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# Effect of Organic and Mineral Nitrogen Sources with and without *Rhizobium* Inoculation on Growth and Yield of Common Bean Plant Using $^{15}$N Tracer Technique

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## ABSTRACT

A Field experiment was conducted to follow up the effect of-organic - and/or mineral N fertilizer either solely or in combinations on seed yield of common bean plant grown on sandy soil using $^{15}$N tracer technique. The experimental design was ~~a~~ Factorial Complete Randomized Block Design (FCRBD) - and treatments were replicated three times. Each experimental plot (10m2) received different proportions of organic and / or mineral N fertilizer up to 100% N in presence or absence of rhizobium inoculants. Results indicated the enhancement of seed yield and N uptake by bacterial inoculation. The overall averages of organic sources revealed the superiority of quite manure over leuceana residues followed by compost treatment. Absolute values of Ndff, Ndfs+ Ndfo and Ndfa were frequently affected by portions of chemical and organic additives whereas Ndff tended to increase with increasing N fertilizer rate. Adversely, Ndfa was negatively affected by increasing N rate. Organic additives enhanced the portions of Ndff and Ndfs+ Ndfo. This holds true with and without Rhizobium inoculation. These portions were markedly increased as affected by the addition of quite manure mixed with moderate N fertilizer rate (50% Chem-N + 50% quite manure-N). Low to moderate quantities of chemical fertilizer in combination with organic additives had enhanced the utilization of chemical-N source comparing to the full chemical fertilized plants.

**Keywords:** Common bean, Rhizobium, Sandy soil, $^{15}$N-Urea fertilizer, Organic compost

## INTRODUCTION

Importance of common bean as an economical vegetable crop has a nutritional value for human and considered main source of protein was reported earlier (CIAT 2001; Broughton *et al*. 2003).

Realistic use of nitrogen by common bean indicates its gluttonous for N fertilizer that may negatively affect the nodule formation and inhibited the biological nitrogen fixation process while low adequate additions may enhanced the process (Brito *et al*. 2011, Soares *et al*., 2006; Souza *et al*. 2011).

Meanwhile, due to the high cost of manufactured chemical fertilizers (Ngetich *et al*. 2012; Fukuda *et al*. 2012), BNF could be considered as low or cheap cost effective way to compensate N for legumes (Belane and Dakora 2009, 2010; Naab *et al*.2009; Nyemba and Dakora 2010; Sprent *et al*. 2010; Pule-Meulenberg *et al*. 2010; Mohale *et al*.2014). In addition, it improves crop yield and replenish soil N budget (Maina *et al*. 2011).

In this respect, Rhizobium leguminosarum biovar phaseoli inoculants widely applied in common bean cultivation to meet plant growth and grain yield (Kellman *et al*. 2005, Kabahuma 2013, Akhter *et al*. 2014; Tabaro 2014). One of the most accurate methods for BNF estimation is the use of $^{15}$N tracer technique (Bambara and Ndakidemi 2010 a,b, Nyemba and Dakora 2010).

Thus, this study aimed at following the performance of different N-fertilizers with or without *Rhizobium* inoculation in improving yield of common bean crop.

## MATERIALS AND METHODS

Field experiment was carried out at~~,~~ experimental farm of Soils and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt.

**Materials:**

**Experimental soil:**

Experimental site is located in Inshas, Sharkia Governorate. For analysis, soil samples were collected from 0-15cm depth layer. The Soil samples were air dried ground and sieved to pass through a 2 mm sieve. Some physical and chemical characteristics of the experimental soil are presented in Table (1).

**Table 1. Some physical and chemical properties of experimental soil.**

| Property | Value |
|---|---|
| Particle size distribution (%) | |
| Coarse sand | 64.1 |
| Fine sand | 26.4 |
| Clay | 6.8 |
| Silt | 2.7 |
| Soil Texture | Sand |
| pH (1:2.5 paste) | 7.97 |
| E.C. (ds $m^{-1}$) | 0.27 |
| Total N (%) | 0.007 |
| Cations (meq 100 $g^{-1}$ soil) | |
| Ca | 1.25 |
| Mg | 1.00 |
| Na | 0.32 |
| K | 0.09 |
| Anion (meq 100 $g^{-1}$ soil) | |
| $SO_4^{--}$ | 0.53 |
| $CO_3^{--}$ | 0.00 |
| $HCO_3^-$ | 0.88 |
| $Cl^-$ | 1.25 |

**Plant seeds:** Seeds of common bean cultivar (*Phaseolus vulgaris* L. Giza 6) was kindly provided by the Agriculture Research Centre (ARC), Giza, Egypt.

**Organic fertilizers:**

**Organic manure sources:** Leucaena residues and quite manure were locally supplied.

**Composting manure**: The organic compost used in this experiment was prepared by composting a mixture of organic manures (1) [cattle +poultry +goat] + plant residues (2) [wheat+ barley + chickpeas + lupine straw]. The mixture consists of half (1) + half (2). To enhance composting 2 kg ammonium sulphate +2 kg of agricultural sulphur + 2 kg Ca-super phosphate were added to each $m^3$ of residues. Composting process was carried out according to Moursy, (2008).

**Table 2. Some chemical characteristics of used organic materials.**

| characteristic | Organic materials | | |
|---|---|---|---|
| | Compost | Luciana residues | Quite manure |
| pH (1:2.5 water) | 6.70 | 6.89 | 6.14 |
| E.C.($dSm^{-1}$)1:1 extract | 12.70 | 10.50 | 12.3 |
| Total N g $kg^{-1}$ | 28.3 | 40.9 | 16.3 |
| Total P g $kg^{-1}$ | 8.4 | 17.8 | 19.1 |
| Total K g $kg^{-1}$ | 6.9 | 8.0 | 9.66 |
| C/N ratio | 12.6 | 13.1 | 15.3 |
| Organic matter % | 57.02 | 59.00 | 43.10 |

**Nitrogen-Fixing Bacteria:**

*Rhizobium leguminosarum* biovar *phasolii* was provided by the Microbiology Department, Soils,Water and Environment Institute, Agricultural Research Center, Giza, Egypt.

**Chemical fertilizer:**

$^{15}$N-labeled urea (5% $^{15}$N atom excess) was applied in equal the doses; the first was three weeks after planting while the second was two weeks after the first one dose.

**Experimental treatments and design:**

**Experimental treatments were randomly arranged as follows:**

1-100% Urea - N as a control.

2- 75% Urea – N + 25% Org – N.

3- 50% Urea - N +50% Org – N.

4- 25% Urea - N + 75%Org – N.

5- 100% Org – N.

**Experimental layout:**

The experiment was carried out under drip irrigation system on sandy soil. The area of each plot was 10 $m^2$ (9.0 X 1.11) and spacing of 10-15 cm between plants inter-row and 55 cm between rows. Fertilization treatments were arranged with and without bacterial inoculation. All treatments were replicated three times. Common Bean (*Phaseplus vulgaris* L. Giza 6) seeds were planted at 18 February 2016. Nitrogen fertilizer was applied at rate of 60kg N $fed^{-1}$ (equal to 143 g N per plot) either added solely or in combination with organic-N. Were thoroughly incorporated into the soil four weeks before sowing. Afterwards, the soil was lightly irrigated to establish good microbial activity for decomposing leucaena residues. Quite manure was applied three weeks before sowing Compost was thoroughly incorporated ~~with~~ into the soil two weeks before sowing. All plots were basically fertilized with 80 kg P $fed^{-1}$ as super phosphate 6.8%P and 50 kg K $fed^{-1}$ as potassium sulphate 40% K. Recommended potassium fertilizer rate was splitted into three equal portions added at 15, 30, and 45 days after sowing while super-phosphate was added once before sowing.

**Methods of analysis:**

Chemical and physical analyses of experimental soil samples were determined according Carter and Greogrich (2008).

Chemical analyses were of plant samples were carried out according to Estefan *et al*., (2013).

**$^{15}$N isotopes dilution technique:-**

Isotope dilution concept and standard equations were used for quantifying nitrogen derived from fertilizer (Ndff%), from soil ( Ndfs%), from air (Ndfa%) and nitrogen use efficiency (NUE%) which recovered by seed plants according to Training Course series No 14, IAEA (2001)

**Statistical analysis:**

The analysis of variance for the final data was assayed using the system ANOVA and the values L.S.D from the controls were calculated at 0.05 levels according to SAS (1987).

## RESULTS AND DISCUSSION

**Seed yield (g $plot^{-1}$)**

Seed yield was significantly fluctuated as affected by fertilization treatments and bacterial inoculation (Table 3). The highest values of seed yield were observed with 100% quite manure as will as 25% Min – N + 75% Leucaena residues under inoculated plants ,which relatively increased by about 27.1%. Over the fully chemical fertilized treatment (100% urea- N On the other hand, the lowest value was detected with the uninoculated plants treated with 50% Min-N+50% compost-N which relatively decreased by about 55.4% under those fully fertilized with urea.

**Table 3. Effect of fertilization treatments on seed yield of common bean in the presence or absence of bacterial inoculation.**

| Treatments | Seeds (g $plot^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Organic Additives | | | | | |
| %Min + %Org. | Inocu. | untreated | Leucaena residues | Compost | Quite manure | Mean |
| 0 + 100 | Inoc | - | 405.00 | 395.00 | 410.00 | 348.89 |
| | unInoc | - | 243.33 | 231.67 | 348.33 | 328.89 |
| Mean | | | 324.2 | 313.3 | 379.2 | 338.9 |
| 25 + 75 | Inoc | - | 383.33 | 345.00 | 236.66 | 309.99 |
| | unInoc | - | 210.00 | 190.00 | 201.66 | 212.22 |
| Mean | | | 296.7 | 267.5 | 219.2 | 261.1 |
| 50 + 50 | Inoc | - | 303.33 | 376.66 | 375.00 | 336.33 |
| | unInoc | - | 257.33 | 183.33 | 373.33 | 286.66 |
| Mean | | | 280.3 | 280.0 | 374.2 | 311.5 |
| 75 + 25 | Inoc | - | 410.00 | 290.00 | 358.33 | 352.78 |
| | unInoc | - | 355.00 | 186.66 | 316.66 | 286.11 |
| Mean | | | 382.5 | 238.3 | 337.5 | 319.4 |
| Averages | | | 320.9 | 274.8 | 327.5 | |
| 100 + 0 | Inoc | 322.67 | | | | |
| | unInoc | 411.00 | | | | |

L.S.D At 5%

| Analysis of Variances | With and without Inoculation | |
|---|---|---|
| Organic Materials (A).71 : | 124.50 | 73.71 |
| Nitrogen (B) : | 106.30 | 62.96 |
| A x B : | 141.60 | 83.84 |

**Mean averages, despite of inoculation, indicated the superiority of fully organic treated plant over those received 75% urea+25% organic followed by 50% urea plus 50% organic the those of 25% urea plus 75% organic. The overall averages of organic sources revealed the superiority of quite manure over leuceana residues followed by compost treatment.**

Generally, inoculated plants produced higher seed yield than the un-inoculated ones. These findings are in harmony with those obtained by Adeyeye, et.al, (2017), who proved that inoculation improved the seed yield of soybean. Similar findings were reported for peanut (Nabil *et al*., 2015), and common bean production (Hungria *et al*., 2003) at low rates of N applied with Rhizobium inoculation.

**N uptake by seeds (g plot$^{-1}$)**.

N uptake by Seed yield was significantly fluctuated as affected by fertilization treatments and bacterial inoculation (Table 4). The highest values of N uptake by seed yield were observed with 100% compost manure as will as 75% Min – N + 25% Leucaena residues under inoculated plants ,which relatively increased by about 18.9%. over the fully chemical fertilized treatment (100% urea- N. On the other hand, the lowest value was detected with the uninoculated plants treated with 50% Min-N+50% compost-N which relatively decreased by about 49.9% under those fully fertilized with urea.

Mean averages, despite of inoculation,–indicated the superiority of fully organic treated plant over those received 75% urea+25% organic followed by 50% urea plus 50% organic the those of 25% urea plus 75% organic. The overall averages of organic sources revealed the superiority of quite manure over leuceana residues followed by compost treatment.

Positive effect of inoculation either applied solely or in combination with moderate amounts of chemical fertilizer on growth, seed yield and N-uptake by snap bean was reported earlier by Mostafa and Zohair *et al*., (2014) under field conditions.

**Table 4. Effect of fertilization treatments on N- uptake by seeds yield of common bean in the presence or absence of bacterial inoculation.**

| Fertilization treatments | N-uptake by seeds (g/plot) | | | | | |
|---|---|---|---|---|---|---|
| | Organic additives | | | | | |
| %Min + %Org. | Inoc | untreated | Leucaena residues | Compost | Quite manure | Mean |
| 0 + 100 | Inoc | - | 11.280 | 12.329 | 11.359 | 11.656 |
| | Uninoc | - | 6.307 | 5.758 | 9.297 | 7.121 |
| | Mean | | 8.794 | 9.044 | 10.328 | |
| 25 + 75 | Inoc | - | 9.210 | 8.587 | 6.708 | 8.168 |
| | Uninoc | - | 6.017 | 5.054 | 5.718 | 5.596 |
| | Mean | | 7.614 | 6.821 | 6.213 | |
| 50 + 50 | Inoc | - | 9.074 | 9.373 | 10.070 | 9.506 |
| | Uninoc | - | 7.067 | 5.181 | 10.120 | 7.456 |
| | Mean | | 8.071 | 7.277 | 10.095 | |
| 75 + 25 | Inoc | - | 12.054 | 7.743 | 9.051 | 9.616 |
| | Uninoc | - | 9.686 | 5.790 | 8.127 | 7.868 |
| | Mean | | 10.870 | 6.767 | 8.589 | |
| 100 + 0 | Inoc | 8.37 | | | | |
| | Uninoc | 8.73 | | | | |

| L.S.D at 5% | | |
|---|---|---|
| Analysis of Variances | With and without Inoculation | |
| Nitrogen (A) : | 1.838 | 1.122 |
| Organic Materials (B) : | 2.152 | 0.958 |
| A X B : | 2.448 | 1.276 |

**Portions of nitrogen derived by seeds**

Nitrogen derived from fertilizer (Ndff), from air (Ndfa) and from soil+ organic (Ndfs+ organic) by seeds (g plot$^{-1}$) were markedly influenced by the additions of organic materials in mixture with mineral N fertilizer and alone with *Rhizobium* inoculation (Table 5).

It is clear that absolute values of Ndff, Ndfs + Ndfo and Ndfa were frequently affected by portions of chemical and organic additives whereas Ndff tended to increase with increasing N fertilizer rate. Adversely, Ndfa was negatively affected by increasing N rate. Highest value of Ndfa was recorded with application of compost at 75% in combination with 25% chemical fertilizer. It seems that organic additives enhanced the portions of Ndff and Ndfs. This holds true with and without Rhizobium inoculation. Nitrogen utilized from fertilizer were 6.712, 4.801 and 4.536 g plot$^{-1}$ observed in the plots received under with Rhizobium inoculation with organic materials mixed with mineral – N fertilizer additions, Leucaena residues, compost manure and quite manure at rates of (25% min + 75% organic manure) respectively, in without Rhizobium inoculation, were 6.420,4.987,4.272 , and 3.094g pot$^{-1}$ observed in the plots received rates of (100% min + 0 organic -N) and (25% min + 75% Leucaena residues -N) ratios, (25% min + 75% compost manure -N) ratios, (25% min + 75% quite manure -N) ratios, respectively.

These findings were recently proved by Ismail *et al*., (2017), who found that, portion of nitrogen derived from fertilizer (% Ndff), soil (%Ndfs+Ndfo) and air (% Ndfa) by shoot and seeds of chickpea plants were markedly increased as influenced by the addition of organic manure mixed with mineral N fertilizer under bacterial inoculation.In the same time, Ndfa found to be enhanced by exogenous application of rhizobium inoculant comparing to the un-inoculated treatment(Bambara and Ndakidemi, 2010).

**Table 5. Nitrogen derived from fertilizer (Ndff), air (Ndfa) and soil+ organic (Ndfs+Ndfo) (g plot$^{-1}$) by seeds as affected by organic additives mixed with chemical N fertilizer with and without *Rhizobium* inoculation.**

| Fertilization treatments | Organic additives | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Untreated | | | Leucaena | | | Compost | | | Quite | | |
| Chem –N% + Org-N% | Portions N derived by seeds (g plot$^{-1}$) | | | | | | | | | | | |
| | Inoculated | | | | | | | | | | | |
| | Ndff | Ndfa | Ndfs+Ndfo | Ndff | Ndfa | Ndfs+Ndfo | Ndff | Ndfa | Ndfs+Ndfo | Ndff | Ndfa | Ndfs+Ndfo |
| 25% + 75% | - | - | - | 4.444 | 1.580 | 3.187 | 3.700 | 1.500 | 3.400 | 2.584 | 1.244 | 2.880 |
| 50% + 50% | - | - | - | 4.751 | 1.152 | 3.171 | 4.300 | 1.432 | 3.640 | 4.801 | 1.444 | 4.127 |
| 75% + 25% | - | - | - | 6.712 | 0.723 | 4.615 | 4.536 | 0.693 | 2.514 | 4.230 | 0.835 | 3.937 |
| 100% + 0% | 5.302 | 0.543 | 0.096 | - | - | - | - | - | - | - | - | - |
| | Un-inoculated | | | | | | | | | | | |
| 25% + 75% | | - | | 3.241 | - | 2.775 | 2.504 | - | 2.550 | 3.055 | - | 2.699 |
| 50% + 50% | | - | | 3.623 | - | 3.444 | 2.690 | - | 2.491 | 5.298 | - | 4.822 |
| 75% + 25% | | - | | 4.987 | - | 3.899 | 3.094 | - | 2.696 | 4.272 | - | 3.855 |
| 100% + 0% | 6.420 | - | 2.310 | - | - | - | - | - | - | - | - | - |

**Nitrogen use efficiency (NUE%)**

Data listed in Table (6), revealed that nitrogen use efficiency (NUE%) by seeds of common bean plants were markedly affected by the additions of organic-N mixed with chemical N fertilizer in different ratios with and without *Rhizobium* inoculation. the highest were 28.2% and 16.8% observed in the plots received 25% chem -N + 75% Leucaena residues - N, while the lowest values recorded 5.9 % and 6.1 % observed in the plots fertilized with 100% Min - N with and without *Rhizobium* inoculation, respectively.

**Table 6. Nitrogen use efficiency (NUE %) by seeds as affected by different organic and chemical fertilizers in the presence or absence of *Rhizobium* inoculation.**

| Fertilization treatments | | Organic additives | | | |
|---|---|---|---|---|---|
| Min% + Org% | Inoculation | untreated | Leucaena residues | Compost | Quite manure |
| 25% Min + 75%Org | Inocu | - | 25.8 | 24.0 | 28.2 |
| | Uninocu | - | 16.8 | 14.1 | 16 |
| | Mean | | 21.3 | 19.1 | 22.1 |
| 50% Min + 50% Org | Inocu | - | 12.7 | 13.9 | 14.1 |
| | Uninocu | - | 9.9 | 7.7 | 14.2 |
| | Mean | | 11.3 | 10.8 | 14.15 |
| 75% Min + 25% Org | Inocu | - | 11.2 | 7.2 | 8.4 |
| | Uninocu | - | 8.4 | 5.4 | 7.6 |
| | Mean | | 9.8 | 6.3 | 8.0 |
| 100% Min + 0 %Org | Inocu | 12.13 | - | - | - |
| | Uninocu | 23.96 | - | - | - |

The results revealed that both inoculation and low to moderate quantities of chemical fertilizer in combination with organic additives had enhanced the utilization of chemical-N source comparing to the full chemical fertilizer plants. Jatav, *et al*, (2016), demonstrated that, NUE was maximum (30 kg seeds N-1) under lower dose of N with Rhizobium (15kg N ha$^{-1}$ + Rhizobium) as compared to only 15kg N ha$^{-1}$ application without Rhizobium (22.13 kg seeds kg N$^{-1}$). Inversely, increasing dose of N decreased the N use efficiency for all the treatments where in per cent decrease in NUE was more when only graded dose of N was applied as compared to its usage on Rhizobium inoculated seeds.

The superiority of one organic source over another was correlated to the amount of chemical nitrogen. On the other turn, the efficient use of chemical-N as affected by organic source is dependent on N rate.

## CONCLUSION

Based on the present findings, we can conclude that leucaena residues and quite manure mixed with mineral N fertilizer in different ratios had a significant effect on the increase of seed yield and N content in plant either inoculated or not. Inoculated plants produced seed yield that is 9.82% higher than non-inoculated ones. Inoculated plants achieved relative increase in N uptake by seeds by about 21.83% over the non- inoculated ones. Considering the nitrogen derived from fertilizer, air, and soil by seeds, as well as nitrogen use efficiency (NUE%) in the presence or absence of *Rhizobium* inoculation, organic type and chemical-N had a remarkable effect on NUE%**.** Efficient use of chemical-N was significantly enhanced by bacterial inoculation.

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## تأثير مصادر نيتروجينية عضوية و معدنية مع وبدون التلقيح بالريزوبيوم على نمو وانتاجية نبات الفاصوليا مستخدما تقانة النظير الثابت - ن15.


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أجريت تجربة حقلية لدراسة تأثير اضافة السماد النيتروجينى العضوى أو االمعدنى منفردين أو فى مخاليط منهما - على نمو وانتاجية نبات الفاصوليا النامي على ارض رملية مستخدما مع تطبيق تقانة النظير المستقر – ن15. رتبت المعاملات فى تصميم عاملي كامل العشوائية وكررت ثلاث مرات. اضيفت المعاملات السمادية فى قطع تجريبية (10م2) - عشوائيا فى ظل التلقيح البكتيرى أو عدمه. اضافة السماد العضوى اوالمعدنى او كليهما معا كان له تأثير كبير بالزيادة والنقصان على النمو والمحتوى من النيتروجين المأخوذ بواسطة حبوب محصول الفاصوليا . اظهرت النتائج ان التلقيح البكتيري ادى الى تحسين انتاجيه البذور والنتروجين الممتص . المتوسطات فى المصادر العضوية كانت اعلى قيمه فى سماد السمان يليه مخلف الليوسينا يليه سماد الكمبوست . بالنسبة للنيتروجين المأخوذ من السماد المعدنى المرقم , المأخوذ من التربة وكذلك من الهواء الجوى تاثرت بكميات النتروجين المعدنى المضاف مع العضوى وكانت الزيادة تزداد تدريجيا مع زيادة النتروجين الكيمائي المضاف بالنسبة للنتروجين المأخوذ من السماد وكان على العكس النتروجين المأخوذ من الهواء الجوي تناقص تدريجيا مع ارتفا كمية النتروجينى الكيمائى المضاف . ادى اضافة الاسمده العضويه الى تحسين البروتين فى التروجين المأخوذ من السماد و المأخوذ العضوى و المأخوذ الهواء الجوى مع جميع المعاملات الملقحة والغير ملقحه بالريزوبيوم .ومن خلال النتروجين المرقم كانت المعامله مع سماد السمان ومعدل 50% سمان+ 50% نتروجين كميائى هى الاعلى.ايضا كانت الكميات القليلة من السماد الكيميائى مع السماد العضوى ادت الى تحسين الاستفادة من النتروجين مقارنة بالكميه الكامله من اضافة السماد الكميائى للنبات