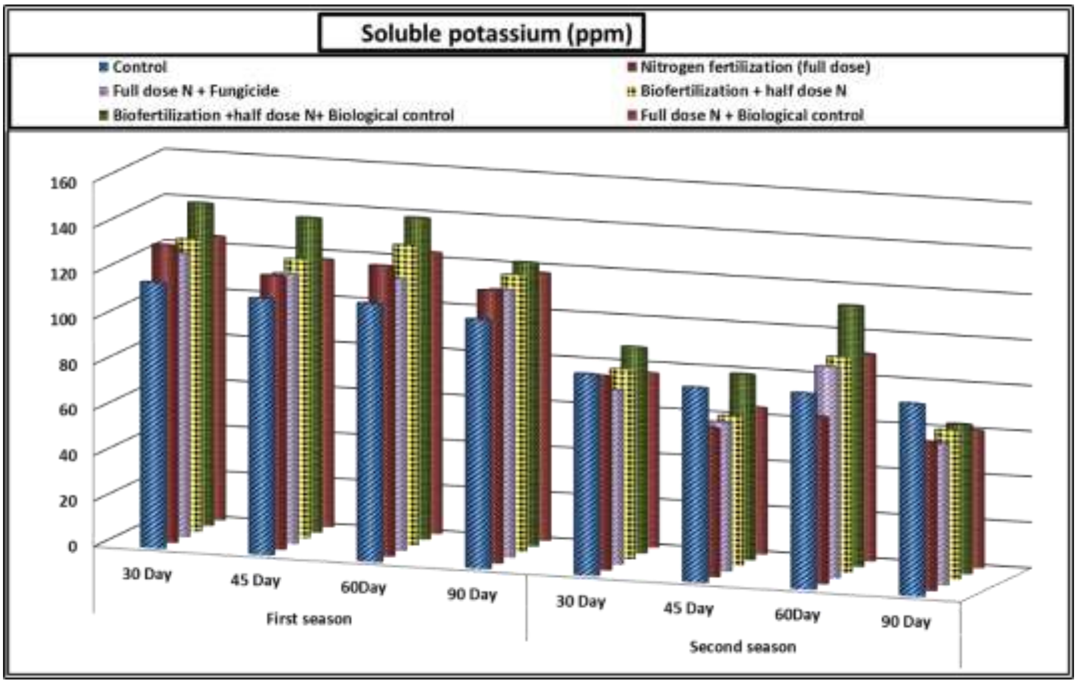



**Fig. (4).** Effect of biofertilization and biological control on soluble potassium in soil cultivated with cowpea

Soil amended with the full dose of chemical fertilizers gave lower record of soluble-K compared with the soil treated with half dose combined with biofertilizers. This result was happened in all growth periods and the two growing seasons. These results are in harmony with **Youssef and Eissa (2014**) who reported that potassium in the soil, increased soil microbial population.

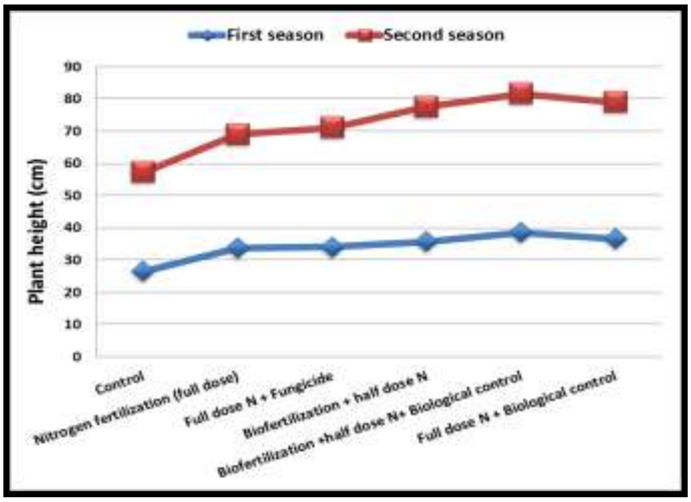
It is worthily to mention that soil amended with the biofertilization + ½ chemical+ biological control treatment showed the highest records of soluble potassium than other investigation treatments.

**Effect of inoculation with biofertilization and biological control on Vegetative growth characteristics.**

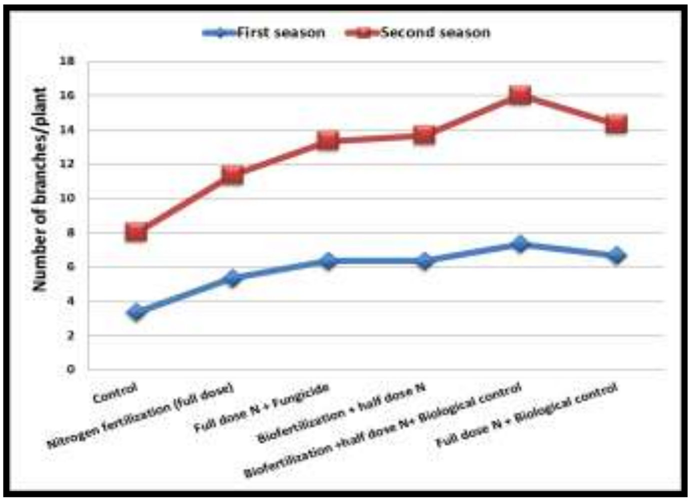
Except the control treatment, data presented in **Fig. 5 (a, b, c, d** and **e)** clearly indicated that the lowest records of cowpea plants growth characteristics, i.e., plant height, number of leaves/plant, number of branches/plant, plant fresh weight and plant dry weight branches/plant and number of leaves/plant were observed in plants fertilized with full dose of chemical nitrogen fertilizers only. This trend was true in the two growing seasons.

In addition to, obtained data revealed that the rhizosphere of cowpea plants treated with full dose of chemical nitrogen fertilization in combination with fungicide emphasized higher records of all estimated parameters as compared to chemical nitrogen fertilization amendment individually.

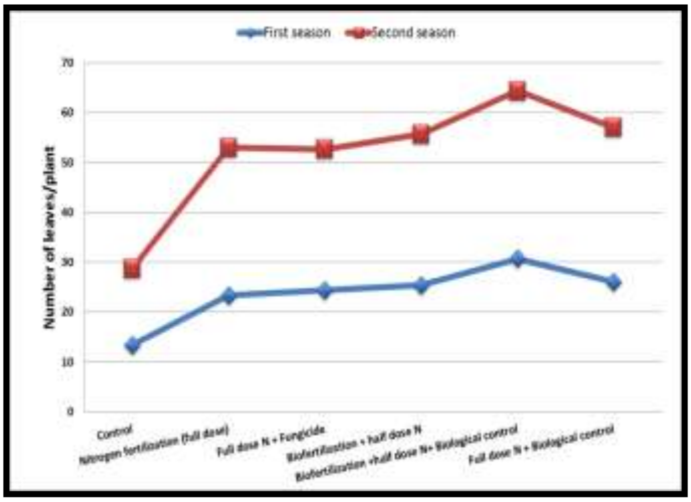
Results also showed that cowpea plants treated with biofertilizers in combination with half dose of chemical nitrogen fertilizers gave higher records of all estimated parameters in comparison with either chemical nitrogen fertilization combined with fungicides or chemical nitrogen fertilization (full dose) amendment only.



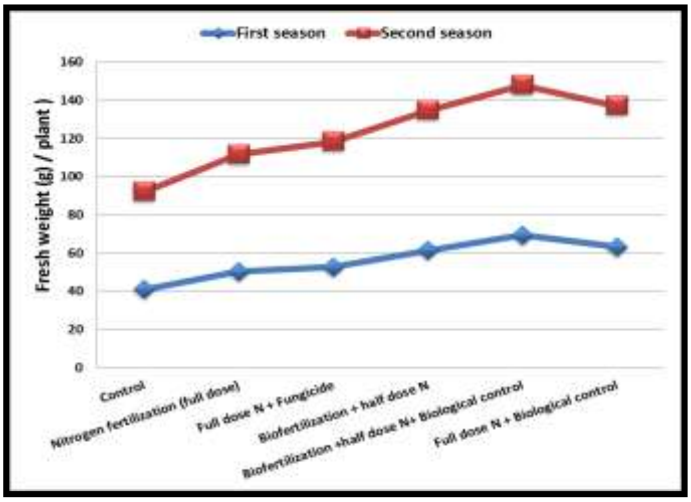

A



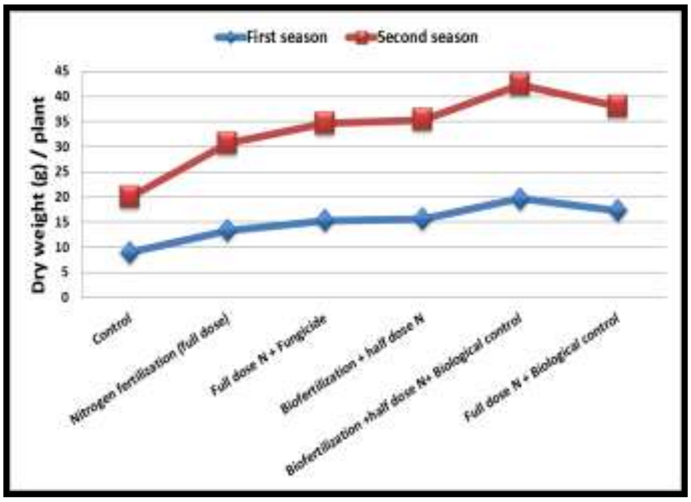

B




C




D




E

**Fig 5. Effect of biofertilization and biological control on (a).plant height (cm) of cowpea.**
**(b) number of branches/plant of cowpea.**
**(c) number of leaves/plant of cowpea.**
**(d) fresh weight (g)/plant of cowpea.**
**(e) dry weight (g)/plant of cowpea.**

This result is in agreement with **Gabr *et al.* (2007)** who reported that these enhancing effects of the different biofertilizers on of pea plants could be due to the efficiency of the different bacterial strains, on $N_2$-fixation, dissolving immobilized P and producing appropriate amounts of phytohormones necessary for activating plant growth parameters. **El-Waraky and Kasem (2007)** mentioned that applied N and inoculation of cowpea seeds with biofertilizer increased plant height, number of leaves and branches. Also, the combination was the best treatment for improving most vegetative growth characters.

**Antoun *et al*. (1998)** and **Helmy *et al*. (2015)** found that rhizobia stimulate plant growth mainly by modifying root development, which in turn improved macro and micronutrients and water uptake in the early of plant development.

The highest records of all estimated parameters were observed in dual inoculation of cowpea with biofertilizers combined with half dose of nitrogen fertilizers and biological control agent. This result was observed during two growing seasons.

Concerning the biological control with *Pseudomonas fluorescenes* effect, data in **Fig. 5 (a, b, c, d** and **e)** showed that the biological control treatment gave slightly higher records of vegetative growth characters than chemical nitrogen with fungicide (Rhizolex-T), this result was observed at all determination periods as well as during the two growing seasons.

**Effect of inoculation with biofertilization and biological control on Yield and its components**

Data in **Fig 6 (a,b,c** and **d)** showed clearly that the lowest total seeds yield and it's components expressed as number of nodules/plant, number of pods/plant, dry weight of 100 seed and yield were observed in the treatment of full dose from chemical nitrogen fertilization amendment. This trend of results was observed during the two growing seasons. In addition to, except the number of nodules/plant obtained data in **Fig 6 (a,b,c** and **d)** revealed that the rhizosphere of cowpea plants treated with full dose of chemical nitrogen fertilization in combination with fungicide emphasized higher records of number of pods/plant, dry weight of 100 seed and yield as compared to chemical nitrogen fertilization amendment individually. These results are in agreement with those obtained by **Hassan *et al*. (1990)** and **Nandi *et al*. (2013)** who found that the increase of dose of nitrogen fertilizer led to marked decrease in nodulation of broad bean and amount of $N_2$- fixe.

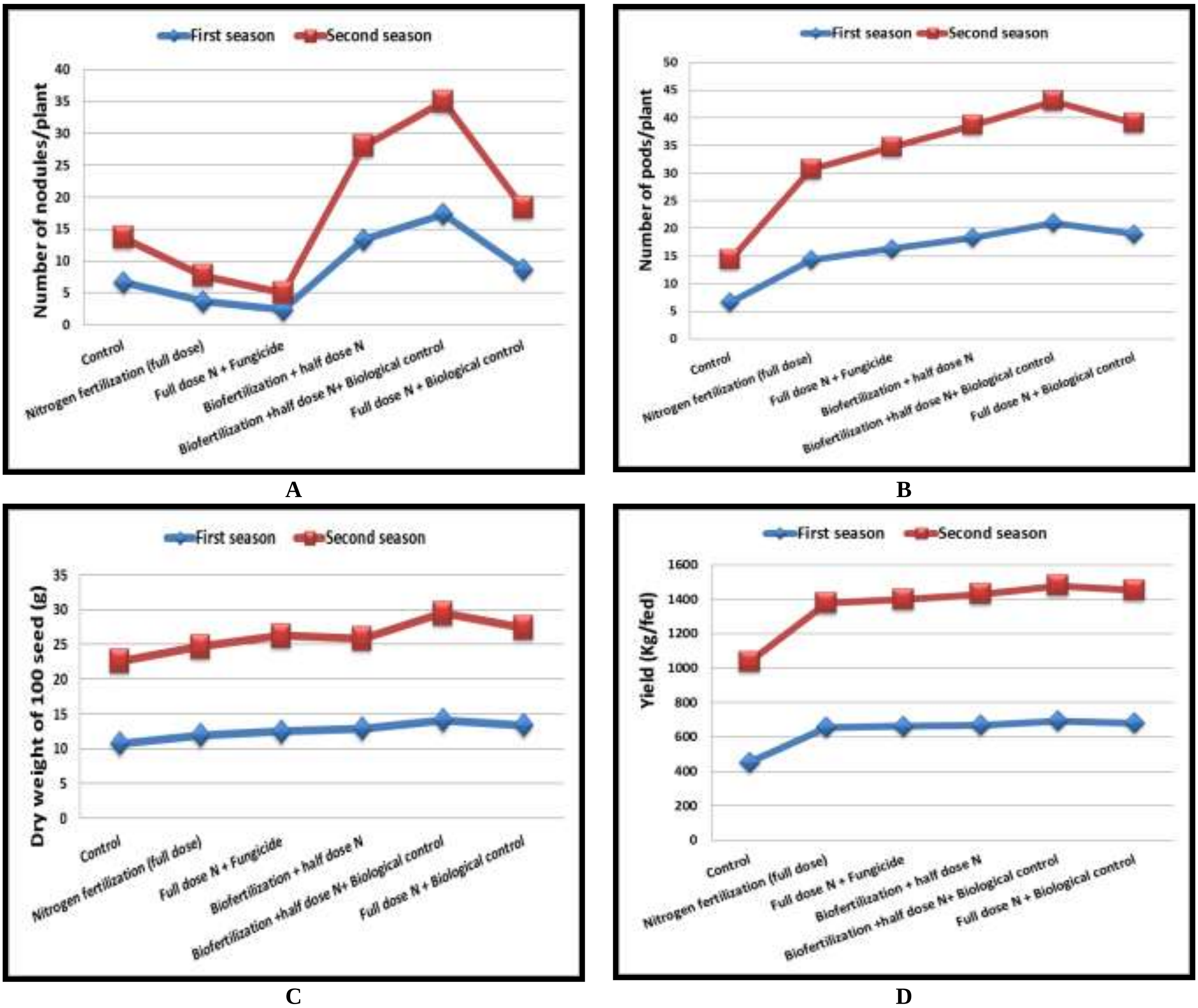



**Fig. 6. Effect of biofertilization and biological control on (a) number of nodules/plant of cowpea (b). number of podes/plant of cowpea (c). dry weight of 100 seeds (g) of nodules/plant of cowpea (d). yield (kg/fed) of cowpea**

Moreover, data showed that the inoculated soil with biofertilizer strains plus half dose of chemical nitrogen fertilizer gave higher records of number of nodules/plant, number of pods/plant, dry weight of 100 seed and yield in comparison with either chemical nitrogen fertilization combined with fungicides or chemical nitrogen fertilization (full dose) amendment only.

These results are in agreement with those obtained by **Askar and Rashad (2010)** who found that the increase of dose of nitrogen fertilizer led to a marked decrease in nodulation of broad bean and amount of $N_2$- fixed. It is noticeable that plants fertilized with chemical NPK made nodules on their roots.

The present results are in line with **EL-Bassiony (2003)** on beans and **Ismail (2002)** on pea who indicated significant positive effects on green pods yield and its components of bean due to the inoculation of seeds with different biofertilizer types. **Musa *et al.* (2011)** postulated that inoculation seed of cowpea with *Bradyrhizobium* strain significantly increased the seed yield.

The interaction effects of biofertilizer types and chemical fertilizer rates on green pods yield and its components, in the two seasons of 2014 and 2015 are presented in **Fig 9 (a,b,c** and **d)** indicated that green pods yield fed$^{-1}$, number of pods plant$^{-1}$, number of seeds pod$^{-1}$ were significantly increased through the inoculation of seeds with different biofertilizers and different N levels, relative to the control treatment.

The combined treatment of chemical nitrogen fertilizers + mixed biofertilizer and biological control gave the highest mean values of green pods fed$^{-1}$ in both seasons. These results might be explained on the basis that the promoting effects of biofertilizer and nitrogen together on growth of cowpea plant were reflected on the increased of green pods yield and its components. Many investigators, working on different vegetable crops, emphasized the beneficial effects of the interaction between inoculation with biofertilizers and mineral nitrogen application on yield and its components as **Abd EL-Mouty (2000)** on cowpea**; Shiboob (2000)** on common bean; **El-Araby *et al.* (2003)** on peas. **Farahvash *et al*. (2010)** declared that significant effect of interactions between the chemical nitrogen and biofertilizers. Maximum number of pods per plant belonged to 50 inoculation seeds of cowpea with *Bradyrhizobium* strain plus N and *Bradyrhizobium* plus P fertilizers, significantly increased seeds yield of cowpea.

Generally, The most favorable treatment of the interaction obtained with the application of biofertilization combined with half dose of nitrogen fertilizers + *Pseudomonas fluorescence* helped in producing the highest records of number of nodules/plant, number of pods/plant, dry weight of 100 seed and yield and this was true during the two growing seasons when compared to the other inoculations and control untreated plants, this results are in agreement with **Abdel-Hady (2009)**. Also, inoculation seeds of cowpea with the *Bacillus megaterium* and *Pseudomonas fluorescens* significant effect on these obtained data compared to uninoculated seeds of cowpea (**Abdel-Aziz and Salem, 2013**). Biological control agents such as *Pseudomonas* spp. have shown potential for practical application in agriculture **(Carisse *et al*., 2001)**.

## Conclusion and Recommendation

In view of the obtained results, it could be concluded that both integrated biofertilization and chemical fertilizers had pronounced effect on growth performance and yield of cowpea plant. Where, the highest values of dehydrogenase, nitrogenase and phosphatase activities were recorded. In addition, $NH_4$-N, $NO_3$-N, available–P and soluble–K values were the highest records. It may be recommended that developing countries should be interesting the use of biofertilizers and biological control agents to promote plant growth that increase crop production, decrease production costs, obtain safety plant and reduce environmental pollution

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## إستخدام التسميد الحيوى والمقاومة الحيوية فى انتاج اللوبيا

**راشد عبد الفتاح زغلول[1] ، حامد السيد أبو على[1] ، هانى محمد أحمد عبد الرحمن[1] ، مخلص عديل أحمد حسن[2]**

1. **كلية الزراعة جامعة بنها**
2. **كلية الزراعة جامعة جنوب الوادى**

أجريت تجربتان حقليتان خلال موسمين متتاليين 2014 و 2015 في كلية الزراعة جامعة جنوب الوادي (محافظة قنا، مصر) لتقييم كفاءة التسميد الحيوى *Bradyrhizobium* sp, *Bacillus megaterium* var. *phosphaticum, Bacillus circulans, Glomus macrocarpum* وعامل المقاومة الحيوية *Pseudomonas fluorescens* لتخفيض جزء من معدل التسميد الكيميائي النيتروجيني و تأثير ذلك على نشاط بعض الإنزيمات، وخصائص النمو، وبعض المكونات الكيميائية الحيوية وكذلك المحصول لنبات اللوبيا ( .*Vigna unguiculata* L.). وأظهرت النتائج التي تم الحصول عليها أن أعلى نشاط لانزيم الديهيدروجينيز، النيتروجينيز ونشاط إنزيم الفوسفاتيز عند معاملة التربة بنصف الجرعة الموصي بها من السماد الكيماوى مع تلقيحها بالسماد الحيوى فى وجود عامل المقاومة الحيوية. وقد أوضحت النتائج أن أعلى تركيز للفسفور الميسر والبوتاسيوم المذاب. ظهر عند التربة الملقحة بالتسميد الحيوى والكيماوى. ومن الجدي بالذكر أن إستبدال نصف الجرعة من التسميد الكيماوى بالتسميد الحيوى فى وجود *Pseudomonas fluorescens* أسفر عن تحسنا ملحوظا جميع صفات النمو الخضري وزياد انتاجية محصول اللوبيا. وعليه فالدراسة توصي بالتوسع في استخدام الاسمدة الحيوية وعوامل المقاومة الحيوية لانتاج غذاء آمن والحفاظ علي خصوبة التربة كهدف من اهداف الزراعة المستدامة.