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# Field Application of Bio-Fertilizers Technology on Faba Bean Growth and Yield.

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**ABSTRACT**

In the last three years different areas of Egypt are rapidly being planted with faba beans *(Vicia faba)* to increase the production of this economically important legume. Understanding the impact of inoculant rhizobia in supplying sufficient nodulation is crucial to optimising N2 fixation by legume crops. Since indigenous populations of rhizobia is absent or low in number in such soil, the objective of the current study was to determine whether inoculation with several strains of *Rhizobium leguminosarum bv. viceae* could successfully increase the growth and production of faba bean. Field experiments were carried out in twenty seven different sites at 6 provinces of three governorates two each; Al-Behaira (8 sites), Al-Daqahlia (11sites), Al-Sharkia (8 sites) Governorates, Egypt in 2014/2015 season. The field experiments consist of three main treatments as follows in each studied site: (a) Un-inoculated un-fertilized treatment (b) Un-inoculated and fertilized with full dose of N (60kg/ acre) as a positive control treatment. (c) Inoculated with *Rhizobium leguminosarum bv. viciae* treatment. The growth of faba beans was increased by inoculation with rhizobia, to a much greater extent than can be expected. Inoculation increased nodule number, shoot dry weight and increased N2 fixation which increased shoot N content from 15.46 to 30.15 kg per acre. On the other hand, nodules dry weight was increased from 25.2 g/plant in the un-inoculated field sites to 65.11 g/plant in the inoculated field sites. Effective inoculation also increased the grain yield, number of pods, seed N content and the weight of 100 seeds. There were no significant differences between the inoculated un-fertilized treatment and the un-inoculated but fertilized with 60 Kg N/acre in their effects on the studied parameters. Results showed that the inoculated rhizobial strains were more competitors to indigenous rhizobia since it occupies the most of the formed nodules. The introduced *Rhizobium leguminosarum bv. viceae* local strain NRC 21 was the most effective of the rhizobial strains used as inoculants. The impact of inoculation strongly influenced grain yield, grain N content and the potential contributions of legume cropping to soil fertility.

**Keywords:** Biofertilizers, Faba bean, *Rhizobium leguminosarum bv. Viciae*, Competition

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## INTRODUCTION

Populations of soil rhizobia often vary considerably in their abundance and effectiveness in nodulating and fixing atmospheric nitrogen (N2) in symbiotic association with their legume host [1, 2]. There is increasing evidence that genetic exchange among rhizobia and other soil microbes has led to populations that vary in their symbiotic effectiveness [3, 4]. Since 50 years ago, Egypt produced high quality effective rhizobial inoculants for use with pasture and crop legumes. Despite this, the extent of inoculation of commercial legume crops with rhizobia is too low, with only 0.2 % of field crops.

Faba bean is one of the most effective nitrogen fixing legumes relative to other crop legumes under Egyptian field conditions; the estimated average amount of atmospheric nitrogen fixed by this crop is 135 Kg $ha^{-1}$. Faba bean serves as an important source of protein for humans, especially for those with low-income. In the four last decades, the area under faba bean decreased considerably due to the competition from other winter crops mainly wheat crop and clover forage. Bio-fertilizers are very important for increasing crop productivity all over the world, in particularly for developing countries with a mainly low input agricultural system of production where chemical fertilizers, if available, may not be affordable [5]. To not only mitigate chemical fertilizers inputs and elevate soil fertility but also to enhance crop production, several studies on bio-fertilizer applications have been conducted [6, 7, 8, 9, 10, 11, 12]. It was found that inoculation of faba bean has been increased the shoot and root dry weights, number of nodules per plant, as well as yield and yield components [5, 13, 14, 15].

Increasing crop production is one of the major targets of agricultural policy in Egypt, which could be achieved by several ways from which the application of potential bio-fertilizers technology. Therefore, the present study aimed to investigate the potentiality of the large application of bio-fertilizers technology on growth and yield of faba bean in three different Governorates of Egypt. This study has also considered the effects of the recommendation additions of fertilizer nitrogen (60 Kg N/acre) as a comparison treatment on the growth, seed yield and nitrogen content of field beans.

## MATERIALS AND METHODS

### Growth of cultures and seed coating

Four efficient *Rhizobium leguminosarum* bv. *viciae* strains (NRC 21, NRC 73, NRC 93 and TAL 1400) characterized by their salt-tolerant (up to 6.5 % NaCl), and high temperature-resistant (up to 50$^{o}$C) were selected on the basis of the previous experiments that carried out at National Research Centre. These strains were grown on YMA medium in incubator shaker at 28 °C to early stationary phase. Then the culture was washed twice using sterile water to remove the YMA medium components that may affect the nodulation of the plants, before the planting date. After the second wash, the cells were suspended in sterile water with a final density of 2 x $10^{8}$ cells $mL^{-1}$. Peat moss was used as a carrier of rhizobial inoculants. The weight of peat moss package *Rhizobium* was 500 g and contained at least $10^{9}$ viable cells of *Rhizobium* per gram of carrier. Seeds were coated with inoculum strains, directly before planting using 40% Arabic gum as sticker, to be ready for planting. Un-inoculated control was prepared with sterile water.

### Site description

Field experiments were carried out in twenty seven different sites at 6 provinces of three governorates two each; Al-Behaira (8 sites), Al-Daqahlia (11sites), Al-Sharkia (8 sites) Governorates, Egypt in 2014/2015 season. These sites were varied one from another in their soil physico-chemical properties.

### Treatments

Super-phosphate and potassium sulfate, at the rate of 200 and 150 kg /acre respectively were incorporated into the soil before planting, in all treatments. A complete randomized block design was used with three replicates. The seeds were planted in the field on October, 2014, first by sowing the un-inoculated control plot and then the inoculated treatment plots. After one week, plants were fertilized once with nitrogen as enhancement dose (15 kg N/ acre) in bio-fertilizer plots, while plants in positive control plots were fertilized with 60 kg N/ acre at three times one month interval. The other agricultural practices commonly used for faba

bean were followed. Plots were irrigated as required and weeded by hand. The field experiments consist of three main treatments as follows in each studied site:

(*a*) Un-inoculated un-fertilized treatment
(*b*) Un-inoculated and fertilized with full dose of N (60kg/ acre) as a positive control treatment.
(c) Inoculated with *Rhizobium leguminosarum* bv. *viciae* treatment

**Faba Bean (*Vicia faba*) Seeds**

Faba bean (*Vicia faba* L.) cultivar Al-Nobaraia2 for the province of Al-Nobaraia region and Giza 843 for the other different provinces were acquired from seeds production unit, Agriculture Research Centre, Egypt.

**Plant sampling**

The plant samples were taken in two stages throughout the experiment in function of growth and development of faba bean. (*i*) At middle of flowering stage, nodules dry weight, shoot dry weight; shoot nitrogen content and nodule serotyping were determined by harvesting in 1m×1m of each plot. (*ii*) At harvesting stage, number of pods, seed yield, total nitrogen in seeds, and weight of hundred seeds were determined.

**Nodule serotyping**

Strain specific fluorescent antibodies (FAs) were used for nodule serotyping. Gelatin-Rhodamine conjugate [16] was used to control non-specific staining and auto fluorescence. Fifty nodules from each treatment were carefully washed and crushed in sterilized water. Smear of sterile crushed nodule was put on the slides then these slides were air dried and heat fixed. With a Pasteur pipette, a drop of gelatin-Rhodamine conjugate was placed on the smears. Before the Rhodamine gel was dried, one drop of FA stain was added. Stained nodule smears were examined with a Zeiss universal microscope equipped for epifluorescence and phase contrast. A strong positive reaction was indicated by brilliant yellow green fluorescence of the smear on a dark purple background. No cells would be visible if the specific strain was not present on the smear. The presence of more than one serogroup per nodule was detected by using the dual lighting system of reflected fluorescent and transmitted phase-contrast light. The switching from phase-contrast to fluorescent light clearly shows the presence of one or more than one strain in the same nodule. The dominance of one strain over the other within the same nodule was based on the ratio between the stained and non-stained cells with each FA. Nodule smears with 5% or more of non-fluorescing cells in the presence of FA-positive cells were considered evidence of a mixed infection.

**Statistical analysis of data**

Yield data from each field harvest and the dry weight of plants in the greenhouse were analyzed statistically using software of SPSS (version 16.0). The least significant difference (LSD) was performed at 5% probability to evaluate the significant differences among the experimental treatments.

## RESULTS AND DISCUSSION

***Nodules dry weight:*** Response of faba bean to inoculation with *R. legurninosarum* bv. *vicaea* in the three governorates under study is given in Table 1. A highly significant response in terms of nodule dry weight was obtained in the inoculated treatments in all governorates as compared to the un-inoculated and the un-inoculated and N-fertilized treatments. Al Daqahliyah governorate gave the highest insignificant nodules dry weight compared with the two other governorates. The inoculation response with effective rhizobial strains would be expected where the native soil rhizobia are insufficient number to nodulate the host adequately and/ or the introduced effective strains were more competitors as compared to the native rhizobial populations [7, 17]. This finding clearly indicates that the infectivity of the introduced strains were greatly higher than those of native rhizobial strains. It was also found that inoculation of Rhizobium increased significantly the nodulation of faba bean [18].

***Shoot dry weight:*** Because of the nodulation success by the introduced rhizobial strains, the inoculation with effective rhizobial strains increased shoot dry weight and shoot N- content of faba plants as did un-inoculated N fertilized plants in all studied governorates. These results proved that we could reach the same shoot dry weight by rhizobial inoculation without any need to add the full dose of N-fertilizers except the initial dose which means saving 75 % of plant N-requirements. In other studies it was found that inoculation of *Rhizobium* increased significantly the plant growth and nodulation of faba bean [18, 19, 20].

***Shoot N-content:*** As a result of the nodulation success (Table 1) the maximum amount of N-fixed and subsequent the shoot N content was found to be the same more or less but insignificant in the inoculated plants and in N-fertilized plants. Shoot dry weight increased in response to inoculation. Previous studies have identified that there are often strong relationships between shoot dry weight and the amount of N2 fixed [21, 22]. Although, the un-inoculated N-fertilized faba bean plants formed a considerable amount of nodules, these nodules fixed the lowest amount of atmospheric nitrogen as compared with the inoculated treatments. This means that the introduced rhizobial strains can fix a considerable amount of atmospheric nitrogen equal or more than the added nitrogen fertilizer. Increasing the number of rhizobia in the rhizosphere of faba bean seed improved N2 fixation, due to increased nodulation. This effect attenuated when the number of nodules was sufficiently high that N2 fixation was no longer limited by nodule mass. It was also found that inoculation with *Rhizobium* significantly increased some of the growth indices such as total N content [19, 20, 23].

**Table 1: Effect of rhizobial inoculation on nodule dry weight, shoot dry weight and shoot N-content, of faba bean grown in different sites of three Governorates.**

| Treatment | Nodule dry weight ( g / plant) | Shoot dry weight ( Kg / acre ) | Shoot nitrogen content ( Kg / acre ) |
|---|---|---|---|
| Al Buhayrah Governorate ( 8 sites) | | | |
| Un-inoculated | 22.025 | 604.4 | 13.30 |
| Un-inoculated and N-fertilized | 25.342 | 1105.8 | 28.75 |
| Inoculated with Rhizobium | 57.275 | 1076.7 | 29.08 |
| Al Daqahliyah Governorate ( 11 sites) | | | |
| Un-inoculated | 24.917 | 800.9 | 16.98 |
| Un-inoculated and N-fertilized | 26.178 | 1192.7 | 31.02 |
| Inoculated with Rhizobium | 70.542 | 1170.5 | 31.61 |
| Al Sharqiyah Governorate ( 8 sites) | | | |
| Un-inoculated | 28.657 | 749.8 | 16.12 |
| Un-inoculated and N-fertilized | 27.245 | 1177.4 | 31.79 |
| Inoculated with Rhizobium | 67.504 | 1144.7 | 29.76 |
| Mean | | | |
| Un-inoculated | 25.200 | 718.367 | 15.467 |
| Un-inoculated and N-fertilized | 26.255 | 1157.633 | 30.520 |
| Inoculated with Rhizobium | 65.107 | 1130.700 | 30.150 |
| L.S.D at 5 % | 18.324 | 159.700 | 11.240 |

***Number of pods:*** The inoculated and un-inoculated and N-fertilized treatments produced a significant effect on number of pods per plants. This increased of pod number from an average of about 16 per plant in the un-inoculated treatments to 19.5 (P = 0.05).

***Seed yield:*** The positive inoculation response was accompanied by significant increases in seed yields compared with un-inoculated plants in all governorates. This represented approximately $USA 200 income per acre for a product investment of just $USA 2 per acre for inoculant, demonstrating the value of inoculation where soil rhizobia are inadequate. The un-inoculated and N-fertilized treatment recorded an increase in seed yield as in case of inoculated treatments which means that the use of full dose of N-fertilizer can be replaced by inoculation with effective strains of rhizobia. Seed yields were increased by 28.3 – 37.1 % in Al Buhayrah governorate, 15.7 – 24.0 in Al Daqahliyah governorate and 33.3 – 38.9 % in Al Sharqiyah governorate. Inoculation with *Rhizobium* increased the seed yield of faba bean in six areas in Australia [8] and in Sudan [23]. Seed inoculation of some native *Rhizobium leguminosarum* bv. *viciae* on faba bean in the south of Iran indicated that seed yield increased from 35% to 69% due to the inoculation [19]. However, Moawad et al. [24] observed responses to N fertilization and rhizobial inoculation with all three winter legumes (berseem clover,

bean and lentil) which indicates inefficient nitrogen fixation by native rhizobia. This was mainly attributed to the lack of rhizobia specific to these crops in Egyptian soils, especially in new reclaimed and salt affected soils (as in Al Kantara east and Gelbana at Sinai) and / or to ineffectiveness of rhizobial inoculation.

***Total nitrogen in seeds:*** In general inoculated treatments averaged 2 % more nitrogen than those relying upon un-inoculated N fertilized treatment but differences were not significant. These results clearly show that the effective inoculation improved the N2 fixation achieved by faba bean. This means that N2 fixation in faba bean seeds can be improved even in the presence of a large soil rhizobia population if sufficient inoculant rhizobia can be applied. The response to inoculation will also depend on the relative effectiveness of soil and inoculant rhizobia, the rate of survival of the inoculants and the positional advantage of inoculant rhizobia [25]. An additional advantage of inoculation is the presence of a large numbers of rhizobia that they may allow nodulation even at relatively high soil N availability [26, 27].

***100 seed weight:*** Table 2 showed that the average 100 seed weight over all treatments was 51.4g. In general differences between treatments were small, but inoculation and un-inoculated and N-fertilized treatments increase significantly the 100 seed weight as compared to the un-inoculated treatments (P = 0.05). It was found that inoculation of faba bean with indigenous *Rhizobium leguminosarum* bv. *viciae* in the south and north of Iran increased significantly the seed yield [19, 20]. Inoculation with *Rhizobium* increased also the biological yield and weight of hundred seeds in Sudan [23].

The current study confirms previous reports that the relationship between a legume and its rhizobial symbiont enhances growth of the host to a great extent. Overall, the benefits in improving nodulation were realised through increased yield, N2 fixation, and in the net contribution of fixed N to soil fertility.

**Table 2: Effect of rhizobial inoculation on number of pods, seed yield, total nitrogen in seeds and weight of 100 seeds of faba bean grown in different sites of three Governorates.**

| Treatment | No. of pods per plant | Seed yield ( Kg / acre ) | Total nitrogen in seeds ( Kg / acre ) | 100 seeds weight (g) |
|---|---|---|---|---|
| Al Buhayrah Governorate ( 8 sites) | | | | |
| Un-inoculated | 13.8 | 1453 | 56.66 | 48.4 |
| Un-inoculated and N-fertilized | 18.3 | 1864 | 80.52 | 55.6 |
| Inoculated with Rhizobium | 17.8 | 1992 | 84.85 | 53.7 |
| Al Daqahliyah Governorate ( 11 sites) | | | | |
| Un-inoculated | 18.2 | 1706 | 66.87 | 49.2 |
| Un-inoculated and N-fertilized | 22.8 | 1973 | 86.81 | 52.7 |
| Inoculated with Rhizobium | 21.4 | 2116 | 89.51 | 53.4 |
| Al Sharqiyah Governorate ( 8 sites) | | | | |
| Un-inoculated | 15.5 | 1359 | 53.68 | 47.6 |
| Un-inoculated and N-fertilized | 19.4 | 1888 | 84.96 | 51.3 |
| Inoculated with Rhizobium | 20.6 | 1812 | 82.91 | 50.8 |
| Mean | | | | |
| Un-inoculated | 15.833 | 1506 | 59.07 | 48.4 |
| Un-inoculated and N-fertilized | 20.167 | 1908 | 84.09 | 53.2 |
| Inoculated with Rhizobium | 19.933 | 1973 | 85.76 | 52.6 |
| L.S.D at 5 % | 2.05 | 152 | 16,82 | 3.1 |

***The competition pattern:*** The competition of introduced and native rhizobia for nodulation faba bean plants is shown in Table (3). The inoculant strains occupied most of nodules formed on faba bean inoculated plants in the three different governorates. The introduced rhizobial strains occupied 95 - 98 % of total nodules formed on inoculated plants versus 3 - 5 % for native rhizobia in different governorates. The results also show that the competitive ability of inoculant strains differed within different governorates. Strain NRC 73 was a highly competitive strain and occupied 62 – 63 % of total formed nodules in different governorates. Whereas, strains NRC 21 and NRC 93 were less competitive and occupied 12 – 14 % for each of total nodules and foreign strain TAL 1400, however, was the least competitor and occupied 7 - 8 % of total nodules in the different governorates. This could be due to the better performance of strain NRC 73 under Egyptian soils conditions

and recommended for faba bean grown under harsh conditions of dryness and salinity. The same finding was achieved previously by Moawad et al. [24] and Al Barakah et al. [28].

**Table 3: Competition between introduced and native rhizobial strains for nodulating faba bean plants in three different governorates.**

| Treatments | % Nodule occupancy | | | | | |
|---|---|---|---|---|---|---|
| | Inoculant strains | | | | | Indigenous strains |
| | NRC 21 | NRC 73 | NRC 93 | TAL 1400 | Total | |
| Al Buhayrah Governorate ( 8 sites) | | | | | | |
| Un-inoculated | 7 | 9 | 5 | 2 | 23 | 77 |
| Un-inoculated and N-fertilized | 5 | 7 | 4 | 2 | 18 | 82 |
| Inoculated with Rhizobium | 12 | 63 | 13 | 7 | 95 | 5 |
| Al Daqahliyah Governorate ( 11 sites) | | | | | | |
| Un-inoculated | 8 | 9 | 8 | 4 | 29 | 71 |
| Un-inoculated and N-fertilized | 5 | 7 | 6 | 6 | 25 | 75 |
| Inoculated with Rhizobium | 13 | 62 | 13 | 7 | 95 | 5 |
| Al Sharqiyah Governorate ( 8 sites) | | | | | | |
| Un-inoculated | 7 | 6 | 7 | 3 | 23 | 77 |
| Un-inoculated and N-fertilized | 6 | 5 | 4 | 4 | 19 | 81 |
| Inoculated with Rhizobium | 14 | 63 | 13 | 8 | 98 | 2 |

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