



Research Article

**APPLICATION OF MYCORRHIZAL TECHNOLOGY FOR IMPROVING YIELD
PRODUCTION OF COMMON BEAN PLANTS**

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Abstract

This study aimed to investigate the effects of arbuscular mycorrhizal (AM) fungi with different levels of NPK fertilizers on yield production of common bean plants which common bean plants were subjected to five levels of NPK fertilizers (0, 25, 50, 75, 100 %). Application of AMF significantly increased the growth and yield components of common beans with minimized the levels of NPK comparing to equivalents non-mycorrhizal ones. The results obtained revealed that inoculation with AMF and the concentrations 50% and 75% of NPK with AMF are the greater than other concentrations and non-mycorrhizal plants. Mycorrhizal Common bean plants had significantly higher number of pods, length of one pod, pods weight, 100 seeds weight, weight of seed/plant and intensity of mycorrhizal colonization(M%) . Concentrations of nutrients (N, P, K, Ca and Mg) and total carbohydrates, crude protein and mycorrhizal dependency of some yield parameters were significantly increased in mycorrhizal plants at different NPK levels when comparing to those of non-mycorrhizal plants particularly at (50% and 75%) concentration of NPK, but lower Na concentration in mycorrhizal common bean seeds than those of non-mycorrhizal.

Keywords: Arbuscular mycorrhiza; (*Phaseolus Vulgaris* L.); Yield components; NPK fertilizers.

Introduction

Recently, consumption of legumes particularly dry beans, has increased in some West European countries and the United States, due to an increased realization of consumers about the nutritional characteristics in foods (Peksen and Artik, 2005). Egypt suffers from food reduction problem as a result for a huge increment of population and the huge loss of agricultural soils due to erosion and desertification problems. Therefore, it is very essential to increase common bean productivity (Abd Allah *et al.*, 2015).

When consumed as seed, beans constitute an important source of dietary protein. In addition to fiber content prohibits blood sugar levels from elevation too rapidly after a meal, making these beans an especially good choice for individuals with diabetes (Celleno *et al.*, 2007).

Egypt faces a noticed shortage in fertile cultivated soils in the old Nile Valley and Delta, which represent about 3 - 4% of the total area of Egypt (Zaki and Radwan, 2006). Due to alkalinity of Egyptian soils, the obtainable phosphorus in the added fertilizer reduces sharply after a short period since application rapidly and transformed to tricalcium phosphate which is unavailable to the plants (khalil, 2013). Also the problem that fertilizer consumer face is to choose fertilizer that can give higher yield but harmful on environment or to

choose fertilizer that can preserve the environment but give slower effect. Biofertilizer can be used to substitute organics and chemical fertilizer, which does not source of pollution like chemical fertilizer and give faster effect compare to organic fertilizer, it should be put at the first place (Norhazimah, 2009).

So, an attention was direct towards using micro-organisms to enhance it through their activities and providing most of the essential nutrients required to crop productivity. Most of the plants form symbiotic relationship with a group of fungi called mycorrhiza (Cardon and Whitbeck, 2007). Arbuscular mycorrhizal fungi enhanced nutrient uptake associated with increase in dry matter yield, typically amounting to several increases for plant species having high dependency on mycorrhiza (Pharudi, 2010). The present work was planned to investigate the effects of AMF on yield of common bean plants grown under different levels of NPK fertilizers.

Materials and Methods

Experimental Design

The experiment was conducted using a randomized complete block design with two mycorrhizal treatments (+AMF,-AMF) combined with 5 concentrations of NPK

fertilizers (0, 25, 50, 75, 100 %) and were replicated 8 times (3 plants /pot) to give 80 pots with (35cm in diameter) containing 10kg sterilized soil (clay : sand 2:1 v/v).. All plants were grown for 3 months and maintained under natural conditions at the Experimental Research Station, Faculty of Agriculture, Mansoura University.

Inoculum Preparation of Arbuscular Mycorrhizal Fungi

The inoculum of arbuscular mycorrhizal species including *Glomus clarum*, *Glomus mosseae*, *Glomus intraradices*, *Gigaspora margarita* and *Gigaspora gigantea* were originally isolated from rhizosphere soil of common bean plants using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963), and then identified by the author.

The identified arbuscular mycorrhizal spores were left to multiply for 6 months on sudangrass plants (*Sorghum halepense* L.) under natural conditions. Plants were irrigated with tap water as needed and the nutrient solutions (Long- Ashton without phosphorus) of plants were provided. A mixture of soil and infected sudangrass root, which contained spores were used as mycorrhizal inoculum. The AM fungal inoculums containing 5 g of rhizosphere soil (approx. 725 spores) and 0.5 g of infected sudangrass root fragments with an infection level of 88.6% was inoculated to each mycorrhizal pot.

Plant and Growth Conditions

Seeds of Common bean (*Phaseolus vulgaris* L.) (Nebraska).which obtained from the Horticultural Research Center, Egypt and used throughout this investigation. Seeds were surface sterilized in 0.01% mercuric chloride for 3 min, subsequently washed with sterilized water.. The results of soil analysis were recorded in (Table 1).

Table 1: physical and chemical analysis of soil used in this study.

Clay (%)	13.85
Silt (%)	37
Sand (%)	49.15
Texture	Loam
Organic matter (%)	1.31
P (ppm)	18.6
K (ppm)	10.2
N (ppm)	59.5
Ca ²⁺ (meq/ 100 g)	0.5
K ⁺ (meq/ 100 g)	0.22
Mg ²⁺ (meq/ 100 g)	0.4
Na ⁺ (meq/ 100 g)	0.35
pH	8.1
EC (dsm ⁻¹)	0.294
TSS (%)	0.094

For mycorrhizal treatments (AMF), each pot was inoculated with 5 g of rhizosphere soil (approx.275 spores/g soil) and 0.5 g of chopped AM fresh sudangrass roots (M = 88.6%).The inoculum was placed 3-5cm below the soil surface of common bean plants to produce mycorrhizal plants. In the non-mycorrhizal treatments (-AMF), each pot received 10 ml filter leaching (Whatman No. 1) from infected roots and sterilized equal amount of soil inoculum to provide the same microorganisms other than mycorrhizal propagules. Each pot received Super phosphate (15% P₂O₅) was used as a source of phosphorus fertilizer, while nitrogen fertilizer used ammonium sulphate (20.5%) and potassium sulfate (48% K) as a source of potassium.

Measurements

Number of pods, length of one pod, pods weight, number of seed/ pod, weight of 100 seeds, and weight of seed/plant were determined immediately after harvest. Harvest index calculated from the following formula (Beadle, 1993) {weight of seeds plant/straw weight plant}×100. Mycorrhizal dependency (MD) was defined as a percentage of a plant growth subjected to AMF application (Menge *et al.*, 1978), and calculated from the following formula as {(M-NM)/NM} ×100, where M is parameter value of mycorrhizal plants and NM is parameter value of non-mycorrhizal plants.

Estimation of mycorrhizal colonization, it were described by (Philips and Hayman, 1970). The intensity of mycorrhizal colonization (M%) of the stained roots were determined according to the method of (Trouvelot *et al.*, 1986).

After digestion by acid, analysed of N, P, K, Ca, Na and Mg contents. Total nitrogen (N) was measured by using Kjeldahl method (Nelson and Sommers, 1973).Phosphorus (P) concentration was determined following ammonium molybdate blue method (Chapman and Pratt, 1961). Potassium (K) and sodium (Na) were assayed using a flame spectrophotometer (Corning 400, UK), and calcium (Ca) and magnesium (Mg) were determined using atomic absorption (PerkinElmer, Model 2308, USA) according to (Allen, 1989).

Total carbohydrates were estimated by anthrone method (Hedge and Hofreiter, 1962). Crude protein according to (AOAC, 2000) calculated by multiplying the total nitrogen by the factor 6.25.

Statistical analysis

Data were subjected to statistical analysis using two-factor analysis of variance (ANOVA) and the least significant LSD at the significance level of P≤0.05 method using the Costat software (Cohort, Berkeley, CA, USA).

Result and Discussions

Yield Parameters

Generally, inoculation of mycorrhizal fungi increased significantly the yield parameters (Number of pods, length of one pod, pods weight, number of seed/ pod, weight of 100 seeds, weight of seed/plant and harvest index) regardless of the NPK level as compared with non-mycorrhizal common bean plants (Table 2) and (Fig 1). Mycorrhizal fungi were significantly greater than those of non-mycorrhizal plants. In this connection, the application of NPK fertilizers to the infected mycorrhizal plants caused a significant increase in yield components particularly at 50 and 75% of NPK fertilizers as compared to the control treatments. These results are agreement with finding of (Millar and Ballhorn, 2013; Adavi and Tadayoun, 2014; Parial *et al.*, 2014) who reported that that 75% fertilizer and AM fungi treated plants was found significantly higher than the plants treated 75% fertilizer alone and almost similar to the plant treated 100% fertilizer alone. Seedlings treated with 50% fertilizer and arbuscular mycorrhizal fungi also showed good results as compared to 50% fertilizer alone on different crops. In addition, Singh and Kallo (2000) had been showed that Inoculation of seedlings with VAM in the main field recorded an additional yield and saved 25 % of recommended dose of NPK with improvement in soil health, vegetable quality and yield. Intensity of mycorrhizal colonization of common bean plants decreased gradually with increasing NPK concentrations in the soil. However, a significantly increased were observed in common bean plants grown either in 50% or 75% concentration of NPK compared with other concentrations. No mycorrhizal

colonization was observed in the non-inoculated. AM colonization can improve the uptake of nutrients that can reflected on the yield components of common bean plants. This result supports the previous findings of (Smith and Gianinazzi-Pearson, 1990; Cavagnaro *et al.*, 2005; Sheng *et al.*, 2013) who indicated that increased soil phosphorus generally reduced AMF development and consequently the mycorrhizal benefits. In addition, these results are in accordance also with Abdullahi and Sheriff (2013) who reported that Root% colonization was affected with increase in fertilizer application in all the treatments. Thus, the high rate of chemical fertilizers application, led to antagonistic interaction with mycorrhiza. Mycorrhizal colonization % level of inoculated plant with increase in fertilizer application is in agreement with the fact that excessive chemical fertilizers have negative effect on AMF colonization (Valentine *et al.*, 2001; Gryndler *et al.*, 2005).



Fig.1: Effect of different levels of NPK fertilizers on seeds yield of non-mycorrhizal and mycorrhizal common bean plants.

Table 2: Yield parameters and intensity mycorrhizal colonization (M%) of mycorrhizal (+AMF) and non-mycorrhizal (AMF) common bean plants with different concentrations of NPK fertilizers.

Treatments		Number of pods/plant	Pod length (cm/pod)	Pods weight (g/plant)	100 seeds weight (g)	Weight of seed (g/plant)	Harvest index %	M%
NPK conc. (%)	AMF status							
0	-AMF	6.00 ^j	8.23 ^j	17.31 ⁱ	43.6 ^j	12.18 ^j	398.99 ^g	0.0
	+AMF	7.33 ⁱ	9.00 ⁱ	25.20 ^h	45.03 ⁱ	17.53 ⁱ	544.17 ^f	35.80
25	-AMF	8.66 ^h	9.43 ^h	29.70 ^{gh}	46.6 ^h	21.69 ^h	652.89 ^e	0.0
	+AMF	9.66 ^g	10.00 ^g	33.08 ^g	48.76 ^g	24.47 ^g	685.54 ^d	33.10
50	-AMF	11.00 ^f	10.40 ^f	39.28 ^f	49.96 ^f	27.49 ^f	687.83 ^d	0.0
	+AMF	19.33 ^b	12.40 ^b	90.33 ^b	57.43 ^b	65.50 ^b	1183.34 ^b	42.26
75	-AMF	12.33 ^e	10.73 ^e	45.45 ^e	52.16 ^e	31.13 ^e	712.89 ^d	0.0
	+AMF	21.66 ^a	13.56 ^a	111.15 ^a	59.36 ^a	80.06 ^a	1374.83 ^a	53.10
100	-AMF	14.66 ^d	11.06 ^d	58.75 ^d	53.26 ^d	38.26 ^d	835.54 ^c	0.0
	+AMF	16.66 ^c	11.40 ^c	72.40 ^c	55.7 ^c	56.27 ^c	1186.51 ^b	37.20

Values in each column followed by the same letters are not significantly different at $P \leq 0.05$. NS: not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Analysis of Some Biochemical Parameters

Our results of nutrient contents were recorded in (Table 3), the mycorrhizal common bean plants had higher contents of N, P, K, Ca, and Mg seed contents than those of non-mycorrhizal plants particularly at 50% and 75% levels of NPK fertilizers, such increases in nutrient contents in response to the mycorrhizal effects were highly associated, respectively, with the intensity of mycorrhizal colonization for each treatment. On the other hand, mycorrhizal inoculated common bean plants had sodium content lower than non-mycorrhizal ones at all treatments. In our study, mycorrhizal Common bean plants accumulated less Na ions compared to non-mycorrhizal plants, this prevents Na ions from interfering in metabolic pathways of growth and yield (Evelin *et al.*, 2012). In contrast, the mycorrhizal colonization enhances uptake of N, P, K, Ca and Mg particularly with decreasing NPK fertilizers concentration. Our results are agreement with Khalil and Yousef (2014); Abdullahi and Sheriff (2013) who reported that nutrients were taken up by the hyphae to the plants, which lead to a very efficient mobilization and uptake of phosphate, nitrogen, potassium, magnesium and other elements that were transported to the plant.

In (Table 4) illustrated that both total carbohydrates content and protein content of mycorrhizal common bean plants fungi were significantly greater than those of non-mycorrhizal plants at all concentrations of NPK. In this

connection, the mycorrhizal colonization and NPK showed a significant increase as compared to those non-mycorrhizal plants in particularly at 50% and 75% concentrations of NPK. However, total crude protein and total carbohydrates of the mycorrhizal and non-mycorrhizal root extracts of common bean plants was generally reduced with increasing NPK addition to the soil. These findings are in accordance with the results of Shinde and Thakur (2015) found that the total carbohydrates and protein content increased of mycorrhizal pea plants in the leaves and seeds as compared to control treatment and Egberongbe *et al.* (2010) who showed that the crude protein, carbohydrate content of soybean and dry matter in all treatments there were significant increases of mycorrhizal plants when compared to other non-mycorrhizal plants.

Mycorrhizal Dependency

There was a positive link between improving most of yield parameters and mycorrhizal colonization of common bean plants (Table 5), MD% Were significantly higher particularly at 50 and 75% concentration than other concentration. In addition, these results are in accordance also with Abdullahi and Sheriff (2013) who reported that Root% colonization was affected with increase in fertilizer application in all the treatments. The positive effects are likely attributed to the improvement of phosphorus absorption that reflected on the yield quality (Asrar *et al.*, 2012).

Table 3: Concentrations (%) of N, P, K, Ca, Mg and Na in mycorrhizal(+AMF) and non-mycorrhizal (-AMF) in seeds of common bean plants.

Treatments		N	P	K	Ca	Mg	Na
NPK conc. (%)	AMF status						
0	-AMF	2.43 ^j	0.298 ^j	2.08 ^j	1.99 ^j	1.32 ^j	1.67 ^a
	+AMF	2.47 ⁱ	0.311 ⁱ	2.18 ⁱ	2.09 ⁱ	1.39 ⁱ	1.58 ^b
25	-AMF	2.53 ^h	0.318 ^h	2.26 ^h	2.19 ^h	1.53 ^h	1.51 ^b
	+AMF	2.59 ^g	0.332 ^g	2.34 ^g	2.27 ^g	1.59 ^g	1.40 ^c
50	-AMF	2.65 ^f	0.340 ^f	2.46 ^f	2.37 ^f	1.69 ^f	1.32 ^{cd}
	+AMF	2.91 ^b	0.383 ^b	2.83 ^b	2.71 ^b	2.07 ^b	0.97 ^f
75	-AMF	2.72 ^e	0.354 ^e	2.57 ^e	2.43 ^e	1.79 ^e	1.23 ^d
	+AMF	2.97 ^a	0.397 ^a	2.92 ^a	2.83 ^a	2.13 ^a	0.89 ^f
100	-AMF	2.78 ^d	0.364 ^d	2.65 ^d	2.52 ^d	1.88 ^d	1.13 ^e
	+AMF	2.84 ^c	0.371 ^c	2.76 ^c	2.62 ^c	1.96 ^c	1.08 ^e

Values in each column followed by the same letters are not significantly different at $P \leq 0.05$. NS: not significant;

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 4: Effect of mycorrhizal colonization on total carbohydrates and crude protein (%) in seeds of common bean plants.

Treatments		Crude protein %	Total carbohydrates %
NPK conc. (%)	AMF status		
0	-AMF	13.95 ^j	19.55 ^j
	+AMF	14.20 ⁱ	20.07 ⁱ
25	-AMF	14.56 ^h	20.57 ^h
	+AMF	14.87 ^g	21.06 ^g
50	-AMF	15.24 ^f	21.64 ^f
	+AMF	16.72 ^b	23.65 ^b
75	-AMF	15.63 ^e	22.17 ^e
	+AMF	17.08 ^a	24.19 ^a
100	-AMF	15.98 ^d	22.63 ^d
	+AMF	16.32 ^c	23.13 ^c

Values in each column followed by the same letters are not significantly different at $P \leq 0.05$. NS: not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 5: Mycorrhizal dependency (MD)* for several yield parameters of common bean plants.

Parameters	0	100	75	50	25	LSD
100 Seeds weight	3.28b	4.56b	13.8a	14.95a	4.65b	1.47
Crude protein%	1.81b	2.11b	9.27a	9.69a	2.15b	0.85
Total carbohydrates %	2.65b	2.2b	9.12a	9.31a	2.41b	0.97
Vitamin C mg/100g	5.95b	4.65b	15.54a	16.63a	4.86b	3.51
Folic acid mg/100g	9.69b	6.47c	27.01a	28.22a	8.85bc	2.38
Vitamin B1 mg/100g	10.98b	6.18b	24.51a	28.13a	7.76b	5.85

Values in each row followed by the same letters are not significantly different at $P \leq 0.05$. * $MD = [(M-NM)/NM] \times 100$, where M is parameter value of mycorrhizal plants and NM is parameter value of non-mycorrhizal plants.

Conclusion

Use of NPK fertilizer is common practice among farming community through the world but application of NPK to legumes is limited especially in the developing countries. The results of this study have shown that inoculation with mycorrhizal fungi can enhance yield production of common bean plants as compared to other treatments. Application of NPK fertilizer at 50% and 75% along with VAM gave a significant increase in yield production and yield components like total carbohydrates, total protein, mineral elements and some vitamins which are the components of grain contribute as compared to non-mycorrhizal ones. These led to increase of economic value of common bean also for sustainable agricultural purposes and healthy food production.

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