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# Estimate Growth, Yield and Pods Quality of Yard Long Bean (*Vigna unguiculata var. sesquipedalis*) by Using Microbial Inoculation under Egypt Condition

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**Abstract:** This experiment was carried out under plastic house without cover during summer seasons of 2015 and 2016 at the Faculty of Agriculture, Cairo University, to study the effect of some microbial inoculation , i.e. Rhizobin (Rhizobium Cowpea strain), Nitrobin is a mixture of non-symbiotic N-fixing bacteria (*Azotobacter* and *Azospirillum*) and Effective Microorganisms (EM) commercial biofertilizer contains many useful microorganisms (photosynthetic bacteria, lactic acid bacteria and yeast) as a soil application and the combination between them compared with the control ( untreated ) on vegetative growth parameters, total yield and its components, pod quality and some associated characterize of Yard long bean (*Vigna unguiculata var. sesquipedalis*) cv. Hotyan Pangon from Chines Academy of Agriculture Sciences (CAAS). Results show that Yard long bean plants inoculated with Rhizobium, Nitrobin and EM significantly increased the plant height in the first season. Plants inoculated with Rhizobium +EM and EM produced highest pod yield followed by inoculated with Rhizobium and Nitrobin in the first season. Plants inoculated with Rhizobium, Nitrobin, EM or Rhizobium +EM and Nitrobin +EM had a significantly increased pod chlorophyll content compared to the untreated plant. It is recommended to add EM because of their role in stimulating plant growth in addition to the inoculation with effective Rhizobium strains .The result may be to be a step to reduce dependence on nitrogen fertilization.

**Key words:** Yard long bean · *Vigna unguiculata* var. *sesquipedalis* · Microbial inoculation · Rhizobium · Nitrobin · Effective Microorganisms (EM)

## INTRODUCTION

Yard long bean is an annual plant and there are two types short and climb plant, grown in the tropical areas and the most vegetable like Cowpea, climbing type often reaches heights of 180 to 360 cm the pods grow to 30 to 60 cm long [1]. The pods harvest at maximum length but are still smooth before maturation the seeds, pods grown from flower to marketable length in about 9-14 days, harvest often begin about 70 days after planting. At this stage pods be snapped to use a green vegetable [2]. Yard long bean grown under wide range of climatic conditions and prefer high temperature condition under which green beans cannot be produced. Yard long bean are grown on pillars to prevent pods from touch the soil and pods grow straight. Yard long bean is called many names, asparagus bean, snake bean and Chinese long bean.it has exposed leaves and flowers white to purple. Yard long bean pods are common vegetable in Southeast Asia, China, Philippines and the Caribbean; edible pods production in china excess of 1000, 000 ton [1]. The tropical leguminous plants are distinguished in their ability for N-fixing in close cooperation with Rhizobium which play significant role in biological nitrogen fixation (BNF) [3, 4]. In general the legume family is rich in many natural compounds that contribute to human nutrition and health such as flavonoids, proteins, mineral nutrients , oils, antioxidant, folic acid and vitamin C [5]. Yard long bean is rich in protein, calcium, iron, riboflavin, phosphorus, potassium, magnesium, manganese, vitamin A and vitamin C [6- 8]. Yard long bean fixes almost 240 kg/hec. N and a large part of fixed N will be available for other crops in the soil [9]. Rhizobia convert atmospheric N to protein in legumes which are important in human nutrition [3, 10]. Nitrogen

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deficiency leads to dwarf plants, yellowing of leaves and lack of photosynthesis [11]. Excessive nitrogen fertilization leads to nitrate leaks into ground water and negatively effects on the environment [12, 13]. The amount of nitrogen required for cultivation is likely to increase, resulting in serious environmental problems [14]. On the whole, a large percentage of fertilizer is lost and the plant absorbs only a small percentage, thus microbial inoculation supports agricultural production and preserves the environment [15]. Biofertilization many provide a solution to the problem of soil fertility and improve crop productivity [16]. Bacterial inoculation with highly efficient strains has many advantages, such as the do not need to repeat fertilization and increase the yield of pods [17].

Effective microorganisms (EM) are commercial biofertilizer contains many useful microorganisms collected from the environment and this mixture improves the yield by producing some bioactive hormones and enzymes that stimulate plant growth and work on analysis of organic matter and resistance of the molds [18]. Biofertilization makes soil rich in various types of microorganisms that produce antibodies and decompose organic matter [19].

Azotobacter and Azospirillum are the most important non-symbiotic bacteria of atmospheric nitrogen fixation and provide nitrogen for plants absorbable [20]. Azotobacter plays important role in nitrogen fixation, produce vitamins and hormones such as indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) [21]. Efficient strains of Azotobacter, Azospirillum and Rhizobacter serve with a significant amount of nitrogen to Rice and *Heliathus annus* and increase the plant growth [22, 23]. Azospirillum free-living mobile bacteria promote the plant growth and the crop yields [24]. However, little studies made to evaluate the effect of microbial inoculation on Yard long Bean under arid land. Hence, the objective of this study was to evaluate the response of Yard long Bean to microbial inoculation on the growth, dry matter accumulation, green pod yield, pod quality P, K, protein, carbohydrate, chlorophyll, carotenoids in pods and some associated characterize .

## MATERIALS AND METHODS

This experiment was carried out under plastic house without cover during summer seasons of 2015 and 2016 at the Faculty of Agriculture, Cairo University, to study the effect of some microbial inoculation, i.e. Rhizobin (Rhizobium Cowpea strain), Nitrobin is a mixture of non-symbiotic N-fixing bacteria (Azotobacter and Azospirillum) and Effective Microorganisms (EM) commercial biofertilizer contains many useful microorganisms (photosynthetic bacteria, lactic acid bacteria and yeast) as a soil application and the combination between them compared with the control (untreated) on vegetative growth parameters, total yield and its components, pod quality and some associated characterize of Yard long bean (*Vigna unguiculata var. sesquipedalis*) cv. Hotyan Pangon from Chines Acadmy of Agriculture Sciences (CAAS). Seeds of yard long bean were sown in 13$^{th}$ and 15$^{th}$ march in 2015 and 2016 seasons respectively. Seeds were sown in hills on two sides of each row and spacing was 15 cm between the seeds. The inoculation process was made a side dressing beside the seed at the sown. Rhizobin, nitrobin and EM were obtained from Budget Fund, Ministry of Agriculture and Reclamation, Egypt.

This experiment included six treatments as follow.

- Inoculation with *Rhizobium leguminosarum* cowpea strain (R).
- Inoculation with *Rhizobium leguminosarum* cowpea strain +Effective Microorganisms (EM) 0.2% (R + EM).
- Inoculation with Nitrobin (*Azotobacter* and *Azospirillum*) (N).
- Inoculation with Nitrobin + EM 0.2% (N+EM).
- EM 0.2%.
- Control (untreated) did not receive any of the nitrogen sources.

All microorganisms were added at sowing, but EM repeated three times during the growing period of Yard long bean at 30, 40 and 50 days after sowing and the phosphorus as super-phosphate was added during soil preparation before planting at the rate of 300 Kg/fed.

These treatments were arranged in a complete randomized block design (RCBD) with three replicates. The area of each plot was 5m$^2$.

**The Following Data Were Recorded:** Vegetative growth: Five plants were taken at 60 days after sowing, from each experimental plot for measuring plant growth characters. Plant height from the soil surface to the highest point of the plant (cm) and total chlorophyll content in newest fully expanded leaves at 60 days was measured as SPAD unit (SPAD - 502).

**Yield and its Components:** Yard long bean was start harvest at 65 days from planting. Yard long bean were picked for estimation of yield parameters:

Pod weight /plant (g) and total yield/plant.
Pods quality characteristics:
Physical properties:

Pod length (cm), pod diameter (mm) and pod T.S.S (total soluble solids) was measured using the digital refractometer

Pods chemical composition:

Dry matter percentage (%), Total chlorophyll content, total carotenoids were determined by using N.N. Dimethyl formamide according to Narnia [25]. Total carbohydrates percentage (%) was determined according to the method described by Dubois *et al*. [26]. Potassium percentage, phosphorus percentage and protein percentage in pods: it was calculated by multiplying the total nitrogen by the factor 6.25, it was determined according to the method described in AOAC [27]. (Carbohydrates, potassium, phosphorus and protein were estimated in the second season only).

Growth formulas: The following growth parameters were calculated at harvest:

Biological yield (W) = Total plant dry matter at harvest.
Economic yield (EY) = Yield of economic part of the plant.
Harvest index (HI) = (EY/W) ×100.

**Statistical Analysis:** Data in the two seasons were subjected to statistical analysis as described by Snedecor and Cochran [28].Treatment mean differences were compared using LSD test to evaluate the significant differences of the data at $p \geq 0.05$.

## RESULTS AND DISCUSSION

**Vegetative Growth:** Data in Table 1 showed that there are significant differences between treatments. Yard long bean plants inoculated with Rhizobium, Nitrobin and EM significantly increased the plant height in the first season, while the plants inoculated with Rhizobium had the highest plant height in the second season. Plants inoculated with Nitrobin had the lowest plant height in the second season and there are slightly significant differences between other treatments in the second season. There were no significant differences between treatments in chlorophyll reading (SPAD) in leaves.

Data in Table 2 showed that Yard long bean plants treated with Rhizobium had the highest pod weight meanwhile; there are slightly significant differences between other treatments especially in the second season. Pods yield per plant, showed that plants treated with Rhizobium +EM and EM produced highest pod yield followed by treatments Rhizobium and Nitrobin in the first season while, there were no significant differences between treatments in the second season.

**Pod Quality:** Data in Table 3 showed that the treatments had a little significant effect on pod length, pod diameter and T.S.S, since most treatments were shared in the same letters. Concerning to dry matter there were no significant differences between all treatments in the pod dry matter content.

Data in Table 4 showed that Yard long bean plants inoculated with Rhizobium, Nitrobin, EM or Rhizobium +EM and Nitrobin +EM had a significantly increased pod chlorophyll content compared to the untreated plant. There are significant increases in carotenoids content in all treatments except untreated plants and plants inoculated with Rhizobium +EM.

Data in Table 5 showed that Yard long bean plants inoculated with EM had the highest significant increase in the protein and phosphorus in the pods while, the plants inoculated with Rhizobium + EM had the highest potassium content. Untreated plants had the highest carbohydrate.

Data in Table 6 showed that there are significant differences between treatments. Yard long bean plants inoculated with EM and untreated plants had the highest Biological yield in the two seasons while, there are no significant differences between other treatments in the first season and slightly significant differences in the second season. Plants treated with EM had the highest Economic yield in the first season while, untreated plants had the lowest value .There are no significant differences between treatments in the second season. Yard long bean plants inoculated with Rhizobium + EM and Nitrobin had the highest significant increase in the Harvest index (HI) in the first season and Rhizobium + EM and Nitrobin+ EM in the second season. While, untreated plants had the lowest Harvest index value in the first season.

Table 1: Effect of microbial inoculation on vegetative growth of Yard long bean plants during summer seasons of 2015 and 2016

| | Season 2015 | | Season 2016 | |
|---|---|---|---|---|
| Treatments | Plant height(cm) | Chlorophyll (SPAD) | Plant height (cm) | Chlorophyll (SPAD) |
| Inoculation with Rhizobium (R) | 248.30 | 75.73 | 247.00 | 72.20 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 212.00 | 64.73 | 219.00 | 69.10 |
| Inoculation with Nitrobin (N) | 242.00 | 60.13 | 203.00 | 63.90 |
| Inoculation with Nitrobin + EM 0.2 % (N+EM) | 215.00 | 69.90 | 207.00 | 69.30 |
| EM 0.2% | 235.00 | 70.20 | 226.00 | 71.53 |
| Control (untreated) | 216.30 | 59.80 | 231.00 | 57.50 |
| LSD 0.05 | 12.53 | N.S | 14.43 | N.S |

Table 2: Effect of microbial inoculation on yield of Yard long bean plants during summer seasons of 2015 and 2016

| | Season 2015 | | Season 2016 | |
|---|---|---|---|---|
| Treatments | Pod weight (g) | Pod yield/plant (g) | Pod weight (g) | Pod yield /plant (g) |
| Inoculation with Rhizobium (R) | 15.23 | 79.17 | 15.81 | 97.29 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 12.85 | 90.04 | 13.18 | 93.23 |
| Inoculation with Nitrobin (N) | 12.67 | 78.31 | 11.88 | 97.10 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 11.85 | 68.03 | 14.79 | 110.60 |
| EM 0.2% | 14.14 | 94.68 | 12.73 | 91.01 |
| Control (untreated) | 12.85 | 62.28 | 14.06 | 110.76 |
| LSD 0.05 | 1.32 | 9.96 | 2.29 | N.S |

Table 3: Effect of microbial inoculation on Pods quality of Yard long bean plants during summer seasons of 2015 and 2016

| | Season 2015 | | | |
|---|---|---|---|---|
| Treatments | Pod length (cm) | Pod diameter (mm) | T.S.S | Drymatter (%) |
| Inoculation with Rhizobium (R) | 48.67 | 6.27 | 6.55 | 15.08 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 45.60 | 5.69 | 7.53 | 15.19 |
| Inoculation with Nitrobin (N) | 43.00 | 5.97 | 7.60 | 16.01 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 46.70 | 5.95 | 6.90 | 15.37 |
| EM 0.2% | 49.00 | 6.61 | 7.70 | 15.66 |
| Control (untreated) | 45.33 | 6.29 | 7.73 | 16.45 |
| LSD 0.05 | 3.97 | 0.66 | 1.09 | N.S |
| | Season 2016 | | | |
| Inoculation with Rhizobium (R) | 49.00 | 6.55 | 5.95 | 15.90 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 48.00 | 6.19 | 7.70 | 15.64 |
| Inoculation with Nitrobin (N) | 45.33 | 5.90 | 7.55 | 16.40 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 48.67 | 6.03 | 7.55 | 15.75 |
| EM 0.2% | 44.60 | 6.32 | 8.00 | 15.51 |
| Control (untreated) | 47.70 | 6.36 | 8.25 | 15.86 |
| LSD 0.05 | 3.07 | 0.41 | 0.55 | N.S |

Table 4: Effect of microbial inoculation on chlorophyll and carotenoids of Yard long bean pods during summer seasons of 2015 and 2016

| | Season 2015 | | Season 2016 | |
|---|---|---|---|---|
| Treatments | Total Chlorophyll (mg/100 g) | Total carotenoids (mg/100 g) | Total Chlorophyll (mg/100 g) | Total carotenoids (mg/100 g) |
| Inoculation with Rhizobium (R) | 34.05 | 37.41 | 30.11 | 32.19 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 39.09 | 23.47 | 27.90 | 21.00 |
| Inoculation with Nitrobin (N) | 42.65 | 38.54 | 43.74 | 40.95 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 42.38 | 38.96 | 49.66 | 44.80 |
| EM 0.2% | 29.27 | 36.62 | 28.39 | 30.18 |
| Control (untreated) | 21.40 | 21.05 | 21.75 | 20.00 |
| LSD 0.05 | 9.72 | 11.64 | 5.96 | 6.11 |

Table 5: Effect of microbial inoculation on chemical properties of Yard long bean pods during summer seasons of 2016

| | Season 2016 | | | |
|---|---|---|---|---|
| Treatments | Total carbohydrate (%) | Protein (%) | Phosphorus (%) | Potassium (%) |
| Inoculation with Rhizobium (R) | 11.40 | 14.31 | 0.28 | 1.65 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 11.30 | 14.31 | 0.26 | 2.03 |
| Inoculation with Nitrobin (N) | 11.60 | 14.50 | 0.27 | 1.73 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 11.70 | 12.31 | 0.31 | 1.85 |
| EM 0.2% | 11.50 | 14.81 | 0.37 | 1.75 |
| Control (untreated) | 12.10 | 12.75 | 0.26 | 1.71 |
| LSD 0.05 | 4.29 | 0.02 | 0.01 | 0.02 |

Table 6: Effect of microbial inoculation on growth formulas of Yard long bean plants during summer seasons of 2015 and 2016

| | Season 2015 | | | Season 2016 | | |
|---|---|---|---|---|---|---|
| Treatments | Biological yield (W) (g) | Economic yield (EY) (g) | Harvest index (HI) (%) | Biological yield (W) (g) | Economic yield (EY) (g) | Harvest index (HI) (%) |
| Inoculation with Rhizobium (R) | 35.38 | 11.97 | 34.13 | 41.61 | 15.50 | 37.22 |
| Inoculation with Rhizobium+ EM 0.2% (R+EM) | 31.36 | 13.68 | 43.66 | 31.27 | 14.55 | 46.52 |
| Inoculation with Nitrobin (N) | 34.32 | 12.54 | 36.60 | 48.40 | 15.91 | 32.86 |
| Inoculation with Nitrobin+ EM 0.2 % (N+EM) | 31.07 | 10.46 | 33.70 | 39.90 | 17.42 | 43.80 |
| EM 0.2% | 43.09 | 14.82 | 34.51 | 45.04 | 14.10 | 31.34 |
| Control (untreated) | 43.72 | 10.27 | 24.06 | 47.51 | 17.69 | 37.35 |
| LSD 0.05 | 6.42 | 2.22 | 8.35 | 10.15 | N.S | 3.08 |

Results show that Yard long bean plants inoculated with Rhizobium, Nitrobin and EM significantly increased the plant height in the first season. Plants inoculated with Rhizobium +EM and EM produced highest pods yield followed by inoculated with Rhizobium and Nitrobin in the first season. Yard long bean plants inoculated with Rhizobium, Nitrobin and EM or the mixture between them had a significantly increased pod chlorophyll content compared to the untreated plants. Yard long bean plants inoculated with Rhizobium + EM and Nitrobin had the highest significant increase in Harvest index (HI) in the first season and Rhizobium + EM and Nitrobin+ EM in the second season. The rhizobium bacteria can live in the soil without the host for up to ten years or more. Inoculation with highly efficient strains may not increase the nitrogen stabilization process [29].

It is Cleary that Rhizobium and EM improved the vegetative and yield characteristics of the yard long bean plants, i.e., the yard long bean plants is very compatible with Rhizobium and EM than other microorganisms. This compatibility led to the yard long bean gets more nitrogen and the EM produce growth stimulator accelerating the growth [18, 30]. Abdel- Wahab and Said [31] indicated that to improve the production, the inoculation must be effective and compatible with cultivation area. Chemining' we *et al.*, [32] concluded that the soil contained many strains may be effective or not. Many environmental stresses such as temperature and dry soil can effect on the effective of inoculation, lead to a lack of responses [33]. Many microorganisms play an important role in the stabilization of atmospheric nitrogen and produce bioactive substances, but vary in their capacity due to differences efficiency between the strains in the soil. There may be competition between endemic strains in soil and additive. Therefore, strains of high efficiency should be selected for soil inoculation; one of the biggest challenges in this field the definition of microorganisms and their basic characteristics, as well as the mechanics of their work [34]. Different soils may vary microorganisms in it as well as efficiency and this was evident in the second season where the treatments did not have a clear effect and was a slightly significant. That explains the differences between the two seasons in some results.

## CONCLUSION

Finally it is recommended to add EM because of their role in stimulating plant growth in addition to the inoculation with effective Rhizobium strains .The result may be a step to reduce dependence on nitrogen fertilization, transition to sustainable agriculture and produce healthy food.

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