*J. Agric. Sci. Mansoura Univ., 34 (2): 781 - 792, 2009*

# FIELD TRAILS TO IMPACT OF COLONOZATION WITH AM-FUNGI, $N_2$-FIXERS AND POTASSIUM SOLUBLIZING BACTERIA ON ONION CROPS (*Allium cepa*)

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## ABSTRACT

Two field experiments were conducted in a calcareous soil at El-Noubaria Research Station (ARC) during two successive seasons (2006 and 2007) to study the effect of biofertilizer inoculation with nitrogen fixing bacteria (*Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa* and *Klebsiella pneumoniae*), K-solubilizing bacteria (*Bacillus circulans*) and Arbuscular mycorrhizal fungi on growth, yield and different storage periods of onion (*Allium cepa*) cultivar Giza 20. The important obtained results are as follows: Plant height and number of tubular leaves, plant dry weight and yield as growth parameters, nitrogenase enzyme activity, dehydrogenase enzyme activity and the percentage of AM-mycorrhizal infection as microbiological parameters and total nitrogen, phosphorus, potassium and carbohydrates as chemical parameters gave more vigor growth and yield due to the inoculation with $N_2$-fixers + *Bacillus circulans* + AM-mycorrhizae + ½ dose of NPK fertilizers compared to control during both tested seasons and that also they recorded best plant growth, harvest bulbs yield (12.7 and 13.7 ton/fed), onion chemical properties as well as increased the tolerance against diseases and maintained healthy bulbs during the different storage periods.

**Keywords:** Onion (*Allium cepa*), *Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa Bacillus circulans, Klebsiella pnumoniae*, AM-mycorrhizae.

## INTRODUCTION

In Egypt, onion (*Allium cepa*) is the second major export crop after cotton. This is mainly due to the in-demand increase of the crop in local markets and for export. Agriculture depends heavily on the use of chemical fertilizers for boosting crop yield. However, these fertilizers are often in short supply and their indiscriminate use has an adverse effect on long-term soil health and environment, which has received global attention. Moreover, chemical fertilizers are costly and hence are hardly affordable by small and marginal farmers, who constitute the majority of the farming community in developing countries. The most realistic solution is, therefore to exploit the possibility of supplementing chemical fertilizers with organic ones, more particularly bio-fertilizers of biological origin. Nowadays, bio-fertilizer have emerged as an important component of integrated nutrient management strategy and had a promise to improve all crops performance yield and nutrient supply. Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers and their role in onion as well as other vegetable production. Onion responds well to $N_2$-fixers like *Azospirillum*, *Bacillus* and *Klebsiella* in general and *Azotobacter* in particular

and a yield increase up to 20 percent has been reported (Meshram and Shende, 1990).

*Azospirillum*, *Azotobacter , Klebsiella* and *Bacillus* are $N_2$-fixing bacteria that can exert beneficial effects on plant growth and yield of onion and many crops. Besides, fixing nitrogen they are able to produce plant hormones such as auxins. (Sabry *et al*., 2000). Therefore those bacteria could enhance plant growth by nitrogen fixation and hormone production.

Arbuscular mycorrhizal fungi are known to stimulate host plant growth mainly by enhancing soil nutrients uptake particularly P uptake (El-Gahdban *et al*., 2003 and Rahal *et al.,* 2006). Although these fungal endophytes are obligate symbionts (Menge, 1983) and their agricultural use remains limited due to the large quantities of inoculum required (Charron *et al*., 2001) and yet many trails are considered very promising solution to this problem like pre-inoculated seedlings that are very tolerant to transplant shock than non-mycorrhizal plants

Therefore, the main objective of this study is to confirm the impact of $N_2$-fixers and *Bacillus circulans* application, which are inoculated with AM-mycorrhizal fungi under calcareous soil conditions on growth, yield and storage of onion.

## MATERIALS AND METHODS

Two field experiments were carried out at El-Noubaria in two successive seasons of 2006 and 2007 to study the response of onion plants to inoculation with different genera of nitrogen fixers, K-solubilizing bacteria and AM-mycorrhizae fungi each alone or in combination. Chemical and physical properties of the used soil were shown in Table (1).

**Table (1): Chemical and physical properties of the experimental soil**

| | | Season | |
|---|---|---|---|
| | | **2006** | **2007** |
| **Particle size distribution (%)** : | | | |
| Coarse sand | | 11.75 | 10.12 |
| Fine sand | | 37.10 | 38.10 |
| Silt | | 18.03 | 16.76 |
| Clay | | 33.12 | 35.12 |
| Texture class | | Sandy clay loam | Sandy clay loam |
| **Chemical analysis :** | | | |
| pH (1:2.5 soil water suspension) | | 8.2 | 8.0 |
| Calcium carbonate (%) | | 25.8 | 24.2 |
| EC. | ($dS\ m^{-1}$) | 1.48 | 1.20 |
| $Ca^{++}$ | (meq/L) | 4.13 | 3.80 |
| $Mg^{++}$ | (meq/L) | 1.21 | 1.52 |
| $Na^{+}$ | (meq/L) | 6.46 | 5.84 |
| $K^{+}$ | (meq/L) | 1.43 | 1.70 |
| $CO_3^{-}$ | (meq/L) | 0.00 | 0.00 |
| $HCO_3^{-}$ | (meq/L) | 1.47 | 1.32 |
| $Cl^{-}$ | (meq/L) | 6.90 | 6.30 |
| $SO_4^{--}$ | (meq/L) | 4.86 | 5.24 |
| Organic matter | (%) | 0.43 | 0.55 |
| Total N | (%) | 0.058 | 0.072 |
| Total P | (ppm) | 616 | 730 |
| Available N | (ppm) | 25.9 | 33.2 |
| Available P | (ppm) | 4.35 | 6.20 |

Seedlings of onion cv. Giza 20 were transplanted in rows (3.2 m in width) at distance of 20 cm apart each. The experiment of each season comprises the following treatments:

1) Nitrogen-fixers (*Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa* and *Klebsiella pneumoniae.*) + $^1/_2$ dose N + full doses P K.
2) AM-mycorrhizae fungi + full doses N K + $^1/_3$ dose P.
3) *Bacillus circulnce* + $^1/_2$ dose K + full doses N P.
4) $N_2$-fixers + AM-mycorrhizae fungi + *Bacillus circulnce* + $^1/_3$ dose P + $^1/_2$ doses N K.
5) Control without any addition.
6) Full doses of N P K.

**1) Inoculum preparation:**

**a) Nitrogen fixers' organisms:**

Strains of *Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa* and *Klebsiella pnumoniae* are locally active strains isolated from the experimental calcareous soil at El-Noubaria Research Station as described by Rahal et al. (2006). Each culture was grown on semi solid malate medium (Dobereiner, 1976) for *Azospirillum*, modified Ashby's medium for *Azotobacter* (Abd El-Malak, 1968), Hino and Wilson medium for *Bacillus* (Hino and Wilson, 1958) and deficient medium of *Klebsiella* (Yoch and Pengra, 1966). The strains were purified and examined for its ability to produce growth regulators i.e., auxins, gibbrilline and cytokinine like substances and their ability to fix atmospheric nitrogen. The obtained data are recorded in Table (2).

**Table (2): Bio-chemical activities of the tested nitrogen fixers strains**

| Strain | Characters | | | | |
|---|---|---|---|---|---|
| | $N_2$-ase (µL $C_2H_4$/L/day) | Exo-polysaccharides (g/L) | phytohormones | | |
| | | | Auxin | Gibberiline | Cytokinine |
| *Azospirillum lipoferum* | 5.28 | 1.22 | ++ | ++ | - |
| *Azotobacter chrococcoum* | 24.72 | 1.81 | ++ | +++ | - |
| *Klebsiella pnumoniae* | 93.60 | 1.07 | ++ | ++ | - |
| *Bacillus polymyxa* | 64.8 | 0.96 | ++ | +++ | +++ |

**b) *Bacillus circulans*:**

A reference strain of *Bacillus circulans* was obtained from the Microbial Culture Collection, Center of Cairo (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

**c) AM-mycorrhizae fungi:**

AM-fungi were brought through Dr. Massoud, Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. They include the following genera: *Glomus, Gigaspora* and *Acaulospora*.

**2) Fertilization:**

Recommended dose of NPK was applied: 100 kg N/fed. As urea (46% N), 50 kg K as potassium phosphate (48% $K_2O$) and 200 kg P as super phosphate (15.5% $P_2O_5$) were added in rates according to the tested treatments. Phosphorus was applied once and mixed with soil before transplanting. Potassium was added once after 60 days from transplanting

whereas nitrogen was applied in three doses; directly after transplanting, 45 and 75 days, respectively.

**3) Inoculation process:**

**a) $N_2$ fixers application:**

Equal amounts of liquid cultures of each diazotrophs; *Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa* and *Klebsiella pnumoniae* (one liter for each, which contains $10^6$ CFU/ml) were mixed together and applied to grounded (shredded) residues of faba bean that previously moistened with sterilized tap water (60-70%) moisture as a carrier, then Arabian gum was added and mixed as an adhesive agent. The inoculum was applied to each ridge at a rate of 100 g/m after 15 and 30 days from transplanting.

**b) AM-mycorrhizae addition:**

Onion surface sterilized seeds (with clorox 0.05% for 2 minutes and washed three times with sterilized tap water) were divided into two equal quantities, one coated with mycorrhizal spores at the rate of 30-50 spores/seed. Mycorrhizal and non-mycorrhizal seeds were sown in onion transplanted area that was divided into two big plots (20 $m^2$ for each), and the growing plantlets were transplanted into the field after 45 days from sowing.

**c) *Bacillus circulans* application:**

The same technique applied to $N_2$-fixers application was carried out with *Bacillus circulans*.

A complete randomized plot design with three replicates was used. Each plot consisted of four rows 5 meters in length and 3.2 m in width. The plot area was 20 $m^2$.

Data were recorded on growth parameters as plant height (cm), number of tubular leaves, plant dry weight (g/plant), whereas chemical and microbiological parameters, i.e., nitrogenase activity was measured as acetylene reduction activity (ARA) according to Somasegaram and Hoben (1994). Dehydrogenase was also determined according to Skujins (1976). Infection rate of AM-mycorrhizae was determined according to Phillips and Hyaman (1970), NPK and total carbohydrates were also determined according to Jackson (1973) and Dubios *et al.* (1956) respectively. Yield parameters (ton/fed) and healthy and viability (storage period) were also determined.

## RESULTS AND DISCUSSION

**1) Plant height and number of tubular leaves:**

Data in (Table 3) indicated that in both experiments (2006 and 2007 seasons) the treatment that received a combination of $N_2$-fixers represented in *Azospirillum*, *Azotobacter, Bacillus* and *Klebsiella* beside they fix nitrogen, they can produce expolysacarides and phytohormons like auxins and gibrilliens, while AM-mycorrhizae fungi and *B. circulans* as biofertilizers caused significant enhancement in values of onion height and number of tubular leaves at 45 and 75 days after transplanting and also at harvest. These high values are more significant than those recorded by the other

treatments including control. The comparison results due to both tested seasons of 2006 and 2007 indicated that the addition of bio-fertilizers resulted in significant increases in both plant length and the number of tubular leaves. These findings exhibited a similar trend in both tested seasons. Some investigators gained results which are in a good agreement with those mentioned here (Yadav *et al.*, 2003) on onion and (Prabu *et al*., 2003) on okra.

**2) Plant dry weight:**

Results obtained in Table (3) revealed that the positive effect of biofertilizers application was supported with ⅓ recommended dose of phosphorus and ½ NK recommended dose on dry weight of onion plants represented in the weight of bulbs and the number of tubular leaves. It was obvious that the NPK treatment gave high dry weight.

**Table (3): Effect of some microorganisms on some plant growth parameters at different periods and yield of onion cv. Giza 20 in 2006 and 2007 seasons**

| Parameter | Plant dry weight (g) | | | | | | Plant length (cm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 45 day | | 75 day | | Harvest | | 45 day | | 75 day | | Harvest | |
| Treatments* | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1)* | 3.00 | 3.17 | 78.77 | 81.23 | 196.00 | 205.00 | 31.47 | 32.07 | 49.50 | 50.93 | 52.43 | 53.13 |
| AM-mycorrhizae (2) | 2.03 | 2.23 | 50.60 | 51.27 | 172.33 | 177.00 | 28.33 | 29.23 | 42.40 | 43.31 | 50.73 | 51.13 |
| *Bacillus circulans* (3) | 1.30 | 1.46 | 34.80 | 39.44 | 161.00 | 162.67 | 26.43 | 27.67 | 47.47 | 48.83 | 48.10 | 49.03 |
| (1) + (2) + (3) | 4.00 | 4.12 | 80.27 | 82.80 | 217.50 | 220.33 | 33.50 | 34.80 | 56.30 | 57.10 | 57.40 | 58.23 |
| Control | 0.70 | 0.87 | 28.40 | 29.50 | 97.33 | 100.67 | 22.47 | 23.50 | 30.63 | 31.87 | 40.40 | 41.10 |
| Full dose NPK | 2.43 | 2.87 | 41.63 | 42.87 | 181.73 | 184.67 | 32.37 | 33.23 | 45.83 | 46.67 | 46.73 | 48.00 |
| L.S.D. at 0.05 | 0.35 | 0.46 | 3.946 | 8.81 | 6.522 | 12.03 | 1.20 | 1.72 | 0.45 | 2.21 | 1.097 | 3.43 |

| | No. of tubular leaves | | | | | | Yield (ton/fed) | |
|---|---|---|---|---|---|---|---|---|
| | 45 day | | 45 day | | 75 day | | | |
| | 2006 | 2007 | 2006 | 2006 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 11.83 | 12.67 | 28.37 | 28.37 | 28.37 | 29.33 | 36.30 | 37.80 |
| AM-mycorrhizae (2) | 10.67 | 11.00 | 24.40 | 24.40 | 24.40 | 25.33 | 32.77 | 33.30 |
| *Bacillus circulans* (3) | 9.53 | 10.00 | 23.37 | 23.37 | 23.37 | 24.00 | 29.63 | 30.47 |
| (1) + (2) + (3) | 15.77 | 16.33 | 32.30 | 32.30 | 32.30 | 33.00 | 38.17 | 39.37 |
| Control | 5.97 | 6.67 | 19.37 | 19.37 | 19.37 | 20.00 | 18.80 | 19.37 |
| Full dose NPK | 7.97 | 8.67 | 29.40 | 29.40 | 29.40 | 30.67 | 34.33 | 35.10 |
| L.S.D. at 0.05 | 0.93 | 1.47 | 0.95 | 0.95 | 0.95 | 2.04 | 1.134 | 2.34 |

*** (1) $N_2$ fixers (*Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa* and *Klebsiella pneumoniae.*) + $^1/_2$ dose N + full doses P K.**
**(2) AM-mycorrhizae fungi + full doses N K + $^1/_3$ dose P.**
**(3) *Bacillus circulans* + $^1/_2$ dose K + full doses N P.**
**(1) + (2) + (3) + (4): $N_2$-fixers + AM-mycorrhizae fungi + *Bacillus circulans* + $^1/_3$ dose P + $^1/_2$ doses N K.**
**5) Control without any addition.**
**6) Full doses of N P K.**

This may due to the impact of mineral fertilization on growth of most vegetable plants. However, the treatment $N_2$ fixers + AM-mycorrhizae fungi + *Bacillus circulans* + $^1/_3$ dose P + $^1/_2$ doses N K that involved combination between three $N_2$ fixers, ⅓ dose of phosphorus and ½ dose of both

potassium and nitrogen recorded the highest values of dry weight, in both seasons the values were 4.0 and 4.12 g plant$^{-1}$ at 45 days of transplanting where as at 75 days they were 80.27 and 82.8 g plant$^{-1}$. The best values of dry weight were at harvest (for the same treatment) were 217.5 g and 220.33 g in first and second season, respectively. Other treatments exhibited fewer results.

**3) Nitrogenase activity:**

Data in Table (4) indicated that, $N_2$ fixers have the ability to fix atmospheric nitrogen. However, a highest activity was obtained with $N_2$ fixers + AM-mycorrhizae + *B. circulans* compared to control recording 54.33 and 57.00 µmole $C_2H_4$/g dry soil/day at 45 days of transplanting during both seasons, respectively. Whereas at 75 days it increased to 63.00 and 67.33 µmole $C_2H_4$/g dry soil/day.

**Table (4): Effect of some microorganisms on nitrogenase activity ($N_2$-ase), dehydrogenase activity (DHA), and infection percentages of mycorrhizae at different periods on rhizosphere of onion plants cv. Giza 20 in 2006 and 2007 seasons**

| Parameter | Nitrogenase enzyme (µ mole $C_2H_4$/g dry soil/day) | | | | | | Dehydrogenase enzyme µ g TPF / g dry soil/day) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 45 day | | 75 day | | Harvest | | 45 day | | 75 day | | Harvest | |
| Treatments | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 43.00 | 47.00 | 48.40 | 50.27 | 17.27 | 19.34 | 15.07 | 15.97 | 18.10 | 18.67 | 7.77 | 8.08 |
| AM-mycorrhizae (2) | 33.33 | 36.13 | 37.67 | 42.00 | 10.57 | 11.97 | 14.27 | 14.73 | 15.97 | 16.73 | 6.73 | 6.93 |
| *Bacillus circulans* (3) | 24.00 | 25.01 | 31.67 | 32.60 | 8.53 | 9.40 | 14.70 | 15.12 | 16.50 | 17.03 | 8.00 | 8.48 |
| (1) + (2) + (3) | 54.33 | 57.00 | 63.00 | 67.33 | 23.10 | 24.57 | 14.97 | 15.63 | 15.77 | 16.80 | 7.33 | 7.67 |
| Control | 16.33 | 19.53 | 22.67 | 25.10 | 7.33 | 7.93 | 7.70 | 8.37 | 9.87 | 10.90 | 6.07 | 6.54 |
| Full dose NPK | 19.74 | 20.43 | 25.67 | 26.90 | 9.07 | 9.67 | 9.33 | 10.20 | 12.80 | 13.00 | 6.40 | 6.92 |
| L.S.D. at 0.05 | 5.10 | 3.26 | 6.48 | 3.13 | 1.61 | 1.20 | 0.84 | 0.42 | 0.57 | 0.69 | 0.67 | 0.67 |

| | Mycorrhizae infection (%) | | | | | |
|---|---|---|---|---|---|---|
| | 45 day | | 75 day | | Harvest | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 56.67 | 59.33 | 70.67 | 72.33 | 72.00 | 75.00 |
| AM-mycorrhizae (2) | 71.33 | 74.00 | 80.67 | 84.67 | 81.67 | 88.67 |
| *Bacillus circulans* (3) | 45.00 | 47.67 | 53.67 | 56.00 | 52.00 | 55.00 |
| (1) + (2) + (3) | 76.67 | 79.33 | 91.33 | 97.67 | 91.33 | 97.67 |
| Control | 35.67 | 37.33 | 38.33 | 41.67 | 39.67 | 42.67 |
| Full dose NPK | 31.33 | 33.00 | 31.33 | 33.67 | 34.00 | 38.67 |
| L.S.D. at 0.05 | 4.55 | 5.00 | 3.43 | 6.27 | 5.03 | 4.51 |

However, the values of nitrogenase were decreased at harvest in both tested seasons. Similarly, as in many crops grown in the field, acetylene reduction was higher during reproductive stage (75 days in particular). This can be explained by an increase in root volume owing to colonization of AM-mycorrhizae. Consequently, the increase in the number of roots that associated with active $N_2$-fixers as *Azospirillum lipoferum*, *Bacillus polymyx*a, *Azotobacter choroccocum* and *Klebsiella pnumoniae* that lead to domination of $N_2$-fixers population, which enhanced their ability to fix the atmospheric nitrogen in presence of AM-fungi which carried the optimum conditions reflected on an increase of nitrogenase activity. The drop of activity of

nitrogenase enzyme at harvest in all treatments are due to the decrease of microbial population as result of drought condition, which in turn caused the roots dryness and consequently, the migration of the most rhizosphere microorganisms to more moist places (Pandey *et al.,* 1988 and Kefologranni and Aggelis, 2002).

**4) The percentage of AM-mycorrhizal infection (%):**

Root colonization in plants inoculated with AM-mycorrhizae fungi that represented in treatment 2 (Table 4) was high where the values were 71.33 and 74.00% at 45 days from planting during both tested seasons respectively. At 75 days the percentage increased to 80.67 and 84.67 compared to control that produced the lowest root colonization. On the other hand, treatment $N_2$-fixers + AM-mycorrhizae fungi + *Bacillus circulans* + $^1/_3$ dose P + $^1/_2$ doses N K that represent the combination between $N_2$-fixers, AM fungi and *Bacillus circulans* had recorded the highest percentage of colonization of AM fungi in onion plants compared to control and the other tested treatments. The corresponding percentages of infection during first season were 67.67 and 79.33 at 45 days after planting, respectively. While, at 75 days it increased to 91.33 and 97.67%, respectively. The per cent of infection was completely decreased at harvest due to the release of resting fungal spores during the final stage of fungal life cycle. The highest infection that occurred with the treatment ($N_2$-fixers + AM-mycorrhizae fungi + *Bacillus circulnance* + $^1/_3$ dose P + $^1/_2$ doses N K) was due to the positive correlation between AM mycorrhizae that enhanced the release of certain nutrient elements (P, Fe, Zn, Mn and K). These elements support the existence of other rhizospheric microorganisms including $N_2$-fixers and *Bacillus circulans* that improve soil fertility and plant growth development (El-Mageed *et al.,* 2004).

**5) Dehydrogenase activity:**

Data in Table (4) show that the highest values of dehydrogenase enzyme activity were recorded with the treatment (1) that involved inoculation of onion plants with $N_2$-fixers only, whereas the combination of $N_2$-fixers, AM-mycorrhizae and *Bacillus circulans* gave slightly less values of dehydrogenase enzyme against those scored by inoculation with $N_2$-fixers and/or control treatment. However, there is a diversity of dehydrogenase activity among the microbial species and the changes in the dehydrogenase activity indicate a change in the composition and activity of soil microorganisms. The increase of dehydrogenase activity in treatment No (1) could be attributed to the intense activity of $N_2$-fixers as a mixture and also to the ability of these $N_2$-fixers microorganisms to mineralize the organic carbon that maintains their growth and this behavior reflected their efficient respiration. These results are in agreement with those obtained by Serra-Wittling *et al*. (1995) and Massoud (2005).

**6) Total nitrogen, phosphorus and potassium (%):**

Supplementation of nitrogen, potassium and phosphorus fertilizers with $N_2$-fixers, AM-mycorrhizal fungi and *Bacillus circulans* inoculation markedly increased total NPK% in onion plants. Supplementing ½ NP doses and ⅓ P through inoculation with a mixture of $N_2$-fixers, AM-mycorrhizal fungi and

*Bacillus circulans* gave a significant increase in the percent of nitrogen, potassium and phosphorus over the control received full dose of NPK fertilizers as well as over all the other tested treatments. Data in Table (5) show that nitrogen, phosphorus and potassium percentages increased during the second season (2007) compared to those recorded by the first season (2006). In concern to the periods, both the percentages of nitrogen and phosphorus scored the highest percentages during 45 and 75 days during both 2006 and 2007 seasons. While for potassium, the percentage was higher at 45 days and decreased gradually till harvest stage. Also, mycorrhizal colonization increased the number of the diazotrophic bacterial count in onion roots. This effect suggests a beneficial action of AM-mycorrhizae in helping the diazotrophic bacteria to penetrate and colonize the plant roots, consequently AM-mycorrhizae fungi and diazotrophs have been shown to promote plant growth and plant nutrients uptake and are particularly important for phosphorus, nitrogen and potassium nutrition (Bolandnuzar *et al.,* 2007).

**Table (5): Effect of some microorganisms on NPK percentage at different periods of onion plants cv. Giza 20 in 2006 and 2007 seasons**

| Parameter | Nitrogen (%) | | | | | | Phosphorus (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 45 day | | 75 day | | Harvest | | 45 day | | 75 day | | Harvest | |
| Treatments | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 2.361 | 2.793 | 2.813 | 3.000 | 1.892 | 2.100 | 0.071 | 0.072 | 1.851 | 2.183 | 1.518 | 1.787 |
| AM-mycorrhizae (2) | 2.089 | 2.233 | 2.228 | 2.533 | 1.499 | 1.667 | 0.097 | 0.103 | 2.191 | 2.513 | 1.923 | 2.000 |
| *Bacillus circulans* (3) | 1.605 | 1.877 | 1.952 | 2.100 | 1.285 | 1.443 | 0.037 | 0.040 | 0.962 | 1.057 | 0.655 | 0.750 |
| (1) + (2) + (3) | 2.856 | 3.100 | 3.097 | 3.467 | 2.293 | 2.567 | 0.074 | 0.083 | 2.294 | 2.670 | 1.844 | 1.923 |
| Control | 1.328 | 1.607 | 1.692 | 1.807 | 1.016 | 1.137 | 0.020 | 0.025 | 0.066 | 0.075 | 1.083 | 0.187 |
| Full dose NPK | 1.975 | 2.110 | 2.095 | 2.383 | 1.926 | 2.133 | 0.044 | 0.053 | 1.111 | 1.367 | 0.167 | 1.167 |
| L.S.D. at 0.05 | 0.345 | 0.142 | 0.193 | 0.209 | 0.232 | 0.147 | 0.009 | 0.019 | 0.182 | 0.208 | 0.182 | 0.122 |

| | Potassium ( %) | | | | | |
|---|---|---|---|---|---|---|
| | 45 day | | 75 day | | Harvest | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 3.063 | 3.600 | 2.133 | 2.243 | 1.213 | 1.300 |
| AM-mycorrhizae (2) | 3.323 | 3.533 | 2.037 | 2.170 | 0.973 | 1.000 |
| *Bacillus circulans* (3) | 3.623 | 3.873 | 2.157 | 2.290 | 1.103 | 1.217 |
| (1) + (2) + (3) | 3.850 | 4.267 | 2.700 | 2.850 | 1.753 | 1.817 |
| Control | 1.993 | 2.017 | 1.186 | 1.217 | 0.607 | 0.723 |
| Full dose NPK | 3.430 | 3.433 | 1.687 | 1.800 | 0.703 | 0.730 |
| L.S.D. at 0.05 | 0.204 | 0.248 | 0.129 | 0.360 | 0.101 | 0.462 |

**7) Total carbohydrates in bulbs:**

Results in Table (6) revealed that dual inoculation of onion with diazotrophus and AM-mycorrhizae plus *Bacillus circulans* had pronounced an influence on biosynthesis of carbohydrates in onion leaves and bulbs. Also, there was a significant increase in total carbohydrates for onion bulbs and the treatment received a combination of diazotrophs, *Bacillus circulans* and AM-mycorrhizae. Treatment 4 had recorded the highest percentage of carbohydrates content for onion leaves and bulbs compared to control and

the other tested treatments. Incidentally it was also reported that the root association of AM-mycorrhizae fungi are known to improve the amino acids and carbohydrates in host plants (Koshyap *et al*., 2004).

**Table (6): Effect of some microorganisms at different periods on total carbohydrates of onion plants cv. Giza 20 in 2006 and 2007 seasons**

| Parameter / Treatments | Carbohydrates (%) | | | | | |
|---|---|---|---|---|---|---|
| | 45 *DAS | | 75 DAS | | Harvest (in bulbs) | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 21.272 | 22.933 | 22.596 | 23.967 | 18.050 | 18.600 |
| AM-mycorrhizae (2) | 19 .447 | 20.033 | 20.451 | 21.267 | 16.934 | 17.967 |
| *Bacillus circulans* (3) | 16.984 | 17.167 | 17.737 | 18.100 | 16.190 | 16.833 |
| (1) + (2) + (3) | 29.263 | 30.033 | 30.744 | 31.800 | 28.143 | 28.687 |
| Control | 14.493 | 15.100 | 14.972 | 15.667 | 14.027 | 14.267 |
| Full dose NPK | 16.842 | 17.867 | 18.067 | 18.800 | 16.714 | 17.033 |
| L.S.D. at 0.05 | 0.549 | 0.661 | 0.530 | 0.417 | 0.718 | 0.542 |

***DAS = Days after sowing**

**8) Onion yield:**

Results in Table (3) indicated that the highest yield were recorded due to the treatments (1+2+3) that included the combination of diazotrophs, *Bacillus circulans* and AM- mycorrhizae fungi. This combination had achieved a significant increase in onion yield (12.70 and 13.1 ton/fed) over the control in both tested seasons, respectively. The trend explained the ability of fungal hyphae to play an important role for the distribution of bacterial population and could therefore act as vectors for bacteria in microbial inocula. Studying the spatiotemporal stability of such bacterial fungal association would provide more information on this potential and improve our understanding of microbial interactions, which is important for the development of sustainable management of soil fertility that lead to increase crop production in general and onion in particular (Johansson *et al*., 2004).

**9) Bulb storage:**

Onion was stored to period of 3 to 10 months and they were treated correctly during and after harvest. The growing conditions will also influence the quality of onions during storage. Generally, onions can grow slowly in cool temperature climates for longer periods than onion grown under irrigation in hot climates.

Some fungal and bacterial diseases that cause severe loss during storage of onion such as blue-green mould that caused by *Pencillium* spp., black mould by *Asperigillus* spp., neck rot by *Botrytis* spp. and fusarium rot by *Fusarium* spp. While bacterial rots caused by *Pseudomonas* spp. and *Erwinia* spp. (Knoxfield, 1996). Data in Table (7) showed that the mixture of diazotrophs, *Bacillus circulans* and AM-mycorrhizal fungi are the best inoculants for onion (*yellow crranex*) where the inoculants increased the tolerance of onion against diseases and maintained the healthy bulbs during storage process.

**Table (7): Effect of storage periods on onion viability (healthy) at different periods of onion bulbs in 2006 and 2007 seasons**

| Parameter / Treatments | Storage | | | | | | Healthy (%) | |
|---|---|---|---|---|---|---|---|---|
| | 3 Months | | 6 Months | | 9 Months | | | |
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| $N_2$-fixers (1) | 10 | 10 | 10 | 10 | 9 | 10 | 90 | 100 |
| VAM-mycorrhizae (2) | 10 | 10 | 10 | 10 | 10 | 10 | 100 | 100 |
| *Bacillus circulans* (3) | 10 | 10 | 8 | 9 | 7 | 8 | 70 | 80 |
| (1) + (2) + (3) | 10 | 10 | 10 | 10 | 10 | 10 | 100 | 100 |
| Control | 10 | 10 | 8 | 7 | 4 | 5 | 40 | 50 |
| Full dose NPK | 10 | 10 | 7 | 7 | 5 | 6 | 50 | 60 |

**In conclusion,** application of diazotrophs combined with AM-mycorrhizae didn't only reduce phosphorus inputs, but also reduced by half the total nitrogen and potassium requirements of onion. Thereby, reducing the fertilizers costs for the farmers. Biofertilization of onion is an eco-friendly nutrient supplement for onion and should be part of onion production system. The same explanation discussed by Rita (1998) and Ram Rao *et al.* (1998). Mycorrhizal fungi modify the quality and the abundance of the rhizosphere microflora assemblage leading to an alteration in the overall rhizosphre microbial activity. Benefits arising from synergistic interactions between AM-mycorrhizae fungi and other rhizosphere microflra such as rhizobia, non-symbiotic diazotrophs, phytohormone producers and phosphate solubilizers are well recognized. The role of AMF-rhizosphere microflora combination appears to be a promising strategy to enhance plant growth or protect plants against pathogens and other environmental stresses (Xavier and Boyetchko, 2002).

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## تجارب حقلية لتأثير فطريات الميكوريزا ومثبتات النيتروچين والبكتريا المذيبة للبوتاسيوم على محصول البصل *Allium cepa*

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**مركز البحوث الزراعية - معهد بحوث الأراضى والمياه والبيئة - قسم بحوث الميكروبيولوچيا الزراعية**

أجريت تجربتان حقليتان فى تربة جيرية بمحطة بحوث النوبارية خلال موسمين متعاقبين ٢٠٠٦ ، ٢٠٠٧ ؛ لدراسة تأثير التلقيح بالأسمدة الحيوية المثبتة لنيتروچين الهواء الجوى *Azospirillum lipoferum, Azotobacter chrococcoum, Bacillus polymyxa and Klebsiella pneumoniae* وكذا البكتيريا المذيبة للبوتاسيوم *Bacillus circulans* ، وفطريات الميكوريزا ، على نمو ومحصول وكفاءة تخزين نبات البصل صنف جيزة ٢٠ ، وكانت أهم النتائج المتحصل عليها هى زيادة فى أطوال النباتات ، وعدد الأوراق الأنبوبية ، والوزن الجاف للنبات ، وكمية المحصول كقياسات مورفولوچية لنمو النباتات ، ونشاط إنزيمى النيتروچينيز والديهيدروچينيز ، ومعدل الإصابة بفطريات الميكوريزا كقياسات ميكروبيولوچية ، والنيتروچين والفوسفور والبوتاسيوم الكلى والكربوهيدرات الكلية كقياسات كيميائية . كل هذه القياسات سجلت نمو ومحصول قوى نتيجة المعاملة بمثبتات النيتروچين + والبكتيريا المذيبة للبوتاسيوم + فطريات الميكوريزا + ٢/١جرعة السماد النيتروچينى والفوسفاتى والبوتاسيومى، مقارنة بمعاملة الكنترول خلال موسمى الزراعة ( ٢٠٠٦، ٢٠٠٧ ) وأعطت أحسن نمو للنباتات ، وأعلى محصول (٧ر١٢، ٧ر١٣ طن / فدان ) وكذا الخواص الكيميائية للمحصول ، وزيادة المقاومة للأمراض ، وثبات للمحصول خلال فترة التخزين.