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# GROWTH, YIELD AND YIELD COMPONENT OF INOCULATED CHICKPEA AND FABA BEAN PLANTS AS AFFECTED BY USING METHYLOTROPHIC BACTERIA [40]

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## ABSTRACT

Two pot experiments were performed in Giza research station, Agricultural Research Center (ARC) using 2 isolates of Pink-Pigmented Facultatively Methylotrophic bacteria (PPFMs) originated from chickpea and Faba bean. Foliar application with PPFM isolates were conjugated with specific rhizobial inoculum and N-fertilization (15 Kg N $Fed^{-1}$). Nodulation status, nitrogen fixation and growth yield and yield component were recorded. Results clearly indicated that Chickpea was superior in its response to foliar application with PPFM.C. As it gave higher records of number and dry weight of nodules, dry matter and N-content of plants as compared to Faba bean. A field experiment was also conducted in sandy loam soil at South EL-Tahreer province to investigate the effect of foliar application with PPFM.C strain + specific Rhizobia and N-fertilization on nodulation, growth and yield of chickpea legume plants. Results indicated that foliar application with PPFM.C in the presence of specific rhizobial inoculation scored significant increases in economic turnover of chickpea in the range of 21-32% as compared to N-fertilization at rate 50 Kg N $Fed^{-1}$. Foliar application with 5 L $Fed^{-1}$ in the presence of 15 Kg N $Fed^{-1}$ and specific rhizobial inoculation led to an increase of 518 kg $fed^{-1}$ productivity of seed yield , with economic turnover of 2491 L.E.


**Keywords**: *Rhizobium leguminosarum*, Nitrogen Fixation, Pink-Pigmented, Facultatively Methylotrophic bacteria (PPFMs), Foliar Application, Economic turnover, Faba bean, Chickpea.

## INTRODUCTION

The extensive use of chemicals for increasing of agricultural production led to increase both production cost and environmental pollution leading to many health hazards. To avoid some of these problems, a wide variety of Plant Growth

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Promoting Rhizobacteria (PGPR) which stimulate of growth of their host by one or more of different mechanisms, these bacteria are called were extensively used **(Frankenberger & Arshad, 1995)**.

The search for PGPR and investigation of their modes of action are increasing to exploit them commercially as biofertilizers fixing nitrogen, increasing the availability of nutrients in the rhizosphere, positively influencing both morphology and growth of roots and promoting other beneficial plant-microbe symbiosis.

One of the most traditionally exploited rhizobacteria are root nodule bacteria is nitrogen fixation in legumes; it depends upon a highly coordinated sequence of interactions between plants of the family *leguminosae* and the genera of rhizobia group, which results in the formation of root nodules in a symbiotic association system **(Arshad and Frankenberger, 1998)**.

Many microbes live on phylloplane and feed on materials leached from the leaf. Phylloplane bacteria produce B-vitamins, auxins and cytokinins among other products. The term methylotrophic is used to describe a wide variety of bacteria, which can utilize single carbon compounds more reduced than carbon dioxide as sole carbon source **(Lidstorm 2002).**

The most abundant group of methylotrophs isolated from surfaces of green plants were Pink Pigmented Facultatively Methylotrophs (PPFMs) **(Holland, 1997a).**

The present work is conducted to study the relationship between methylotrophic bacteria (PPFMs) and inoculated legume plants with rhizobia under greenhouse and field conditions.

## MATERIAL AND METHODS

### 1. Soil used

The soil used in greenhouse experiments were collected from El-Tahady sector, South El-Tahreer Province, Behera Governorate. The field experiment was conducted in the same site. Mechanical and chemical analysis of the soil sample in both experiments was carried out according to **Jackson (1973)** at soil analysis Lab., Soils, Water and Environment Research Institute, ARC, Giza, and was shown in **Table (1).**

### 2. Seeds used

Seeds of Faba bean (*Vicia faba*) variety Nubaria 1 and chickpea (*Cicer arietinum*) variety Giza 195 were used in greenhouse and field experiments. They were kindly supplied with Field Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

### 3. Bacterial strains and isolates used

Four strains of *Rhizobium leguminosarum* bv. *viciae* namely ICARDA 31 and NIFTAL 1148 specific to chickpea and ICARDA 441 and ARC 207 specific to Faba bean were obtained from biofertilizers production unit, Agricultural Microbiology Dept., Soils, Water and Environment Research Institute, ARC, Giza, Egypt, and two PPFM strains (PPFM.C & PPFM.F) which were isolated and identified by **Orf, *et al* (2005) .**

**4. Greenhouse experiments**

Seeds of chickpea (Giza 195) and faba bean (Nubaria 1) were planted in sterilized pots of 30 cm diameter each filled with 10 kg washed and sterilized sandy loam soil, seedlings were thinned after two weeks to three seedlings per pot.

The seeds of chickpea and faba bean were inoculated twice, at planting and after two weeks with 10 mL of liquid mixture of rhizobial strains(containing $3x10^9$ cells $mL^{-1}$) specific to both legumineous plants, which were enrichment with YEM broth medium **(Vincent, 1970) .**

The inoculation with the PPFMs bacteria was carried out by applying both soaking and foliar methods,which were sub cultured on Met-AMS broth medium according to **Holland and Polacco (1994)** .

The layout of the experiments **(Fig. 1)** consisted of 16 treatments with 3 replicates in a complete randomized block design.

The recommended doses of NPK fertilizers were used as follows: super phosphate (15.5% $P_2O_5$) and potassium sulphate (48%$K_2O$).

N-Fertilization as Ammonium sulphat, (20.5% N) was applied at rates of 0, 75 and 250 kg $fed^{-1}$ in two equal spilt doses 3 and 4 weeks after planting. Plants were sampled after 75 days old to determine number (No.Nod. $plant^{-1}$) and dry weight of nodules (mg $plant^{-1}$), plant dry weight (g $plant^{-1}$) and plant nitrogen content (mg $plant^{-1}$).

Table 1. Mechanical and chemical analysis of soil

| Analysis | Values |
|---|---|
| Coarse sand % | 45.11 |
| Fine sand % | 40.03 |
| Silt % | 6.45 |
| Clay % | 8.41 |
| Texture | Sandy loam |
| Calcium carbonate % | 4.24 |
| Water holding capacity % | 16.81 |
| Saturation percentage (Sp) % | 22.33 |
| pH | 7.81 |
| E.C. ($dSm^{-1}$) | 1.40 |
| Organic matter % | 0.55 |
| Total nitrogen % | 0.011 |
| Soluble cations (meq $L^{-1}$) | |
| $Ca^{++}$ | 4.20 |
| $Mg^{++}$ | 2.31 |
| $Na^{+}$ | 3.99 |
| $K^{+}$ | 1.80 |
| Soluble anions (meq$L^{-1}$) | |
| $Co_3^{--}$ | 0.00 |
| $HCo_3^{--}$ | 1.0 |
| $Cl^{-}$ | 2.51 |
| $So_4^{--}$ | 8.79 |

**5. Field experiment**

Seeds of chickpea variety (Giza 195) at rate of 35 kg $fed^{-1}$ were drilled in rows at 20 cm apart.

Layout **(Fig. 2)** of 8 treatments with 3 replications in a complete randomized block design.

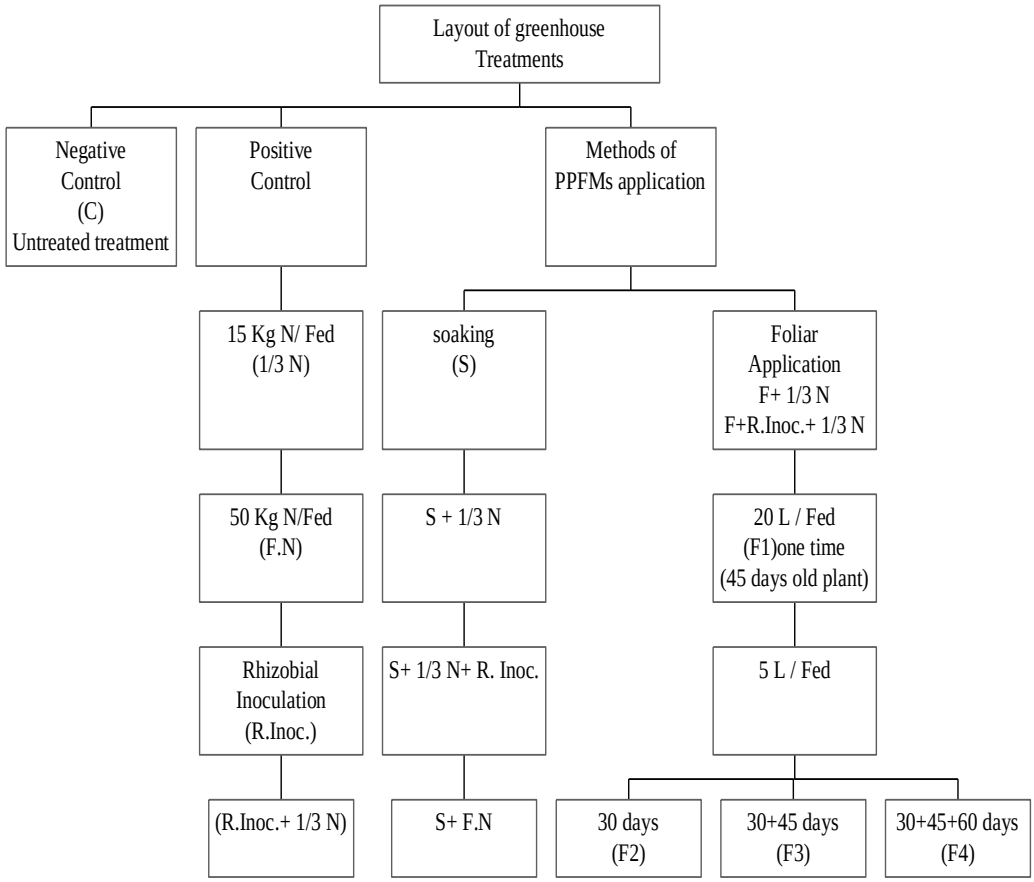


Fig 1. Layout of greenhouse experiments

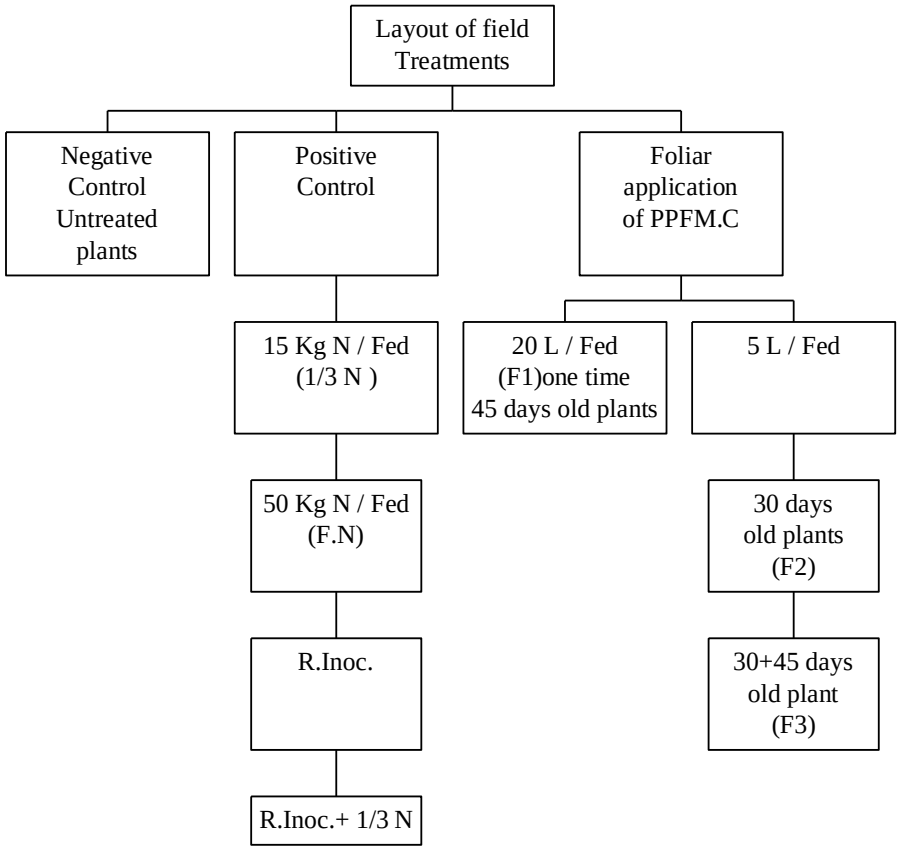


Fig 2. Layout of field experiment.

The recommended doses of P and K fertilizers:

100 Kg superphosphate (15.5% $P_2O_5$ $fed^{-1}$) and 50 Kg potassium sulphate (48% $K_2O$ $fed^{-1}$) were added to soil, during land preparation, N-fertilization as ammonium sulphate (20.5% N) was applied at the rates of 15 and 50 Kg N $fed^{-1}$ added in two equal spilt doses 3 and 4 weeks after planting. The seeds were inoculated with peat based inoculum containing rhizobial strains (ICARDA 31 and NIFTAL 1148) specific to chickpea.

Plants were sampled were taken at 60 days old plant to determine number (No. nod.$plant^{-1}$) and dry weight (mg $plant^{-1}$) of nodules, plant dry weight (g $plant^{-1}$) and plant nitrogen content (mg $plant^{-1}$). At harvest stage, total biological yield, seed yield (kg $plot^{-1}$ and kg $fed^{-1}$, nitrogen content (%) at seed and straw and seed index (g 100 $seeds^{-1}$) were determined.

### 6. Statistical analysis

Results were statistically analyzed by the least significant difference test (LSD) at P<0.05 by using MSTAT Microcomputer Statistical Program **(Steel and Torrie, 1980).**

## RESULTS AND DISCUSSION

### 1. Greenhouse experiments

### 1.1. Nodulation status

Data in **Table (2)** show the number of nodules and nodules dry weight, untreated treatment and fertilized treatments have no nodules formed. Rhizobial inoculation scored the lowest number and dry weight of nodules for Faba bean and chickpea (28, 15 and 120, 22 mg plant$^{-1}$, respectively). N-Fertilization at rational dose added with inoculation led to significant increases in both nodules number and nodules dry weight and such increases ranged between 54-40 % and 57-41 % in same order as compared to inoculated ones.

PPFMs inoculation used as soaking in presence of 15 Kg N Fed$^{-1}$ and rhizobial inoculation of two legume crops used gave significant increases in both number and dry weight of nodules as compared with rhizobial inoculation as such and ranged from 53-57% for nodules number and 55-63% for nodules dry weight.

Foliar application of PPFMs at rate 20L Fed$^{-1}$ one time (45 days old plant)recorded no significant increases as compared to inoculated treatment + 15 Kg N Fed$^{-1}$ and/or PPFMs soaking treatment in case of chick pea plant. In contrast, faba bean plants showed an positive trend.

Foliar inoculation with PPFMs at application rate 5L Fed$^{-1}$ in presence of 15 Kg N Fed$^{-1}$ and rhizobial inoculation emphasized the superiority of twice treatment at 30 and 45 days from planting against one or three times ones. Moreover, the above-mentioned treatment recorded the highest number of nodules (30-49 plant $^{-1}$) and nodules dry weight (45-220 mg plant$^{-1}$) for chickpea and faba bean, respectively.

Irrespective to the rates and times of applied foliar inoculation of PPFMs, soaking in PPFMs recorded higher values for both number and dry weight of nodules (44 and 196) against foliar application (42, 174) in case of faba bean plants. In case of chickpea plants foliar application exhibited a positive effect and recorded higher increase ranged from 10 to 13% against soaking treatment in the same two tested parameters.

These results could be attributed to that the ability of the inoculation with PPFMs strains as helper bacteria to release cytokinin, which play a role in increasing the nodulation of both plants.

These results are in agreement with **Truchet *et al* (1984); Holland (1997b); Joshi *et al* (2000) and Stougaard (2000)** who reported that PPFMs plays very important role in root nodule initiation, development and function of many legume plants ,*i.e.* alfa alfa and soybean.

### 1.2. Dry weight and N-content

Data presented in **Table (3)** show that high significant differences in plant dry weight and plant N-content were evident among treatments.

Table 2. Effect of PPFM bacterial strains (PPFM.F & PPFM.C),*Rhizobium* inoculation and N-fertilization on nodulation status of faba bean and chickpea plants grown in greenhouse (75 days old plant)

| Parameter / Treatment | Faba bean | | Chickpea | |
|---|---|---|---|---|
| | No .of nodules (plant$^{-1}$) | Dry weight of nodules (mg plant$^{-1}$) | No .of nodules (plant$^{-1}$) | Dry weight of nodules (mg plant$^{-1}$) |
| $T_1$C | 0 | 0 | 0 | 0 |
| $T_2$ 1/3N | 0 | 0 | 0 | 0 |
| $T_3$ F.N | 0 | 0 | 0 | 0 |
| $T_4$ R. Inoc. | 28 | 120 | 15 | 22 |
| $T_5$ R. Inoc. + 1/3N | 43 | 188 | 21 | 31 |
| $T_6$ S + 1/3N | 0 | 0 | 0 | 0 |
| $T_7$S+R.Inoc.+1/3N | 44 | 196 | 23 | 34 |
| $T_8$S+ F.N | 0 | 0 | 0 | 0 |
| $T_9$ F1+1/3N | 0 | 0 | 0 | 0 |
| $T_{10}$F1+R.Inoc.+1/3N | 39 | 139 | 24 | 37 |
| $T_{11}$F2+1/3N | 0 | 0 | 0 | 0 |
| $T_{12}$F2+R.Inoc.+1/3N | 39 | 146 | 23 | 28 |
| $T_{13}$ F3+1/3N | 0 | 0 | 0 | 0 |
| $T_{14}$F3+R.Inoc.+1/3N | 49 | 220 | 30 | 45 |
| $T_{15}$F4+1/3N | 0 | 0 | 0 | 0 |
| $T_{16}$F4+R.Inoc.+1/3N | 41 | 192 | 27 | 40 |
| L.S.D.$_{0.05}$ | 5 | 15.7 | 5 | 8.2 |

T: Treatment
C: Control (untreated treatment) F.N: Full Nitrogen (50 Kg N Fed$^{-1}$)
1/3N: 15 Kg N Fed$^{-1}$ R. Inoc.: Rhizobial inoculation
S: Soaking treatment
$F_1$: Foliar application 20L Fed$^{-1}$ PPFM at 45 days from planting
$F_2$ : Foliar application 5L Fed$^{-1}$ PPFM at 30 days from planting
$F_3$ : Foliar application 5L Fed$^{-1}$ PPFM at 30+45 days from planting
$F_4$ : Folar application 5L Fed$^{-1}$ PPFM at 30+45+60 days from planting

The untreated treatment recorded the lowest values of plant dry weight (5.20 and 0.45) and plant N-content (109 and 5.32 mg plant$^{-1}$) in faba bean and chick-pea plants respectively.

The percentage significant increases of plant dry weight and plant N-content among various treatments as compared to untreated ones also found in **Table (3)**.

Table 3. Effect of PPFM bacterial strains (PPFM.F &PPFM.C), *Rhizobium* inoculation and N-fertilization on plant dry weight, N-content and percentage of increases of faba bean and chickpea plants grown in greenhouse (75 days old plant)

| Parameter / Treatment | Faba bean | | | | Chickpea | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant dry weight | % increase | N content | % increase | Plant dry weight | % increase | N content | % increase |
| $T_1$C | 5.20 | - | 109 | - | 0.45 | - | 5.32 | - |
| $T_2$ 1/3N | 6.60 | 27 | 203 | 86 | 0.49 | 9 | 6.57 | 23 |
| $T_3$F.N | 14.50 | 179 | 415 | 281 | 1.04 | 13 | 18.63 | 250 |
| $T_4$R. Inoc. | 8.03 | 54 | 244 | 124 | 0.64 | 42 | 8.63 | 62 |
| $T_5$ R. Inoc. +1/3N | 10.50 | 102 | 284 | 160 | 0.67 | 49 | 9.11 | 71 |
| $T_6$S + 1/3N | 6.8 | 31 | 199 | 82 | 0.66 | 47 | 9.49 | 78 |
| $T_7$S+R.Inoc. +1/3N | 10.30 | 98 | 310 | 184 | 0.73 | 62 | 11.38 | 114 |
| $T_8$S+ F.N | 13.40 | 158 | 414 | 280 | 0.66 | 47 | 10.20 | 92 |
| $T_9$F1+1/3N | 8.33 | 60 | 259 | 138 | 0.76 | 69 | 12.24 | 130 |
| $T_{10}$F1+R.Inoc. +1/3N | 12.50 | 140 | 361 | 231 | 0.81 | 80 | 13.05 | 145 |
| $T_{11}$F2+1/3N | 8.37 | 61 | 299 | 174 | 0.81 | 80 | 13.30 | 150 |
| $T_{12}$F2+R.Inoc +1/3N | 14.00 | 169 | 412 | 278 | 0.82 | 82 | 14.30 | 169 |
| $T_{13}$ F3+1/3N | 12.80 | 146 | 388 | 256 | 0.86 | 91 | 15.02 | 182 |
| $T_{14}$F3+R.Inoc. + 1/3N | 13.70 | 163 | 411 | 277 | 0.87 | 93 | 15.10 | 184 |
| $T_{15}$F4+1/3N | 13.03 | 151 | 42.3 | 288 | 0.89 | 97 | 15.66 | 194 |
| $T_{16}$F4+R.Inoc. +1/3N | 14.90 | 187 | 487 | 347 | 1.10 | 144 | 19.14 | 260 |
| L.S.D.$_{0.05}$ | 1.44 | | 61.27 | | 0.24 | | 4.24 | |

T: Treatment
Plant dry weight: (g plant$^{-1}$). Plant N-content: (mg plant$^{-1}$).
% of increase $= T_x - T_1 / T_1 \times 100$ x: number of any treatment

A part from levels of N-fertilization, rates and times of applied foliar inoculation of PPFMs, data presented in **Table (4)** show that the mean plant dry weight of N-fertilized, soaking application of PPFMs and foliar application of faba bean and chick pea plants increased by 14, 10 and 31% for faba bean and 17, 3 and 32% for chickpea plants against rhizobial inoculated treatments. The corresponding percentages of plants N-content were 17,7 and 44% for faba bean and 42, 17 and 66% for chickpea plants in the same order. Moreover foliar application of PPFMs increased plant dry weight (20 and 28%) and plant N-content (23 and 42%) against soaking application treatments for faba bean and chickpea plants, respectively.

Under greenhouse conditions inoculation of faba bean and chickpea plants with respective PPFMs significantly improved nodule status, plant growth and plant N-content, especially when the foliar inoculation with both strains of PPFMs was used. Inoculation with rhizobial strains and foliar application of PPFM strains combined with reasonable dose of N-fertilizer gave the best results comparing to the other treatments, specially the inoculation with rhizobial strains to the same N-fertilizer treatment.

This result could be attributed to the role of the PPFM strains as helper bacteria in stimulating symbiosis system between *Rhizobium* spp. and the legume plants.

These data were in harmony with those obtained from different reports; **Corpe and Basile (1982)** who reported that PPFMs stimulate seed germination and plant development.

**Holland (1997a)** stated that, the activities of the PPFMs could make a biochemically measurable and physiologically meaningful contribution to plant metabolism. **Holland (1997b)** reported that, application of inoculation with PPFMs resulted increasing of plant dry weight of soybean plants as compared to untreated ones.

**Omer (2004)** revealed that PPFMs produced cytokinin and other phytohormones, which stimulate the plant growth and development.

Table 4. Increase of plant dry weight and plant N-content of faba bean and chickpea plants grown in greenhouse as affected by N- fertilization and PPFMs bacterial strains

| Parameter | Faba bean | | | | Chickpea | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant dry weight | | N-content | | Plant dry weight | | N-content | |
| Treatment | g plant$^{-1}$ | % | mg plant$^{-1}$ | % | g plant$^{-1}$ | % | mg plant$^{-1}$ | % |
| **R*.Inoc. | 9.27 | - | 264 | - | 0.66 | - | 8.87 | - |
| N-Fertilization | 10.55 | 14 | 309 | 17 | 0.77 | 17 | 12.60 | 42 |
| Soaking PPFMs | 10.17 | 10 | 308 | 17 | 0.68 | 3 | 10.36 | 17 |
| Foliar PPFMs | 12.19 | 31 | 380 | 44 | 0.87 | 32 | 14.73 | 66 |

* Rhizobial inoculation

## 2. Field experiment

### 2.1. Growth stage

#### 2.1.1. Number and dry weight of nodules

Data in **Table (5)** revealed that, the untreated treatment recorded the lowest nodules number and dry weight and these values were, 8 No plant$^{-1}$ and 19 mg plant$^{-1}$, respectively.

Application of N-fertilization achieved no significant differences in two tested parameters as compared to untreated ones.

Inoculation with specific *Rhizobium* scored higher significant increases as compared to the treatments fertilized with 15 kg N Fed$^{-1}$, in both nodules number (59-12) by 4 and nodules dry weight (252-25 mg plant$^{-1}$) by 9-folds.

No significant increases were found in case of foliar application with PPFM.C at rate 20 L. Fed$^{-1}$ (45 days from planting) in both nodules number and nodules dry weight as compared to inoculated plants with specific *Rhizobium* combined with 15 kg N Fed$^{-1}$.

Table 5. Nodulation status, plant dry matter and plant N-content of chickpea plants as affected by N-fertilization, Rhizobial inoculation and foliar inoculation of strain PPFM.C (60 days old plant) grown under field conditions

| Parameters / Treatments | No. of nodules (plant$^{-1}$) | Dry weight nodules (mg plant$^{-1}$) | Plant dry matter (g plant$^{-1}$) | Plant N-content (mg plant$^{-1}$) |
|---|---|---|---|---|
| Control | 8 | 19 | 1.80 | 22.51 |
| 15 kg N Fed$^{-1}$ | 12 | 25 | 2.70 | 57.93 |
| 50 kg N Fed$^{-1}$ | 7 | 13 | 3.86 | 80.29 |
| R. Inoc. | 59 | 252 | 3.13 | 64.58 |
| R. Inoc.+ 15 kg N Fed$^{-1}$ | 68 | 280 | 3.26 | 88.02 |
| $F_1$+R. Inoc.+15 kg N Fed$^{-1}$ | 89 | 300 | 3.93 | 91.63 |
| $F_2$+R. Inoc.+15 kg N Fed$^{-1}$ | 97 | 333 | 3.90 | 102.73 |
| $F_3$+R. Inoc.+ 15 kg N Fed$^{-1}$ | 121 | 401 | 4.30 | 109.95 |
| L.S.D.$_{0.05}$ | 27 | 73 | 0.34 | 10.91 |

C: Control (untreated treatment) R.Inoc.: Rhizobial inoculation
$F_1$: Foliar application of PPFM.C at rate 20L Fed$^{-1}$ (45 days from planting)
$F_2$ : Foliar application of PPFM at rate 5 L Fed$^{-1}$ (30 days from planting )
$F_3$ : Foliar application of PPFM at rate 5 L Fed$^{-1}$ (30+45 days from planting )

Application of PPFM.C at rate 5 L $Fed^{-1}$ (30 and 45 days old plant) caused significant difference for nodules number as compared to inoculated and fertilized chickpea plants.

Moreover, PPFM.C at rate 5 L $Fed^{-1}$ (30 and 45 days old plant) scored significant increases as compared to applied PPFM.C at rate 20 L $Fed^{-1}$ (45 days old plant); such increases were 36% (121-89) for nodule number and 34% (401-300 mg $plant^{-1}$), for nodules dry weight.

### 2.1.2. Dry matter and N-content

As shown in **Table (5)** inoculation of chickpea plants enhanced plant growth and increased their N-contents, however it showed significant positive variations as compared to fertilized plants with 15 Kg N $Fed^{-1}$.

Application of PPFM.C at rate 5 L $Fed^{-1}$ (30 and 45 days from planting) caused significant increases as compared to applied PPFM.C at rate 20 L $Fed^{-1}$ (45 days old plants) and such increases were 9% for plant dry matter and 20% for plant N-content. In conclusion the foliar application of the PPFM strain combined with the inoculation with *Rhizobium* sp. inoculation and soil amendment with 15 Kg N $Fed^{-1}$ (the reasonable dose) increased plant dry weight and nitrogen content of chickpea plants after 60 days compared to the same treatment without PPFM application. The bi-application of the helper bacteria (PPFM.C strain) after 30 and 45 days gave significant increase in plant dry weight and nitrogen content.

The above mentioned results are in agreement to **Corpe & Basile (1982); Truchet *et al* (1984); Holland (1997 a&b) and Omer (2004)** who reported that PPFM bacteria enhanced nodule formation, plant growth and development and play very important role in plant nitrogen content.

### 2.2. Harvest stage

**Table (6)** presents total biological yield and seed yield (Kg $plot^{-1}$ & Kg $Fed^{-1}$) seed index (g100 $seed^{-1}$), nitrogen and protein percentage for both straw and seed of chickpea crop of the various treatments. Application of 50 Kg N $Fed^{-1}$ treatment markedly recorded significant increases in total biological yield as compared to untreated and inoculated chickpea plants combined with or without 15 Kg N $Fed^{-1}$ and such increases were, 115, 97, 81 and 33% for untreated ones, rational dose (15 Kg N $Fed^{-1}$), inoculated plant and fertilized with 15KgN $Fed^{-1}$, respectively.

The corresponding percentages increases for seed yield Kg $plot^{-1}$ were 173, 99, 93 and 16% in the same order. Strain PPFM.C applied at rate 20L $Fed^{-1}$ supported exceptional seed yield (0.581 Kg $plot^{-1}$) for fertilized plant (50 Kg $Fed^{-1}$) and inoculated plants and fertilized with (15 Kg N $Fed^{-1}$) with respective increases of 20 and 39%, respectively.

The highest seed yield (0.617 Kg $plot^{-1}$) was obtained by PPFM.C at rate of 5 L $Fed^{-1}$ (30 and 45 days from planting) against 20L $Fed^{-1}$ (45 days old plants) for seed yield, (0.581Kg $plot^{-1}$).

In respect to the main effect of PPFM.C, different levels **(Table 6)** on seed yield (Kg $Fed^{-1}$), results that the seed yield (Kg $Fed^{-1}$) of chickpea plants increased obviously with increasing the applied different levels from PPFM.C.

The same trend was obtained at seed index (g100 $seed^{-1}$).

Table 6. Yield parameters of chickpea as affected by N-fertilization, rhizobial inoculation and foliar application of PPFM.C strain grown under field conditions

| Parameters / Treatments | Total biological yield | | Seed yield | | Seed index (g100 seed$^{-1}$) | Seed protein % | Straw N-content% |
|---|---|---|---|---|---|---|---|
| | Kg plot$^{-1}$ | Kg Fed$^{-1}$ | Kg plot$^{-1}$ | Kg Fed$^{-1}$ | | | |
| Control | 0.80 | 672 | 0.177 | 149 | 15.73 | 24.51 | 1.57 |
| 15 Kg N Fed$^{-1}$ | 0.870 | 731 | 0.243 | 204 | 18.60 | 25.92 | 1.71 |
| 50 Kg N Fed$^{-1}$ | 1.717 | 1442 | 0.484 | 407 | 20.10 | 26.19 | 1.83 |
| R.Inoc. | 0.950 | 798 | 0.251 | 211 | 17.87 | 26.19 | 1.88 |
| R.Inoc.+15 Kg N Fed$^{-1}$ | 1.295 | 1084 | 0.419 | 352 | 18.47 | 26.29 | 1.91 |
| $F_1$+ R.Inoc.+15 K NFed$^{-1}$ | 1.547 | 1299 | 0.581 | 488 | 19.50 | 26.22 | 1.94 |
| $F_2$+ R.Inoc.+15 Kg NFed$^{-1}$ | 1.800 | 1512 | 0.604 | 507 | 19.73 | 26.31 | 1.98 |
| $F_3$+ R.Inoc.+15 Kg NFed$^{-1}$ | 2.083 | 1750 | 0.617 | 518 | 20.13 | 26.35 | 2.01 |
| L.S.D$_{0.05}$ | 0.417 | 243 | 0.037 | 51 | n.s | - | - |

C: Control (untreated treatment)
R. Inoc.: Rhizobial inoculation
$F_1$ : Foliar application of PPFM.C at rate 20L Fed$^{-1}$ (45 days from planting)
$F_2$ : Foliar application of PPFM at rate 5 L Fed$^{-1}$ (30 days from planting)
$F_3$ : Foliar application of PPFM at rate 5 L Fed$^{-1}$ (30+45 d days from planting)

Moreover, the highest values (20.13 g 100 seed$^{-1}$) were obtained by PPFM.C at rate 5L Fed$^{-1}$ (30 and 45 days old plants).

Variations in percentage of seed protein content among the different treatments were comparable to those recorded with straw N-content. The lowest seed protein content and straw N-content (24.51 and 1.57%) were recorded for untreated plants. Increases up to 8 and 28% for seed protein content and straw N-content, respectively, were attributed to PPFM.C application.

The obtained data are in agreement to **Polacco and Holland (1994)** and **Holland (1997b)** they reported that the activities of PPFMs could make a biochemically measurable and physiologically meaning full contribution to plant nitrogen accumulation and metabolism.

Again, the highest yield and yield components of chickpea were obtained by intensive inoculation (*Rhizobium* and PPFM.C at rate 5 L Fed$^{-1,}$ 30 and 45 days from planting) in presence of 15 Kg N Fed$^{-1}$. In this respect, **Joshi *et al* (2000)** suggested that the use of PPFMs inocula as biofertilizer in soybean production in arid environments.

### 2.3. Economic turnover

The economic management of desert soil (South El Tahreer) requires complementation of intensive bacterial inoculation (*Rhizobium* + PPFM.C) together with adequate regime of N-fertilization. As shown in **Table (7)** inoculated plants and combined with 15 Kg N $Fed^{-1}$ recorded the lowest economic return (1711 L.E) and decreased by 9% as compared to fertilized plant with 50 Kg N $Fed^{-1}$. Irrespective to PPFM.C amount and times of application, PPFM.C led to increases in yield economic return and such increases ranged from 21 to 32% as compared to applied 50 Kg N $Fed^{-1}$ treatment.

Table 7. Economic turnover of chickpea yield as affected with various treatments

| Parameters / Treatments | Cost (L.E) | Yield (Kg $fed^{-1}$) | Economic return (L.E) | Increases (%) |
|---|---|---|---|---|
| fertilization 50 KgN$fed^{-1}$ | 150 | 407 | 1885 | - |
| R.Inoc. +15 Kg N $fed^{-1}$ | 49 | 352 | 1711 | -9 |
| $F_1$+R.Inoc. +15 Kg N $fed^{-1}$ | 149 | 488 | 2286 | +21 |
| $F_2$+ R.Inoc. + 15 Kg N $fed^{-1}$ | 74 | 507 | 2461 | +31 |
| $F_3$+ R.Inoc. + 15 Kg N $fed^{-1}$ | 99 | 518 | 2491 | +32 |

According to commercial market (2005):
N (Kg $fed^{-1}$) = 3.0 L.E
R. Inoc. = 4.0 L.E
PPFM.C $L^{-1}$ = 5.0 L.E
Ton (seed) of chickpea = 5000 L.E

From the present work it could be concluded that, pot and field experiments demonstrated that, under sandy loam soil conditions, necessity exists for inoculation with symbiotic $N_2$-fixing bacteria and PPFMs as plant growth promoting bacteria to maximize the development and yield production of tested legumes.

Under Egyptian field conditions, PPFMs did support nodulation, biological nitrogen fixation process, plant growth and plant yield component. The highest yield was obtained, when rhizobial inoculation, foliar PPFMs application and 15 kg N $fed^{-1}$ were applied. In economic terms, PPFMs demonstrated (2491 L.E) gains in seed yield productivity.

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# تأثير استخدام البكتريا المتغذية على الميثانول على النمو والحصاد ومكونات المحصول لنباتات الحمص والفول البلدى

# [40]

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أجريت تجربتى صوبة بمركز البحوث الزراعية (محطة بحوث الجيزة) وذلك باستخدام عزلتي PPFM.C (المعزولة من الحمص)، PPFM.F (المعزولة من الفول البلدى) و التى تم عزلها وتعريفها كسلالة . *Methylobacterium radiotolerans* و *M. mesophilicum* كما نشر فى بحث آخر وذلك بهدف دراسة العلاقة بين البكتريا المتغذية على الميثانول والنباتات البقولية الملقحة بالريزوبيا تحت ظروف الأراضى المصرية. أظهرت النتائج المتحصل عليها تفوق استجابة نباتات الحمص لاستخدام مزارع PPFM.C رشاَمقارنة باستجابة محصول الفول البلدي لمزارع PPFM.Fوذلك في أعداد و أوزان العقد البكتيرية والوزن الخضري الجاف للنباتات وأيضا المحتوي النيتروجيني. أجريت تجربة حقلية بموقع جنوب التحرير (أرض رملية طميية) بهدف دراسة تأثير استخدام الرش بعزلةPPFM.C والتلقيح بالريزوبيا المتخصصة في وجود التسميد النيتروجيني بمعدل 15 كجم نيتروجين/ فدان وذلك على التعقيد والنمو والمحتوى النيتروجينى وأيضا على الحصاد ومكونات المحصوللنباتات الحمص. و كانت أهم النتائج المتحصل عليها حدوث زيادة معنوية فى العائد الاقتصادى للحمص تتراوح بين21-31 % اذا ما قورنت باستخدام التسميد المعدنى بمعدل 50 كم نيتروجين /فدان. أظهر استخدام الرش بمزارع PPFM.C بمعدل 5 لتر / فدان في وجود التلقيح بالريزوبيا المتخصصة والتسميد المعدني بمعدل 15 كجم نيتروجين/ فدان الى تعظيم الناتج المحصولي (518 كجم / فدان) وزيادة العائد الاقتصادي للحمص ( 2491 ج.م) و تقليل التلوث الناتج عن استخدام التسميد المعدني في الزراعة المستدامة.

**تحكيم:** ا.د مجدي إسماعيل مصطفي ا.د فكري محمد غزال