MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

The effect of arbuscular mycorrhizal fungi on growth of *Phaseolus vulgaris* under varying NPK levels

Gamal Abd Elfatah Owf[1], Samia Ali Haroun, Amr Mohammed[2] Hassan, Esraa Hussien El-said[3]

[1]Botany Dept., Faculty of Science, Mansoura Univ., Dakahlyia, Egypt Emeritus Prof. Mansoura Faculty of Science.,

[2]Associate Prof. Mansoura Faculty of Science

[3]researcher in Mansoura university Faculty of Science

Received:18/6/2020
Accepted:28/7/2020

Abstract: Common bean (*Phaseolus vulgaris* L.) is economically one of the major vegetable crops in Egypt for local consumption as well as for the exportation. The over-use of chemical fertilizers such as NPK negatively affects soil properties making it unsuitable for raising crop plants. The intensive use of these inputs has also resulted in serious health and environmental hazards. For these reasons, biofertilizers used to replace chemical fertilizers. A pot experiment was carried out to explore the effect of 0% NPK, 25% NPK & 100% NPK in combination with arbuscular mycorrhizal fungi (AMF) on *P. vulgaris* growth during vegetative stage. As compared to –v control value, the application of AMF significantly increased growth parameters (at least 117% root length, 110 % shoot length, 117% fresh &dry weight, and 104% water percentage/ plant), photosynthetic pigments (at least 118% total pigments), carbohydrates fractions(at least 160% total carbohydrate), nitrogen, fractions (at least 135%), total protein (at least 135%) and some elements (N "at least 300%", P (at least 457%) & K) of common bean plants grown at 25% NPK and 100% NPK; especially the former. Such stimulations were positively linked with the mycorrhizal colonization in the common bean root tissue.

keywords: Arbuscular mycorrhizal fungi (AMF), *Phaseolus vulgaris* and NPK**.**

1.Introduction

In recent years, legumes consumption in particular, dry beans have increased in many parts of the world due to high nutritional value. The population increase along with desertification has reduced the agricultural production in Egypt leading to food shortage **[42]**.

Common beans are considered as a good and cheap source for protein, trace minerals such as molybdenum and iron **[50]**.

N, P as well as K enhance plant ability to resist both biotic and abiotic stresses **[67]**. However, their excessive use not only useless for cultivated plants but also, they cause soil degradation and pollution of underground water. Chemical fertilizers and their exploitation resulted in air and groundwater pollution by water bodies being eutrophised **[74]**.

Chemical fertilizers could not be totally avoided but the emerging use of biofertilizers beside them lead to the intended integrated nutrient management to improve crop yields through environmentally friendly way **[16]**. Biofertilizers or bio-stimulants refer to the fertilizers that contain plant growth promoting micro-organisms or their products to increase the availability and uptake of mineral nutrients for plants **[70]**.

AM fungi caused an enhancing in plant water and nutrient uptake **[26]**. The most effective advantage of AM fungi is their ability to scavenge phosphorus available through their hyphae, which have large surfaces on which the extra radical hyphae act as a bridge between the plant roots and the soil **[41, 11]**.

Mycorrhizal hyphae penetrate the walls of root cortex cells to form a common mycorrhizal network within and between plant roots that

serves as the key nutrient exchange sites between the plant and the champignon **[47, 62]**.

The combined use of AMF along with optimized dose of NPK chemical fertilizer could be the appropriate integrated nutrient management practice. This experiment aims to evaluate the response of *P. vulgaris* plant to fertilization with 0% NPK, 25% NPK & 100% NPK plus AMF, during vegetative stage.

2.Material and method:

Plants used and growth conditions:

Pure and homogenous seeds of *P. vulgaris* var. Nebraska were supplied by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The chemicals utilized in this investigation were 25% NPK: (0.3g N, 0.4g P and 0.1g K)/pot and 100% NPK: (1.2g N, 1.6g P and 0.4g K)/pot.

Time course of experiment:

A homogenously-sized lot of *P. vulgaris* var. Nebraska (beans) homogenous seeds were subjected to surface sterilization in 0.01% $HgCl_2$ solution for 3 minutes. Before sowing, the seeds washed thoroughly with tap water.

The seeds were then divided into two groups as the following;

1) The seeds under three levels of NPK (nitrogen, phosphorous and potassium) fertilizers 100%NPK and 25%NPK as well as 0%NPK to serve as –ve control.

2) The seeds under the three levels of NPK fertilizers (0%NPK, 25%NPK and 100%NPK) in addition to arbuscular mycorrhizal fungi for each.

Half of the pots received arbuscular mycorrhizal inoculum at the rate of 10 g of trapped soil (about 50 spores / one gram soil) and 0.5 g of sudan grass infected roots (M = 70%) colonized by stock culture of mycorrhizal fungi per pot. The inoculum was placed 5 cm below garlic cloves before sowing to produce mycorrhizal treatment. Non-mycorrhizal plants received equal amount of autoclaved inoculum to produce the same nutrients without mycorrhizal propagules.

P. vulgaris var. Nebraska seeds were cultivated at 3 December 2018. The pots (five seeds/pot) were kept in field; at the open garden of Faculty of Science Mansoura University, under normal day/night conditions and irrigated when required with equal amounts of tap water.

The collected samples at the vegetative stage were used for assessment of growth parameters (shoot length، root depth, plant fresh & dry weights and plant water percentage)، as well as some metabolic components; pigments, carbohydrates constituents, nitrogen fractions, total nitrogen, some anti-oxidant enzymes (polyphenol, peroxidase & catalase), some elements (N, P & K) in addition to evaluation of (VAM) inoculum.

Analysis of the data was carried out by least significant difference (L.S.D) test at probability of 0.05. ANOVA analysis was done with the IBM SPSS-20 statics software **[63]**.

1-Estimation of photosynthetic pigments:

Leaf pigments chlorophylls and carotenoids were determined by using spectrophotometer according the method adopted previously **[8]** for chlorophylls and carotenoids **[32]** as adopted by **[38]**.

2-Estimation of carbohydrates:

The methods used for extraction of different carbohydrate fractions were essentially those of **[73]** and **[29]**. The contents of glucose were estimated by the O-toluidine procedure of **[22]** and modified by **[52]**. Sucrose content was determined using modification of the procedures of **[29]**. The total soluble sugars in plant extracts were estimated by the anthrone reagent according to modification of the procedures of **[73]**. The method used for estimation of polysaccharides (starch) in the present study was as described previously **[65]**. Total carbohydrates were calculated as the totaling of the amount of total soluble sugars and polysaccharides of the same sample.

3-Estimation of nitrogenous constituents:

The method of extraction was essentially that adopted by **[72]**. Ammonia-N fraction was measured spectrophotometrically using Nessler's reagent **[17]** that modified by **[46]**. The method used for estimation of amide-N was that recommended by **[46]**. The method used for estimation of amino-N in the present study was designed by **[45]**. Protein extraction and determination in the plant extract was determined spectrophotometrically according to **[12]**. The total nitrogen was calculated by

Kjeldahl's process of semi-micropropagation **[51]** and described by **[30]**.

4-Estimation of antioxidant enzymes activity:

Fresh tissue (0.2 g) were homogenized and extracted according to **[7]**. POX activity was assayed by monitoring the increase in the absorbance at 420 nm **[18]**. PPO activity was assayed as the increase in absorbance at 420 nm due to the formation of purpurogallin **[18]**. Catalase activity was assayed by the method of **[59]** that modified by **[25]**.

5- Estimation of elements:

Estimation of phosphorus:

In presence of a reducing agent, this method relays on the formation of a blue color complex of phosphate and molybdic acid following the method of **[40]** as described by **[33]** and adopted by **[30]**.

Estimation of K:

For K estimation, a known dry matter was digested according to the method described previously **[14]**.

6-Evaluation of vesicular-arbuscular mycorrhizal inoculums:

Isolation of Mycorrhiza:

A mixture of arbuscular mycorrhizal (AM) spores, extracted from the rhizosphere of common bean plants in Dakhalia region by using the wet sieving and decanting technique **[23]**.

Multiplication of VAM inoculums:

The collected spores were surface sterilized as described by **[3]** to eliminate carryover of contaminating microorganisms. These spores were inoculated in onion seedlings (Allium cepa) (as standard) planted in sterilized plastic pots (30 cm in diameter) filled with sandy clay soil. The plants were watered when necessary. After three months of planting, the spores were extracted as described before. Inoculums from it can be used to establish a large number of stock plants to provide inoculums for further experiments. Subsequent inoculation of stock can be done with small amount of soil from the purified cultures. These form inoculums rapidly produce new infections.

Staining of VAM infected root:

The roots of common bean roots were gently detached and washed several times by tap water. Then the roots were cut into small segments (0.5-1.0 cm) and heated at 900C for 45 min., in 10% KOH to remove host cells cytoplasm and nuclei. Root pieces were then immersed in 0.05% trypan blue (Sigma) in lacto-phenol **[49]** for l5 min. at 90^0C. After removing excess stain, 30 randomly selected root segments were mounted in Lacto-glycerol, squashed and microscopically examined.

Estimation of AM Infection:

The rate of AM infection was microscopically estimated according to the method of **[66]**. This method calculates three parameters of infection as follows:

F: Frequency of root colonization.

M: Intensity of cortical infection.

A: Arbuscule frequency in roots.

Statistically Analysis:

Data were subjected to (stat graphic- vers-4-2 Display ANOVA), followed by mean separation using the LSD at $P<0.05$

Results and discussion

Changes in growth parameters:

The changes in the determined physical growth parameters of *P. vulgaris* plant, at the vegetative stage, in response to different treatments are presented in Tables (1 & 2).

The shoot length, fresh and dry weight, root length and fresh and dry weight of *P. vulgaris* increased significantly in response to the used treatments. On the other hand, the shoot water percentage of *P. vulgaris* plant increased significantly in response to treatment with 100% NPK+AMF and non-significantly increase with all treatments except, in case of treatment with 100% NPK where a non significant decrease was observed, as compared to –v control value. Concerning the root water percentage, of *P. vulgaris* plant non significantly increase with the used treatments was detected except, in case of treatment with 25% NPK+AMF where a non-significant decrease was observed, as compared to –v control value.

In the majority of cases, the application of 25% NPK+AMF was the most effective

treatment as compared to other treatments, on the other hand, the application of recommended dose 100% NPK was more effective than other treatments (See tables 1 & 2). Other study showed that the AMF are the most important symbiotic association for the majority of plants that improves their nutrient content, growth, and productivity of the host plants **[27, 10]**. And this could be attributed to the improvement of P and N by the interaction between plant roots and their hyphal networks. This response pattern for mycorrhizal infection in low P soils is fully consistent with previous studies **[4, 60, 55]**.

AMF are biotrophic in nature which enhances plant nutrient status through symbiotic association with the living plant root system. In this mutualistic symbiotic relationship, AMF provide phosphorus and other nutrients for the plant in return for photosynthesis **[53]**.

AMF form a symbiotic association with higher plants that facilitating nutrient uptake of phosphorus, zinc, copper, etc. Phosphorus plays an important role in plant metabolism, particularly in cell division, growth, photosynthesis, sugar breakdown, nutrient transport and metabolic pathway regulation **[62]**.

Colonization of plant roots by AMF significantly increased phosphorus and nitrogen uptake in the plant. AM fungi's most prominent contribution was by extra-radical mycorrhizal hyphae **[42]**.

Changes in photosynthetic pigments:

The changes in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, total chlorophylls, chlorophyll a/b and total pigments) of *P. vulgaris* plant, at the vegetative stage, in response to different treatments are presented in Table (3).

In relation to the -ve control value, the chlorophyll a, carotenoids, total chlorophylls (a+b) and total pigments contents of *P. vulgaris* plant significantly increased in response to the used treatments, except for the non significant decrease which detected in case of treatment with 100% NPK in chlorophyll b and with 25% NPK+AMF and 100%NPK+AMF for chlorophyll a / b fraction, as compared to –v control value.

In the majority of cases, the application of 25%NPK+AMF was the most effective treatment as compared to the other treatments, on the other hand, the application of recommended dose 100% NPK was the second effective (See tables 3 & 4). In this concern, the effects of mycorrhizal fungi on increasing of chlorophyll content in plants may be due to increase the uptake of phosphorous and photosynthetic activity of the plants **[35]**. Some species of mycorrhizae caused an increase of the chlorophyll a, b and total chlorophyll in *Pogostemon patchouli* **[54]**.

Common bean plants inoculated with mycorrhizal fungi showed a significant increase in photosynthetic pigments content, this could be attributed to greater rate of photosynthesis in inoculated plants due to the increased content of nitrogen and magnesium (major components of chlorophyll molecules) in mycorrhizal plants. According to **[39]** who have been stated that the mycorrhizal fungi can be attributed to increase Mg and Fe uptake which are essential for chlorophyll biosynthesis.

Changes in carbohydrate contents:

Glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates of *P. vulgaris* plant, at the vegetative stage of growth, in response to different treatments are presented in Table (4).

Throughout the vegetative stage of plant growth, a general significant increase above the -ve control levels were observed for glucose, sucrose, polysaccharides and consequently total carbohydrates content in *P. vulgaris* plant in response to the used treatments. Concerning the total soluble sugars content of *P. vulgaris* plant, this parameter increased significantly in response to 100% NPK treatment but, non significant increases were detected in response to the other used treatments. The increase in carbohydrate fractions for AMF-treated plants might be explained as a response of plants to modulate its photoassimilates toward AM roots to sustain intraradical as well as extraradical mycelia.

Bago *et al*, [9] reported that the central to the flow of carbon in the AM symbiosis may be the glyoxylate cycle. In this study, using $^{13}C3$ to analyze the structural carbohydrates provided to AM suggested that the provided glycogen

performs four roles in AM symbiosis; host sequestration of hexose; long-term storage of spores; translocation from intra-radical mycelium to extra-radical mycelium; and buffering for intracellular hexose levels throughout the life cycle.

AMF have been shown to act as improving to the soil structure **[44**] and have a great importance due to their good capability to increase the plant growth and yield through efficient nutrient uptake **[2, 68]**.

Changes in nitrogen content:

The changes in ammonia-N, amino-N, amide-N, total proteins and total-N of *P. vulgaris* plant, at the vegetative stage of growth, in response to different treatments are presented in Table (5).

Throughout the vegetative stage of plant growth a significant increase above the -ve control levels, was observed for ammonia-N, amino-N, amide-N, total proteins and total-N content in *P. vulgaris* plant in response to the used treatments.

Dumas *et al*, [19] reported that protein content increased in *Nicotiana tabacum* and *Allium cepa* roots in response to inoculation with AMF.

The total protein content of mycorrhizal infected common bean plants compared with non mycorrhizal plants was greater significantly. These findings are in harmony with the results of **[57]** who stated that the total carbohydrates and total protein content increased in mycorrhizal pea plants in the leaves and seeds comparing to control treatment and **[20]** who reported that the crude protein and carbohydrate content of soybean as well as dry matter in all treatments were increased significantly in mycorrhizal plants when compared to other non-mycorrhizal treated plants.

Changes in anti-oxidant enzymes activity:

The changes in the activity of the anti-oxidant enzymes (polyphenol, peroxidase and catalase) of *P. vulgaris* plant, during vegetative stage, in response to different treatments are presented in Table (6).

In relation to the –ve control value, the polyphenol, peroxidase and catalase enzymes activity of *P. vulgaris*, a general significant decrease in response to the used treatments was detected except, in case of treatment with 100% NPK+AMF where a non significant increase was detected.

Stress conditions lead to the accumulation of ROS and consequently antioxidant enzymes are the defending compounds that actively participate in the detoxification of ROS such as superoxide and H_2O_2 **[21, 34]**. Catalase for example is involved in scavenging H_2O_2 **[31]** and thus contributes to the plant defense mechanism against oxidative stress **[24, 15]**.

AMF can retared the level of reactive oxygen species (ROS) by enhancing the activities of antioxidant enzymes (SOD, POD and CAT) under stressful conditions **[75, 71]**. Furthermore, it has been reported that the *Glomus mosseae* stimulated the accumulation of enzymes and amino acids resulting in resistance development of the host plants. He also suggested that *Glomus mosseae* is known to stimulate the development of the enzymes and the accumulation of arginine, which produces host plant resistance **[64].**

Changes in some elements content (N, P and K):

The change in N, P and K content of *P. vulgaris* plant, at the vegetative stage, in response to different treatments are presented in Table (7). It is apparent from the tabulated data that, N and P content of *P. vulgaris* increased significantly in response to the used treatments, as compared to the –ve control value. Throughout the vegetative stage of plant growth a non significant increase, in general above the -ve control levels, was observed for K content in response to the used treatments of *P. vulgaris* plant.

AMF stimulates the absorption of nutrients leading to an increase in dry matter, typically representing several increases for plant species with high mycorrhiza dependency **[48]**. Furthermore, in harmony with **[37, 5]** who stated that nutrients were taken up by the hyphae to the plants, AMF treatment led to a very efficient uptake and mobilization of phosphate, nitrogen, potassium, magnesium and other elements which were transported to the plant.

These results are confirmed by **[36]** who reported that nutrients were absorbed by the

plant via AM hyphaea, which lead to a very efficient mobilization and uptake of phosphate, nitrogen, potassium, magnesium, copper, zinc, boron, sulphur and other elements that were transported to the plant.

Mycorrhizal colonization:

Mycorrhizal colonization as frequency of root colonization (F%), intensity of cortical colonization (M%) and arbuscules frequency in roots (A%) are given in Table (8). The results showed that, the level of mycorrhizal root colonization continued to increase with the increase in plant growth in the used treatments and vesicles were showed in colonized root. At the same time the mycorrhizal infection were significantly reduced by increasing the concentration of NPK fertilizers at 100% concentration while significantly increased at 25% and 0% concentrations of NPK fertilizers. Thus, it could be concluded that the high doses of chemical fertilizers negatively affect AMF colonization. In low amount of added chemical fertilizer, mycorrhizal fungi were able to increase the root infection significantly.

Many studies showed that, the increased levels of P in soil generally decrease the colonization of plant roots by vesi- cular-arbuscular mycorrhizal (VAM) fungi **[1]**. Also, **it** has been shown that the control of mycorrhizal colonization is linked to P in the host plants **[43]**. Moreover, **[58]** it has been reported that the use of VAM led to an increase in yield and reduced 25% of the used NPK.. The intensity of mycorrhizal colonization of common bean plants was retarded gradually with increasing NPK concentrations in the soil. However, a significant increment was observed in common bean plants grown either in 50% or 75% concentration of NPK compared with other concentrations.

AMF can stimulate the nutrient uptake leading to the yield significant increase. This result supports the findings of previous studies **[61, 13, 56]** those indicated that increased soil P generally decrease AMF development.

In addition, these results are in agree with a previous study that concluded root% colonization increased in response to fertilizer application in all the used treatments **[6]**. Thus, the high conc. of chemical fertilizers caused antagonistic interaction with mycorrhiza. Further studies supported this finding **[69, 28]**.

In conclusion: From the aforesaid results, this study confirmed that co-evolution between AMF and *P. vulgaris* plants stimulate the determined parameters under the used NPK fertilization especially 25% NPK and thus the bio-fertilizer (AMF) could replace the chemical fertilizer (100% NPK).

Table (1): The effect of arbuscular mycorrhizal fungi (AMF) on growth parameters of *P. vulgaris* during vegetative stage.

Parameters		Shoot Length (cm)	Shoot Fresh Weight (g)/Plant	Shoot Dry Weight (g)/Plant	Shoot Water Percentage (%)
NPK (%)	Mycorrhizal status				
0	- AMF(-v control)	12.93	3.47	0.37	89.24
	+ AMF	14.40*	5.40*	0.53*	90.12
25	- AMF	13.70	5.93*	0.53*	91.00
	+ AMF	15.70*	6.60*	0.63*	90.39
100	AMF-	16.03*	7.03*	0.80*	88.62
	+ AMF	14.33*	5.47*	0.37	93.15*

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (2): The effect of arbuscular mycorrhizal fungi (AMF) on growth parameters of *P. vulgaris* during vegetative stage.

Parameters		Root Length (cm)	Root Fresh Weight (g)/Plant	Root Dry Weight (g)/Plant	Root Water Percentage (%)
NPK (%)	Mycorrhizal status				
0	- AMF(-v control)	27.30	0.60	0.17	72.22
	+ AMF	34.47*	1.20*	0.23	80.26
25	- AMF	32.83*	1.43*	0.30	79.36
	+ AMF	38.07*	1.57*	0.50*	65.17
100	AMF -	36.48*	2.53*	0.50*	79.96
	+ AMF	32.20*	0.90	0.20	76.33

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (3): The effect of arbuscular mycorrhizal fungi (AMF) on photosynthetic pigments (mg/g fresh weight) of *P. vulgaris* during vegetative stage

Parameters		Chl a	Chl b	Carotenoids	Chl a +Chl b	Chl a/Chl b	Total Pigments
NPK (%)	Mycorrhizal status						
0	- AMF(-v control)	0.75	0.56	0.29	1.31	1.33	1.60
	+ AMF	0.92*	0.60	0.35*	1.53*	1.54*	1.88*
25	- AMF	0.98*	0.71*	0.39*	1.69*	1.38	2.08*
	+ AMF	1.17*	0.95*	0.56*	2.11*	1.24	2.67*
100	AMF -	1.15*	0.52	0.45*	1.68*	2.22*	2.13*
	+ AMF	0.97*	0.85*	0.49*	1.83*	1.14	2.32*

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (4): The effect of arbuscular mycorrhizal fungi (AMF) on carbohydrates contents (mg/g dry weight) of *P. vulgaris* during vegetative stage.

Parameters		Glucose	Sucrose	Total Soluble Sugars	Polysaccharides	Total Carbohydrates
NPK (%)	Mycorrhizal status					
0	- AMF(-v control)	7.12	27.24	40.11	22.41	96.88
	+ AMF	29.09*	54.91*	48.58	46.38*	178.97*
25	- AMF	13.62*	46.22*	47.62	25.47	132.93*
	+ AMF	39.77*	75.29*	52.12	63.36*	230.54*
100	AMF -	42.09*	67.03*	70.79*	59.80*	239.71*
	+ AMF	19.19*	40.54*	44.51	50.00*	154.24*

Significant increase or decrease at 0.05 LSD.

Table (5): The effect of arbuscular mycorrhizal fungi (AMF) on nitrogen fractions (mg/g dry weight) of *P. vulgaris* during vegetative stage.

Parameters		N-ammonia	N-amide	N-amino	Total-N	Total Protein
NPK (%)	Mycorrhizal status					
0	- AMF(-v control)	5.92	1.69	31.18	259.00	161.88
	+ AMF	12.35*	4.05*	82.09*	360.00*	225.00*
25	- AMF	11.22*	3.70*	64.08*	385.00*	240.63*
	+ AMF	19.71*	5.61*	97.73*	390.00*	243.75*
100	AMF -	23.34*	8.18*	106.07*	420.00*	262.50*
	+ AMF	10.41*	2.86*	56.11*	350.00*	218.75*

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (6): The effect of arbuscular mycorrhizal fungi (AMF) on anti-oxidant enzymes activity (µ mole/g fresh weight/min) of *P. vulgaris* during vegetative stage.

Parameters		Polyphenol	Peroxidase	Catalase
NPK (%)	Mycorrhizal status			
0	- AMF(-v control)	4.05	1.84	8.96
	+ AMF	2.37*	1.71	4.60*
25	- AMF	3.23*	1.57*	8.19
	+ AMF	1.45*	1.56*	2.25*
100	AMF -	2.53*	1.60*	4.08*
	+ AMF	4.06	2.42*	11.87*

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (7): The effect of arbuscular mycorrhiz

al fungi (AMF) on some elements contents (N, P and K) (mg/g dry weight) of *P. vulgaris* during vegetative stage.

Parameters		N	P	K
NPK (%)	Mycorrhizal status			
0	- AMF(-v control)	1.00	0.35	0.37
	+ AMF	7.00*	2.03*	0.38
25	- AMF	3.01*	1.75*	0.39
	+ AMF	11.01*	2.80*	0.44
100	AMF -	10.02*	3.08*	0.51
	+ AMF	4.02*	1.61*	0.36

Least significant difference (LSD) increase or decrease at $p<0.05$

Table (8): Effect of NPK fertilizers on the level of mycorrhizal colonization (F%), intensity of mycorrhizal colonization (M%) and arbuscular frequency (A%) of *P. vulgaris* during vegetative stage.

Parameters		F%	M%	A%
NPK (%)	Mycorrhizal status			
0	- AMF(-v control)	0.00	0.00	0.00
	+ AMF	90.00*	11.80*	9.30*
25	- AMF	0.00	0.00	0.00
	+ AMF	90.00*	23.40*	18.70*
100	AMF -	0.00	0.00	0.00
	+ AMF	70.00*	11.00*	8.60*

4. References

1. Abb-ott L K and Robson A D, (1984): The effect of VA mycorr- hizae on plant growth, pp. 113-130. In VA Mycorrhiza. Eds. C L1 Powell and D J Bagyaraj. CRC Press, Boca Raton, Florida.
2. Abd Allah M. M. S., El-Bassiouny H. M. S., Bakry B. A. and Sadak M. S. (2015). Effect of arbuscular mycorrhiza and glutamic acid on growth, yield, some chemical composition and nutritional quality of wheat plant grown in newly reclaimed sandy soil. *Journal of Pharmaceutical, Biological and Chemical Sciences*, **6**, 1038-1054.
3. Abdel-Fattah G. M. (1991). Some ecological and physiological studies on vesicular arbuscular mycorrhizal fungi. Ph. D. Thesis, Botany. Departement, Faculty of Science. Mansoura University. Egypt, p 202.
4. Abdel-Fattah G. M. and Asrar A. W. A. (2011). Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (Triticum aestivum L.) plants grown in saline soil. Acta Physiologiae Plantarum, **34**, 267-277.
5. Abdullahi R and Sheriff HH (2013) Effect of Arbuscular Mycorrhizal Fungi and Chemical Fertilizer on Growth and shoot nutrients content of Onion under Field Condition in Northern Sudan Savanna of Nigeria. IOSR-JAVS. **5** (**3**): 85-90.
6. Abdullahi R and Sheriff HH (2013) Effect of Arbuscular Mycorrhizal Fungi and Chemical Fertilizer on Growth and shoot nutrients content of Onion under Field Condition in Northern Sudan Savanna of Nigeria. IOSR-JAVS. **5 (3):** 85-90.
7. Agarwal, S. and Shaheen, R. (2007): Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of Mormoordica charantia. Braz. *Journal of Plant Physiology*, **19**: 149-161.
8. Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta vulgaris. Plant Physiol., 24: 1-15.
9. Bago,B., Shachar-Hill, Y.and Pfeffer, P.E., (2000): Carbon metabolism and transport in arbuscular mycorrhizae, Pl.Physiol., **124:**949-957.
10. Berendsen R. L., Pieterse C. M. and Bakker P. A. (2012). The rhizosphere microbiome and plant health. Trends in Plant Science, **17**, 478-486.
11. Bianciotto V, Bonfante P. (2002). Arbuscular mycorrhizal fungi: a specialized niche for rhizospheric and

endocellular bacteria. Anton van Leeuwenhoek. **81**:365- 371.
12. Bradford, M.M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein-dye-binding. Anal. Biochemistry, **72**: 248-251.
13. Cavagnaro TR, Smith FA, Smith S E and Jackobsen, I (2005) Functional diversity in arbuscular mycorrhizas: Exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell and Enviro. **146**:485-491. DOI: 10.1111/j.13653040.2005.01310.x
14. Chapman, H.D. and Pratt, P.F. (1978). Method of Analysis for Soil, Plant and Water, California University, Division Agrc. Sci., Priced Publication, pp 50,169.
15. Cheynier V, Comte G, Davies K M, Lattanzio V and Martens S (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol. Biochem. 72,1–20.doi:10.1016/j.plaphy.2013.05.009
16. Das, K., R. Dang, T. N. Shivananda, and N. Sekeroglu. 2007. "Influence of bio-fertilizers on the biomass yield and nutrient content in Stevia rebaudiana Bert. grown in Indian subtropics". *Journal of Medicinal Plant Research*, **1**:5–8.
17. Delory, M. (1949). Colourimetric estimation of ammonia. In: Vogel, H. J. (ed.): Inorganic chemistry. Pp. 126-132. Longman, London.
18. Devi, P. (2002): Principales and Methods in plant molecular biology, biochemistry and Genetics. Agro biology, India, pp .41.
19. Dumas,G.E., Guillaume, P.,Tahiri, A.A.,Gianinazzi-Pearson, V. and Gianinazzi, S., (1994), Changes in polypeptide patterns in tobacco roots by *Glomus* species, Mycorrhiza, 4: 215-221.
20. Egberongbe HO, Akintokun AK, Babalola OO and Bankole MO (2010) The effect of Glomus mosseae and Trichoderma harzianum on proximate analysis of soybean (Glycine max (L.) Merrill.) seed grown in sterilized and unsterilised soil. *JARED***. 4(2):** 54-58.
21. Esfandiari, E., Shekari, F., Shekari, F., and Esfandiari, M. (2007). The effect of salt stress on antioxidant enzymes activity and lipid peroxidation on the wheat seedling. Notulae Botanicae Horti Agrobotanici Cluj Napoca **35**, 48–56. doi:10.1021/acs.jafc.6b03665
22. Fetris, A.W. (1965): A serum glucose method without protein precipitation. Amer. *J. Medical Tech***. 31**, 17-21.
23. Gerdemann J. W. and Nicolson T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society, **46**, 235-244.
24. Gill S S, and Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.doi:10.1016/j.plaphy.2010.08.016
25. Gopalakrishnan V.K and T. Starlin : ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT PROPERTIES OF Tylophora Pauiflora Wight and Arn. aE "AN IN VITRO STUDY". *Asian Journal of Pharmaceutical and Clinical Research*, vol.**6**, no.8, sept.2013, pp. 68-71.
26. Gosling P, Hodge A, Goodlass G, Bending GD. 2006. Arbuscular mycorrhizal fungi and organic farming. Agric Ecosystems Environ. **113**:17-35.
27. Grover M., Ali S. Z., Sandhya V., Rasul A. and Venkateswarlu B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World *Journal of Microbiology* and Biotechnology, 27, 1231-1240.
28. Gryndler M , Larsen J, Hrselová H, Rezácová V, Gryndlerová H and Kubát J (2005) Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in long-term field experiment .Mycorrhiza. **16(3)**:159-166. DOI: 10.1007/s00572-005-0027-4
29. Handel, E.V. (1968): Direct micro-determination of sucrose. Analyt. Biochem., **22**: 280-283.
30. Haroun, S.A .(1985). Studies on adaptation of plants to water stress. Ph. D. Thesis, Bot. Department, Fac. Sci. Mans. Univ. Mansoura. Egypt.
31. Horemans N, Foyer C H, Potters G and Asard H. (2000). Ascorbate function and associated transport systems in plants.

Plant Physiol. Biochem. **38**,531–540. doi:10.1016/S0981-9428(00)00782-8

32. Horvath, G.; Kissimon, J. and Faludi, D. A. (1972): Effect of light intensity of carotenoids in normal and mutant leaves. Phytochem. 11: 183-187.
33. Humphries, E.C. (1956). Mineral components and ash analysis. In modern method of plant analysis .Vol. 1, (edited by K. Paech and M.V. Tracey). 468–502. Berlin: Springer Verlag.
34. Jaleel C A, Gopi R, Manivannan P and Panneerselvam R. (2007). Antioxidative potentials as a protective mechanism in Catharanthus roseus (L.) G. Don. plants under salinity stress.Turk. *J. Bot*. 31,245–251
35. Karthikeyan B., Joe M. and Cheruth A. (2009). Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations. *Journal of Scientific Research*, **1**, 381-386.
36. Khalil AA (2013) Significance of Some Soil Amendments and Phosphate Dissolving Bacteria to Enhance the Availability of Phosphate in Calcareous Soil. ISRN Soil Science. (2013):1-7. DOI: 10.1155/2013/438949
37. Khalil SE and Yousef RMM (2014). Interaction Effects of Different Soil Moisture levels, Arbuscular Mycorrhizal Fungi and Three Phosphate Levels on: I-Growth, Yield and Photosynthetic Activity of Garden Cress (*Lepidium sativum* L.) plant. *IJAR*. **2(6):**723-737.
38. Kissimon, J. (1999): Analysis of the photosynthetic pigment composition. Inter. Workshop and training course on Microalgal Biol. and Biotech. Mosonmagyarouar, Hungary, pp: 13-26.
39. Krishna H., Singh S. K., Sharma R. R., Khawale R. N., Grover M. and Patel V. B. (2005). Biochemical changes in micropropagated grape (Vitis vinifera L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during ex vitro acclimatization. Scientia Horticulturae, **106**, 554-567.
40. Kuttner, T. and Lichtenstein, L. (1932): Micro-colorimetric studies: Estimation of organically-bound phosphorus. A system of analysis of phosphorus compounds in blood. *J. of biological chem*., **95**: 661-670.
41. Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL. 2000. Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maize (Zea mays L.) grown in soil at different P and micronutrient levels. Mycorrhiza. **9**:331-336.
42. Makoi, J.H.J.R. and Ndkakidemi, P.A. (2009). The agronomic potential of vesicular-arbuscular mycorrhiza (VAM) in cereals- legume mixtures in Africa. African Microbiological Research, **3**: 664-675.
43. Menge J A, Steirle D, Bagyaraj D J, Johnson E L V and Leonard R T, (1978): Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol. **80**, 575-578.
44. Miller R. M. and Jastrow J. D. (2000). Mycorrhizal Fungi Influence Soil Structure. Arbuscular Mycorrhizas: Physiology and Function. Springer Science, p3-18.
45. Muting, D. and Kaiser, E. (1963). Spectrophotometric method of determination of α-amino-N in biological material by means of the ninhydrin reaction. Hopper Seyler's *Z. J. physiol. Chem*.**, 332:** 276-289.
46. Naguib, M.I. (1964). Effect of sevin on carbohydrates and nitrogen metabolism during the germination of cotton seeds. *Ind. J. Exp. Biol*., **2** :149-155.
47. Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Reviews Microbiology, **6**: 763–775.
48. Pharudi JA (2010) Effect of mycorrhizal inoculation and phosphorus levels on growth and yield of wheat and maize crops grown on a phosphorus deficient sandy soil. M.Sc. Thesis, College of Agriculture, University of Stellenbosch.South Africa.
49. Phillips J. M. and Hayman D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, **55**, 158-161.

50. Queiroz K. d. S., Oliveira A. C. d., Helbig E., Reis S. M. P. M. and Carraro F. (2002). Soaking the Common Bean in a Domestic Preparation Reduced the Contents of Raffinose-Type Oligosaccharides but Did Not Interfere with Nutritive Value. *Journal of Nutritional Science and Vitaminology*, **48**, 283-289.
51. Rees, M.W. and Williams, E.F. (1943): The total nitrogen content of egg albumin and other proteins.*JournalofBiochemistry*, **37:** 354-359.
52. Riazi, A.; Matsuda, K. and Arslan, A. (1985). Water stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. *J. Exp. Bot.* , **3**6: 1716-1725.
53. S. E. Smith and D. J. Read, (1997). Mycorrhizal symbiosis, 2nd End. Academic Press, London.
54. Selvaraj T., Nisha M. C. and Rajeshkumar S. (2009). Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of Pogostemon patchouli Pellet. Maejo International *Journal of Science and Technology*, **3**, 222-234.
55. Sheng M, Lalande R, Hamel C and Zladi N (2013) Effect of longterm tillage and mineral phosphorus fertilization on arbuscular mycorrhizal fungi in a humid continental zone of Eastern Canada. Plant Soil. 369:599-613. DOI: 10.1007/s11104-013-1585-4
56. Sheng M., Lalande R., Hamel C. and Ziadi N. (2013). Effect of long-term tillage and mineral phosphorus fertilization on arbuscular mycorrhizal fungi in a humid continental zone of Eastern Canada. Plant Soil, **369**, 599-613.
57. Shinde BP and Thakur J (2015) Influence of Arbuscular mycorrhizal fungi on chlorophyll, proteins, proline and total carbohydrates content of the pea plant under water stress condition. *Int.J.Curr.Microbiol.App.Sci*. **4(1):** 809821.
58. Singh KP and Kallo G (2000) Nutrient management in vegetable crops. Fetil. News. **4 (45)**: 77-81.
59. Sinha. AK (1972): Colorimetric assay of catalase. Anal Biochem, **47**: 389-394.
60. Smith S. E., Jakobsen I., Grønlund M. and Smith F. A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology, **156**, 1050-1057.
61. Smith SE and Gianinazzi-Pearson V (1990): Phosphate uptake and arbuscular activity in mycorrhizal Allium cepa L.: Effect of photon irradiance and phosphate nutrition. Aust. *J. Plant Physiol*. **17**:177-188. DOI: 10.1071/PP9900177
62. Smith, S.E. and D.J. Read, (2008). Mycorrhizal Symbiosis. 3rd ed. Academic Press New York, USA.
63. Snedecor, G.W. and Cochran, W.G. (1982): Statistical Methods. The owa State University Press 7th Edit, 2nd Printing. 507 pp. Soilm Sci., **81**: 173–192.
64. Subba Rao, N.S, (1993), In: Biofertilizers in Agriculture and forestry, Oxford and IBH Publishing Co.Pvt.Ltd.,New Delhi,136-149.
65. Thayermanavan, V. and Sadasivam, S. (1984). Quoted from Biochemical Methods. (Sadasivam, S. and A. Manikam, eds) 2 nd ed., 11-12. New Ag. Inter. Limit. Publ. New Delh I, India.
66. Trouvelot A., Kough J. L. and Gianinazzi-Pearson V. (1986). Mesure des taux de mycorhization VA d'un systeme radiculaire. Recherche de methode d'estimation ayant une signification fonctionnelle. In: Physiological and Genetical Aspects of Mycorrhizae (De by V. Gianinazzi-Pearson & S. Gianinazzi). Institut National de la Recherche Agronomique Press, Paris, France, p217-221.
67. Tsai, S.H., Liu, C.P. and Yang, S.S. (2007). "Microbial conversion of food wastes for biofertilizer production with thermophilic lipolytic microbes". Renewable Energy, **32(6)**, 904-915.
68. Turkmen O., Sensoy S., Demir S. and Erdinc C. (2008). Effects of two different AMF species on growth and nutrient

content of pepper seedlings grown under moderate salt stress. African *Journal of Biotechnology*, **7**, 392-396.
69. Valentine AJ, Osborne BA and Mitchell DT (2001) Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. Sci. Horti. 88: 177-189. DOI: 10.1016/S0304-4238(00)00205-3
70. Vessey, J.K. (2003). "Plant growth promoting rhizobacteria as biofertilizers". Plant Soil, **255**:571-586.
71. Yang Y, Han X, Liang Y, Ghosh A, Chen J, Tang M. (2015).The combined effects of arbuscular mycorrhizal fungi (AMF) and lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in Robinia pseudoacacia L. Plos One. **10(12):** e0145726.
72. Yemm, E. W. and Willis, A. J. (1956). The respiration of barely plants. IX. The metabolism of roots during the assimilation of nitrogen, New Phytol. ,P., **55**, 229-252.
73. Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates by *anthrone. Biochem. J.* , 57: 508-514.
74. Youssef, M.M.A. and Eissa, M.F.M. (2014). "Biofertilizers and their role in management of plant parasitic nematodes. A review". E3 *Journal of Biotechnology and Pharmaceutical Research*, **5**:1–6. 53.
75. Zhu X, Song F, Xu H. (2010). Influence of arbuscular mycorrhiza on lipid peroxidation andantioxidant enzyme activity of maize plants under temperature stress. Mycorrhiza. **20**:325–332.