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Synergistic Effects of inoculating Arbuscular Mycorrhizal Fungi and Foliar Iron Fertilizer on Broad Bean Growth and Yield Under North Sinai Conditions

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ABSTRACT

This work has been done at the Experimental Farm of Fac. Environ. Agric. Sci., Arish University, North Sinai, Egypt. The experiment was run for two successive growing winter seasons during 2016-2018. The synergistic effects of Arbuscular mycorrhizal fungi (AMF) inoculation and foliar iron fertilizer on plant growth and yield parameters of broad bean plant (*Vicia faba* L.), grown in sandy loam soil, were conducted. Mycorrhizal colonization and their spore intensity, nodule formation and their number were also investigated. Two mycorrhizal treatments (Inoculum AMF and native AMF) and five foliar Fe-concentrations (0.0, 300, 400, 500 and 600 ppm) were applied. The interaction between different categories of AMF and foliar Fe-fertilization was also evaluated. The results obtained revealed that the application of inoculated AMF and spray with Fe at 400 ppm increased spore count and root colonization. However, total number of active nodules/plant increased with inoculated AMF and spraying iron at 600 ppm. Results indicated the superiority interaction treatment of inoculated AMF with the foliar Fe at the concentration of 400 ppm and/or 500 ppm by which it recorded the highest values of plant growth traits; viz, root length, number of branches/plant, total dry weight/plant and photosynthetic pigments content in both seasons. Moreover, the same treatment recorded the highest values with all pod characters and yield, except weight of number of green seeds/pod in first season and average pod weight (g) in the second one.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Broad bean, foliar iron, Plant growth and Yield

INTRODUCTION

Egypt is located in arid and semi-arid region. The total area of arable land is $3x10^{10}$ m^2, which is only 3% of the total area of Egypt and the rest is desert. Agricultural land in Egypt is considered one of the world's most intensive agricultural systems (Gheda and Ahmed, 2015; Salama *et al.*, 2017).

Legumes are the major direct source of protein for both human and livestock, especially in poor countries, where animal protein is expensive (Hubbell and Gerald, 2003). Broad bean (*Vicia faba* L.) is one of these legumes grown as winter vegetable crop in the Mediterranean region and has considerable importance as a low-cost food enriched protein and carbohydrates (Sepetoglu, 2002). It is one of the most important vegetable crops in Egypt. Its seeds contain a high percentage of carbohydrates in addition to mineral elements, fibers, and vitamins (Gao and Shi, 2007). The seeds also contain high percentage of protein ranging between 25-40% (Natalia *et al.*, 2008) that providing an average of 33% to 60% of humans' dietary nitrogen (O'Rourke *et al*., 2014).

Mycorrhiza is the most widespread symbiotic interactions between microorganism and higher plants (Marschner, 2002; Bonfante and Genre 2008). Arbuscular mycorrhizal fungi (AMF) are obligatorily biotrophic and form mutual symbiosis with about 80% of vascular plant species in all major terrestrial biomes (Barea and Jeffries, 1995; Feddermann *et al*., 2010 and Smith *et al.*, 2010). AMF have been reported to have positive influence on plant growth and tolerance to diseases (Pozo *et al*., 2002; Garmendia *et al*., 2004 and Wang *et al*., 2012). AMF can consider as an essential factors for functioning and sustainability of the agro-ecosystem (Abd-Alla *et al*., 2014 and Nafady *et al.*, 2018). AMF alleviate soil stresses on plant and increased the plant growth and its tolerance (Kumar *et al*., 2014). AMF also increased nutrient uptake, accumulation of osmoregulatory compounds, increase in photosynthetic rates, and decrease root respiration and water use (Abdel Latef and Chaoxing, 2011; Porcel *et al*., 2012). On the other hand, mycorrhizal fungi develop a network of hyphae that link between the soil, the nutrient reservoir, and the plant roots. This hyphal structure is more efficient for phosphorus and iron absorption than root hairs (Dorneless *et al*., 2001).

Iron is important element in plant biochemical reactions; it can directly or indirectly improve the growth performance of crops, especially the legumes (Zarghamnejad *et al*., 2015). Many of metabolic pathways and enzymes are activated by iron that plays an important role in plant growth by stimulating cell division (Rout and Sahoo, 2015). Therefore, iron is considered as a necessary element for the most crops, in particularly legumes since it participates in atmospheric nitrogen fixation process. Moreover, iron has several important functions for plant growth and productivity, including photosynthesis and respiration processes and chlorophyll synthesis which they are functional tools for plant productivity and yield (Houimli *et al.*, 2015; Ferhi *et al*., 2017; Mann *et al*., 2017 and Ren *et al*., 2017). Since iron is impaired in alkaline soil due to the presence of $CaCO_3$, foliar-Fe fertilizer is highly recommended.

Microelements applied in foliar were much more useful than direct applied in soil where in alkaline soil it considered unavailable form (Heidarian *et al*., 2011). Iron as foliar spraying used as chemical fertilizer for different crops, reduce the effects of salinity, adjusts

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the soil pH, ameliorate the negative effect of salt stress on the growth and production, increasing plant growth, and yield and its quality (El-Mansi *et al*., 2005; Tantawy *et al*., 2013; Al Janabi, 2016 and Houimli *et al.,* 2016). The main objective of the current work was to study the synergistic effect of inoculation AMF and foliar iron concentrations on the growth characters, yield and its components of broad bean crop using drip irrigation system under North Sinai conditions.

MATERIALS AND METHODS

Study location and physico-chemical properties of soil and irrigated water used

The study was conducted at Experimental Farm of the Environmental Agricultural Sciences Faculty, Arish University, North Sinai Governorate, Egypt. The experiment was run for two consecutive winter seasons of 2016/2017 and 2017/2018. The physico-chemical properties of soil and irrigated water were analyzed following the method of Piper (1947) and Jackson (1958). The physico-chemical properties were recorded in average of the two working seasons (Tables 1 and 2).

Experimental design

To evaluate the effect of AMF or/and foliar iron fertilizer on plant growth and in sequence its yield, a randomized complete block design, with three replica in split design system, was applied. Ten treatments, in which inoculation with propagated AMF verses native occurring AMF (native AMF), spraying with Fe-foliar fertilizer with five different concentrations (0.00, 300, 400, 500, and 600 ppm) and the interaction between the two major factors were established.

Treated *Vicia faba* seedlings with AMF (factor A) were randomly settled in the main plots, meanwhile spraying with foliar-iron fertilizer (factor B) were randomly arranged in the sub plots. Seeds of broad bean cv."Luzde otono" imported from Fito Semillas Co., Turkey, were sown on 1st November in both season around emitters of two dripper lines in hills at 30 cm between each two hills (the experimental unite was 8 m length and 0.9 m wide). Total plot area was 14.40 m^2. The area around one dripper line (7.2 m^2) was used to estimate growth parameters and the other area was used for estimating yield.

Isolation, purification and identification of AMF from different plants

AMF spores were collected and isolated from the rhizosphere of several vegetable plants, cultivated in the experimental farm, located at Faculty of Environ. Agric. Sci., Arish Univ., including tomatoes, pepper, eggplant and cucumber plants. Rhizosphere soil samples (100g) of each plant were collected by wet sieving and decanting through a series of wire meshes, having a mesh size of 355-25µm sieves (Gerdemann and Nicolson, 1963). The sieved soil samples were centrifuged at 1000 rpm for 15 min following the method modified from INVAM (2020). To facilitate rapid determination of spore density, spore suspensions were filtered through 7 cm diameter filter paper Whitman No 1, marked with small squares (1:1cm) to examine spores. For AMF identification, spores were stained with Meltzer's reagent and then examined microscopically, using differential interference contrast microscope, to screen the different morphological properties based on spore shape, color, longest dimension, wall thickness, and hyphal existence and morphology. The diversity of AMF spores was recorded after mounted in poly-vinyl alcohol-lactic acid-glycerol (PVLG) to make permanent slides (Gerdemann and Trappe, 1974; INVAM, 2020).

Trap culture and inoculation technique of AMF

To increase the AMF propagules, maize (*Zea mays* L.) was used, as a recommended trap plant, in an autoclaved soil for a period of 4 months to increase spore density. Spores were surface sterilized as described by Ravolanirina *et al*. (1987). Heavily colonized adventitious roots developed were chopped into small fragments and mixed thoroughly with the associated rhizosphere soil. The examined inoculum was containing roots as hyphae, vesicles, arbuscules and spores. AMF inoculum was added, after standardized at the rate of 50g of inoculum, which contains spores and colonized maize root fragments. Inoculum was applied to the experimental field soil and at a depth of 2-3 cm below broad bean seedlings following the method described by Menge and Timmer (1982).

Table (1): The physical and chemical properties of the experimental soil* (average of two seasons)**.**

Property	Average of two seasons (2016-2018)
Physical properties	
Texture	Sandy loam
Chemical properties	
pH	7.8
EC (dSm^{-1})	1.3
Total N (ppm)	16.22
Total P(ppm)	0.33
Total K (ppm)	0.79

* Soil sample was taken at 25 cm from the soil surface.

Table (2): Initial chemical analyses of irrigation water (average of two seasons).

pH	EC (ppm)	Soluble ions (meq.l^{-1})							
		Cations					Anions		
		Mg^{-1}	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	SO_4^{--}
7.3	3513	4499	16.33	17.6	35.87	0.27	42.26	6.13	21.41

Determination of AMF colonization

Mycorrhizal root colonization was evaluated by collecting root samples from experimental plants. The roots were divided into 1-2 cm segments after being cleaned, hydrolysed in 10% KOH solution, neutralized using 10% HCl and then stained with 0.05% glycerol-trypan blue solution for observation of AMF root-colonization following the method of McGonigle *et al*. (1990). A hundred stained root samples were randomly selected and observed under a light microscope to determine the intensity of the AMF colonization (Trouvelot *et al*., 1986). The percentage of AMF colonization in root was calculated by the gridline intersects method of Giovannetti and Mosse (1980) according to the following equation:

AMF colonization (%) =

$$\frac{\text{Total number of root segments colonized}}{\text{Total number of root segments studied}} \text{ X } 100$$

Root colonization was estimated at different time period of the experiment. Meanwhile, number of spores/100 g soil was also estimated at different interval of 30 days after sowing, flowering, and yield stages, respectively for two successive seasons.

Isolation and inoculation of *Rhizobium leguminosarum* inoculant

Rhizobium sp. used for broad bean inoculation was isolated from fresh surface-sterilized nodules collected from broad bean roots that previously cultivated in the farm of vegetables and crops, Faculty of Environ. Agric. Sci., Arish Univ. To select the compatible native *Rhizobium* strain, a side trail was established in which *Vicia faba* seedlings were inoculated with different isolated *Rhizobium* cultures. The isolate, that showed higher effectivity for *V. faba* seedlings, was selected to precede the study. For inoculum preparation, the selected *Rhizobium* sp. was propagated on yeast mannitol agar medium (Somasegaran and Hoben, 1985). After 3–5 days of incubation at 28^{o}C, the growing *Rhizobium* was suspended in sterilized saline solution (0.8%) and diluted to reach 1×10^{8} CFU/ml^{-1}. The seeds of broad bean were soaked in this suspension for 30 min. and then mixed with a sterilized carrier mixture, consists of peat and vermiculite, to be ready for field cultivation.

Determination of *Rhizobium*-inoculum efficiency

Efficiency of *Rhizobium*-inoculum was determined by counting the total number of Nodules developed per plant, number of active Nodules verses to non-active nodules/ plant. This was done by randomly collecting three root samples, per treatment, at 30 and 45 days after planting for the two seasons. Each plant was washed by water to remove all the soil particles attached to the roots to enhance visualization of nodules. (Ngakou *et al*., 2009).

Foliar iron application

In this study, Van Iperen Oligo Standard Iron-EDDHA 6% was used as foliar-iron fertilizer. This type was selected for its high purity with a high percentage of ortho-ortho that dissolves rapidly and completely. The fertilizer doses applied were 0.0, 300, 400, 500, and 600 ppm. The fertilizer was sprayed once every 21 days and started at day 30 of sowing. Distilled water was used as a dose 0.0 (control treatment).

Plant growth parameters

Samples of three plants from each experimental unit were randomly taken at 90 days after sowing and the following data, in mean, were recorded for each treatment: a. Stem length, root length and number of both branches and leaves/ plant; b. Total fresh and dry weight/plant.

Measurement of leaf chlorophyll content

Since chlorophyll (Chl) is an important photo-synthetic pigment to the plant that principally determining photosynthetic capacity and in sequence the plant growth, therefore, Chl a and Chl b were measured for each experimental treatment. Ten-disc samples of the fourth upper leaf of each plant tip were randomly taken, from each experimental unit at 90 days from sowing, followed by acetone extraction and measured spectrophotometry (Wettestein, 1957).

Yield performance under different treatments

To evaluate the different experimental treatments, mature green pods were harvested at proper maturity stage and then counted and weighed for each experimental unit. Meanwhile, number of pods/plant, pod length, number of green seeds/pod, weight of green seeds/pod, and average pod weight (g) were measured. Yield (ton /faddan) of each treatment was also calculated.

Statistical analysis

Statistical analysis of the obtained data were performed according to statistical analysis of variance (Snedecor and Cochran (1980). Duncan's multiple range tests was applied for comparison among means (Duncan, 1958).

RESULTS

Isolation, purification and identification of AMF

Data obtained from roots and soil rhizosphere of different treatments at the Experimental Farm of Vegetables, Faculty of Environ. Agric. Sci., Arish University, revealed the existence of three genera of AMF (native AMF). The genera represented by 4

species coexist with various crops (cucumber, eggplant, pepper and tomato) at four different locations (Table 3). According to the frequency of occurrence, the most dominant species were belonging to different taxa that came in the sequence order: the most abundant genus was *Funneliformis mosseae* (Nicolson and Gerd.) Walker & Schüßler - Synonym: *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe (50.0%), followed by *Rhizophagus clarus* (Nicolson and Schenck) Walker and Schüßler, Synonym: *Glomus clarum* Nicolson and Schenck (20.0%), *Gigaspora margarita* Becker and Hall (18.0%) and *Acaulospora laevis* Gerd and Trappe (12.0%).

Increasing soil AMF by trapping culture

The inoculum potential of AMF in soil as spores has been increased by growing maize to increase spore density. The two elements related to the inoculum potential of AMF namely; spore density (as count/100 g) and root colonization rate were used. Data present in table 3 indicated that average spore counts were 20.75 spores/100 g dry soil and 13.00 % average of root colonization. This result is considered very low because these soils are classified as arid and semi-arid reflecting very poor nutrients. After four months from using maize plant, the spore counts reached 192 spores/100 g dry soil and 76.00 % of root colonization.

Effect of AMF inoculum and foliar-Fe concentrations and their interaction on spore counts and total root colonization (%) of *Vicia faba* plant

Spore counts and root colonization (%) in the soil under investigation showed great variability with inoculum AMF verses native AMF alone and with different concentrations of foliar-Fe application. Samples taken at three stages (30 days after sowing, flowering and yield stages) revealed higher significant differences ($p \leq 0.05$) in spore count and total root colonization (%) recorded in 2nd season than those recorded in 1st season for all investigated stages (Table 4).

Inoculation with AMF verses native AMF showed significant ($p \leq 0.05$) increase in spore numbers and the percentage of root colonization at all stages during plant growth for the studied seasons (Table 4). At yield stage, significant ($p \leq 0.05$) increase was highly recorded for the measured parameters. Second season also recorded higher spore density (353.80/100g soil verses 409.68/100g soil for 1st and 2nd season, respectively) and root colonization (77.55 % and 79.88 % for 1st and 2nd season, respectively).

For foliar-Fe fertilization, data represented showed significant ($p \leq 0.05$) increase in AMF spore density as well as root colonization by AMF, at all growth stages, compared to those without fertilization (Table 4). However, foliar-Fe fertilizer at 400ppm recorded high significant differences ($p \leq 0.01$) compared to the rest of fertilizer doses. At yield stage, spore density, represented in number/100g soil, was significantly higher in 2nd season compared to 1st season at the same dose of Fe-fertilizer (253.84 and 315 per 100g soil for 1st and 2nd season, respectively). Meanwhile, percentage of root colonization recorded the highest value at flowering stage for both studied seasons (57.70 and 60.92 for 1st and 2nd season, respectively). Fe-fertilizer at dose 500 ppm also recorded high percentage values (56.69 and 59.01 for 1st and 2nd season, respectively) but significantly less that Fe-fertilizer at 400 ppm (Table 4). In general, spore densities significantly increased as the plant proceed for maturity stages and AMF-root colonization recorded the highest value at flowering stage (Table 4).

For the interaction effect between AMF inoculum and application of foliar-Fe fertilizer, the results indicated that the combination between AMF and different concentrations of foliar-Fe application resulted in a distinctly high number of spores count and total root colonization (%) compared with native AMF at different concentrations of foliar Fe and control in both seasons. Data in Table 4 also revealed that, soils with inoculated AMF and sprayed with 400 ppm Fe concentration showed the highest number of spores/100g soil at all stages in both seasons 136.7, 380 and 466 g soil at 30 days after sowing, at flowering and yield stages in 1st season and 166, 421.3 and 578/100g soil in 2nd season at the same stage. The same result was repeated with total root colonization (%) with 400 ppm foliar-Fe dose, where the highest percentage was 47.63 %, 85.25 % and 67.33 % at 30 days after sowing, flowering and fruiting stages in 1st season, respectively

Table (3): Native AMF genera associated with cultivated plant species and their spore numbers and root colonization percentage.

Plant species	Family	Species of AMF genera	Spores count	Root colonization (%)	Mycorrhizal status		
					Hyphae	Vesicles	Arbuscule
Cucumber (*Cucumis sativus* L.)	*Cucurbitaceae*	*F. mosseae* *G. margarita* *R. clarus*	17	9.00	+	+	+
Eggplant (*Solanum melongena* L.)	*Solanaceae*	*F. mosseae* *G. margarita* *A. laevis*	22	14.00	+	+	+
Pepper (*Capsicum annuum* L.)	*Solanaceae*	*F. mosseae* *G. margarita*	19	12.00	+	+	+
Tomatoes (*Solanum lycopersicum* L.)	*Solanaceae*	*G. margarita* *R. clarus*	25	17.00	+	+	+
Average			**20.75**	**13.00**	+	+	+

Table (4): Effect of AMF inoculum, foliar iron (Fe) concentrations and their interaction on spore counts and total root colonization (%) at different stages of *Vicia faba* plant growth**.**

Treatments†		Number of spores/ 100g soil			Total root colonization (%)		
		Harvested Growth stage					
		30 days after sowing	flowering stage	yield stage	30 days after sowing	flowering stage	yield stage
Inoculation AMF verses Native AMF		**1st Season**					
AMF Inoculum		97.54 a	294.00 a	353.80 a	41.31 a	77.55 a	57.26 a
Native AMF		11.00 b	28.73 b	44.60 b	11.50 b	30.34 b	18.37 b
		2nd Season					
AMF Inoculum		130.93 a	310.24 a	409.68 a	43.40 a	79.88 a	59.42 a
Native AMF		14.33 b	33.27 b	53.80 b	12.80 b	32.49 b	20.35 b
Doses of foliar-Fe fertilizers (ppm)		**1st Season**					
0.0		31.00 e	129.50 e	150.19 e	21.88 e	49.04 e	32.92 e
300		39.34 d	135.34 d	170.52 d	25.30 d	51.68 d	35.10 d
400		74.19 a	205.00 a	253.84 a	29.68 a	57.70 a	42.11 a
500		65.35 b	186.02 b	233.65 b	28.58 b	56.69 b	41.43 b
600		61.49 c	150.99 c	187.82 c	26.58 c	54.64 c	37.54 c
		2nd Season					
0.0		51.00 e	127.84 e	161.35 e	24.17 e	50.03 e	35.17 d
300		67.19 d	143.99 d	195.82 d	26.38 d	53.76 d	36.68 c
400		90.34 a	227.99 a	315.00 a	31.63 a	60.92 a	44.42 a
500		81.99 b	197.99 b	257.19 b	30.00 b	59.01 b	44.16 a
600		72.67 c	160.99 c	229.35 c	28.31 c	57.23 c	39.00 b
Effect of their interaction							
AMF	**Foliar-Fe conc. (ppm)**	**1st Season**					
AMF Inoculum	**0.0**	55.33 e	245.0 d	270.7 e	35.67 d	72.44 c	50.04 e
	300	68.67 d	253.0 d	305.7 d	39.71c	75.83 b	53.15 d
	400	136.7 a	380.0 a	466.0 a	47.63 a	85.25 a	67.33 a
	500	116.7 b	326.7 b	405.3 b	43.04 b	77.81 b	59.47 b
	600	110.3 c	265.3 c	321.3 c	40.48 c	76.42 b	56.33 c
Native AMF	**0.0**	6.67 h	14.00 g	29.67 h	8.09 h	25.63 g	15.79 h
	300	10.00 g	17.67 g	35.33 gh	10.89 g	27.53fg	17.05 h
	400	11.67 fg	30.00 f	41.67 g	11.73 fg	30.15ef	16.89 h
	500	14.00 f	45.33 e	62.00 f	14.11 e	35.56 d	23.39 f
	600	12.67 f	36.67 ef	54.33 f	12.67 ef	32.85de	18.74g
		2nd Season					
AMF Inoculum	**0.0**	92.67 e	236.00 e	290.70 e	38.67 e	72.75 d	53.33 d
	300	120.70 d	263.30 d	353.30 d	41.33 d	78.07 c	54.37 d
	400	166.00 a	421.30 a	578.00 a	49.67 a	89.48 a	69.50 a
	500	146.30 b	347.30 b	435.70 b	44.33 b	80.35 b	61.90 b
	600	129.00 c	283.30 c	390.70 c	42.98 c	78.75 c	58.00 c
Native AMF	**0.0**	9.33 g	19.67 i	32.00 i	9.67 i	27.30 i	17.00 g
	300	13.67 f	24.67 h	38.33 i	11.42 h	29.45 h	18.98 f
	400	14.67 f	34.67 g	52.00 h	13.59 g	32.35 g	19.33 f
	500	17.67 f	48.67 f	78.67 f	15.67 f	37.67 e	26.42 e
	600	16.33 f	38.67 g	68.00 g	13.63 g	35.70 f	20.00 f

† AMF Inoculum, propagated AMF isolated from different plants by trap culture and inoculated to broad bean; Native AMF, AMF exist in soil as native AMF, no inoculation. Values followed by the same letter (s) in a column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

(Table 4). Recoded results in 2nd season showed non-significant increase in total root colonization compared with 1st season (49.67%, 89.48% and 69.50%) at the same stages. Contradictory, native AMF treatment and blank foliar-Fe application showed the lowest spore counts and total root colonization (%) in all growth stages for both seasons 6.67/100g soil and 8.09 % at 30 days after sowing, 14/100g soil, 25.63 % at flowering stage and 29.67/100g soil and 15.79 % at yield stage, respectively in 1st season. The data in 2nd season recoded non-significant increments of total root colonization compared to the 1st season (9.33/100g soil and 9.67 % at 30 days after sowing, 19.67/100g soil and 27.30 % at flowering stage and 32/100g soil and 17.00 % at yield stage, respectively, at the same stages.

Effect of AMF inoculum, foliar-Fe concentrations and their interaction on nodules formation on *Vicia faba* roots

Nodule formation along broad bean was highly influenced by different treatment investigated in this study (Table 5). Inoculation with AMF showed positive effect on nodule formation and a significant, at $p \leq 0.05$ level, increase in their number was recorded. Native AMF recorded less significant nodule counts verses external inoculation with propagated AMF (20.19 and 11.76 for inoculated AMF and native AMF, respectively). At flowering stage of *Vicia faba*, number of nodules were increased and recorded higher significant values in second season that those in investigated in first season (37.17, 22.21, 40.58 and 25.79 for AMF verses native AMF in 1st and 2nd seasons, respectively).

For foliar-Fe treatment, nodule formation recorded significant ($p \leq 0.05$) increase as the plants proceed for flowering stage at all foliar-Fe doses applied (Table 5). However, at 600 ppm of foliar-Fe doses exhibited the best concentration for higher nodulation rate for the two successive season periods of the study (35.51 and 39.59 for 1st and 2nd seasons, respectively). The results in 2nd season also increase active nodules/plant 36.06 nodules/plant at the same stages. On the contrary, the number of non-active nodules decreased compared with other foliar Fe concentrations 2.85 nodules/plant in 1st season while the same result was repeated at all stages in 2nd season.

For AMF and foliar-Fe fertilizers and their influence on nodule formation, table 5 showed synergistic effects where inoculation with propagated AMF and foliar-Fe at 600 ppm recorded the highest significant ($p \leq 0.05$) nodulation rate and reached its maximum value at flowering stage. Native AMF and foliar-Fe fertilizer at the same dose also recorded significant differences among the other fertilizer doses but less significant compared to applied AMF inoculum. Data recorded for second season also showed higher significant nodulation rate and highest number of nodule developed per plant (34.09 and 47.95 for 30 days after sowing and at flowering stage, respectively). The number of active nodules/ plants at 600 ppm Fe as foliar spray and inoculated AMF showed the highest value compared with all treatments at the 1st and 2nd season. Wherever, the interaction of control treatment (native AMF with 0.0 foliar Fe) gained the highest value of number of non-active nodules 8.01 nodules/plant at flowering stage, in the 1st season. In the 2nd season, the results showed the same effect 10.32 nodules/plant at the same stages. The same behaviour was recorded with native AMF and foliar-Fe fertilizer at 600 ppm (Table 5) and recorded 15.65, 29.38, 21.14 and 31.22 at 30 days after sowing and flowering stage for 1st and 2nd seasons, respectively.

Effect of AMF inoculum, foliar-Fe concentrations and their interaction on *Vicia faba* growth

Different growth parameters of *Vicia faba* plant were highly affected by different treatments examined in this study (Table 6). The data reported revealed that inoculated AMF significantly achieved the highest growth parameters in the time period of the study for the two successive seasons. Meanwhile, native AMF recorded the lowest values for all plant growth traits. Data recorded in table 6 also showed that the Foliar-Fe application of at 500 ppm was the superior treat-ment for all measured growth parameters during the two growing seasons except for number of bran-ches/plant in the second season, where Fe fertilizer at 400 ppm recorded the highest values. On the other hand, all plant growth traits were significantly different ($p \leq 0.05$) than control for both seasons.

It is obvious from the data presented in table 6 that the increments in plant growth characters convolved in stem length, root length; number of both branches and leaves/plant and total of both fresh and dry weight/plant were fluctuated between inoculated AMF in combination with foliar Fe concentrations of 400 or 500 ppm in both seasons.

Inoculation with AMF and Fe-fertilizer treatments showed significant synergistic interaction on shoot biomass including stem length, number of leaves/plant and fresh weight/plant in both studied seasons. The interaction treatment (synergistic effects of inoculated AMF and foliar-Fe at 500 ppm) increased stem length, number of leaves/ plant and fresh weight/plant in both seasons. However, combination between inoculated AMF with foliar Fe at 400 ppm resulting in the highest values of root length and total dry weight / plant in both seasons was obtained. On the other hand, number of branches/ plant gives highest value with using inoculated AMF and foliar Fe at 500 ppm in the first season while in the second season inoculated AMF and foliar Fe at 400 ppm had the highest number of branches/ plant.

Effect of AMF inoculum, foliar-Fe concentrations and their interaction on Leaf chlorophyll a and b content

Inoculation with AMF along with foliar-Fe concentrations showed significant positive effect on chlorophyll content of the *V. faba* plants verses to those without inoculation (native AMF). Data represented in Figs 1 and 2 showed AMF and 400 ppm are significantly the highest in chlorophyll content (Chl a and b for 1st and 2nd seasons, respectively). On the other

Table (5): Effect of AMF inoculum, foliar iron (Fe) concentrations and their interaction on nodule formation at different stages, 30 days of sowing and flowering stages, of *Vicia faba* plant.

Treatments†		Total number of nodules/plant		Nodule activity (Number plant $^{-1}$)			
				Active nodule		Non- active	
		Harvesting Time					
		30 Days after sowing	Flowering stage	30 Days after sowing	Flowering stage	30 Days after sowing	Flowering stage
Inoculation AMF verses Native AMF		1st Season					
AMF Inoculum		20.19 a	37.04 a	17.30 a	34.16 a	2.89 b	2.88 b
Native AMF		11.76 b	22.08 b	8.61 b	18.13 b	3.15 a	3.95 a
		2nd Season					
AMF Inoculum		28.83 a	40.58 a	25.31 a	37.17 a	3.52 b	3.41 b
Native AMF		16.45 b	25.80 b	12.64 b	20.91 b	3.81 a	4.89 a
Doses of foliar-Fe fertilizers (ppm)		1st Season					
0.0		10.95 e	21.06 e	7.53 e	15.61 c	3.42 a	5.45 a
300		13.73 d	27.56 d	10.73 d	24.56 d	3.00 b	3.00 b
400		15.03 c	30.28 c	12.05 v	27.37 c	2.98 c	2.91 c
500		18.91 b	33.43 b	16.00 b	30.54 b	2.91 c	2.89 c
600		21.25 a	35.51 a	18.47 a	32.66 a	2.78 c	2.85 c
		2nd Season					
0.0		15.41e	23.56 e	11.30 e	16.95 e	4.11 a	6.61 a
300		21.45 d	32.00 d	17.89 d	28.44 d	3.56 b	3.56 b
400		23.23 c	34.00 c	19.73 c	30.44 c	3.50 b	3.56 b
500		25.51b	36.82 b	21.93 b	33.31 b	3.58 b	3.51 b
600		27.62 a	39.59 a	24.04 a	36.06 a	3.58 b	3.53 b
Effect of their interaction							
AMF	**Foliar-Fe conc. (ppm)**	1st Season					
	0.0	14.96 de	31.23 e	12.25 de	28.35 d	2.71 b	2.88 b
	300	17.74 c	35.01 d	14.75 c	32.16 c	2.99 b	2.85 b
AMF Inoculum	**400**	18.00 c	37.77 c	14.95 c	34.27 b	3.05 b	2.87 b
	500	23.36 b	40.21 b	20.49 b	37.32 a	2.87 b	2.89 b
	600	26.83 a	41.64 a	24.04 a	38.72 a	2.79 b	2.92 b
	0.0	6.93 h	10.88 j	2.80 h	2.87 i	4.13 a	8.01 a
	300	9.73 g	20.09 i	6.72 g	16.95 h	3.01 b	3.14 b
Native AMF	**400**	12.06 f	23.40 h	9.14 f	20.46 g	2.92 b	2.94 b
	500	14.45 e	26.64 g	11.51 e	23.76 f	2.94 b	2.88 b
	600	15.65 d	29.38 f	12.89 d	26.59 e	2.76 b	2.79 b
		2nd Season					
	0.0	23.23 e	33.40 d	19.74 e	30.51 c	3.49 b	2.89 bc
	300	26.90 d	38.45 cd	23.34 d	34.86 bc	3.56 b	3.59 b
AMF Inoculum	**400**	28.56 c	39.55 c	25.08 c	36.04 b	3.48 b	3.51 b
	500	31.33 b	43.55 b	27.82 b	39.99 a	3.51 b	3.56 b
	600	34.09 a	47.95 a	30.55 a	44.44 a	3.54 b	3.51 b
	0.0	7.57 h	13.70 i	2.85 i	3.38 g	4.72 a	10.32 a
	300	16.00 gh	25.53 h	12.44 h	22.01 f	3.56 b	3.52 b
Native AMF	**400**	17.89 g	28.44 g	14.37 g	24.83 e	3.52 b	3.61 b
	500	19.67 f	30.09 f	16.03 fg	26.63 de	3.64 b	3.46 b
	600	21.14 f	31.23 e	17.52 f	27.68 d	3.62 b	3.55 b

† AMF Inoculum, propagated AMF isolated from different plants by trap culture and inoculated to broad bean; Native AMF, AMF exist in soil as native AMF, no inoculation. Means followed by the same letter (s) per column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

Table (6): Effect of AMF inoculum, foliar iron (Fe) concentrations and their interaction on *Vicia faba* growth at harvesting time, 90 days after seed sowing.

Treatments[†]		Plant growth parameters					
		Stem length (cm)	Root length (cm)	Number of branches/Plant	Number of leaves/Plant	Total fresh weight/Plant (g)	Total dry weight/ plant(g)
Inoculation AMF verses Native AMF				**1st Season**			
AMF Inoculum		95.40 [a]	38.73 [a]	9.73 [a]	113.30 [a]	800 [a]	215 [a]
Native AMF		75.70 [b]	24.56 [b]	7.07 [b]	81.20 [b]	547 [b]	150 [b]
				2nd Season			
AMF Inoculum		94.00 [a]	39.93 [a]	8.73 [a]	109.33 [a]	833 [a]	227 [a]
Native AMF		72.10 [b]	23.67 [b]	6.67 [b]	79.20 [b]	576 [b]	160 [b]
Doses of foliar-Fe fertilizers (ppm)				**1st Season**			
0.0		63.67 [e]	22.50 [e]	4.50 [e]	61.67 [e]	468[e]	126 [e]
300		72.67 [d]	25.50 [d]	7.50 [d]	96.17 [c]	566[d]	145 [d]
400		92.17 [c]	36.00 [b]	10.0 [b]	121.2 [b]	754[b]	208 [b]
500		105.2 [a]	42.00 [a]	11.5 [a]	133.2 [a]	925[a]	249 [a]
600		94.17 [b]	32.25 [c]	8.5 [c]	74.17 [d]	655[c]	186 [c]
				2nd Season			
0.0		63.17 [e]	22.50 [e]	5.50 [c]	59.17 [e]	492.0 [e]	127.0 [e]
300		73.17 [d]	25.50 [d]	7.50 [b]	90.67 [c]	591.0 [d]	160.0 [d]
400		85.17 [c]	36.00 [b]	9.50 [a]	119.7 [b]	785.0 [b]	225.0 [b]
500		101.70 [a]	42.00 [a]	9.00 [a]	131.20 [a]	963.0 [a]	263.0 [a]
600		92.17 [b]	32.25 [c]	7.00 [b]	70.67 [d]	694.0 [c]	192.0 [c]
Effect of their interaction							
AMF	**Foliar-Fe conc. (ppm)**			**1st Season**			
AMF inoculum	**0.0**	67.00 [h]	25.33 [f]	5.33 [e]	73.33 [f]	511 [g]	134.6 [g]
	300	81.00 [f]	30.33 [e]	9.33 [c]	133.30 [b]	662 [e]	165.0 [e]
	400	111.00 [b]	49.33 [a]	10.33 [b]	134.30 [b]	964 [b]	281.0 [a]
	500	117.00 [a]	48.33 [b]	13.33 [a]	150.30 [a]	1046 [a]	267.0 [b]
	600	101.00 [c]	40.33 [c]	10.33 [b]	75.33 [e]	817 [c]	226.0 [d]
Native AMF	**0.0**	60.33 [j]	19.67 [j]	3.67 [f]	50.00 [h]	424.0 [j]	118.0 [i]
	300	64.33 [i]	20.67 [i]	5.67 [e]	59.00 [g]	470.0 i	124.0 [h]
	400	73.33 [g]	22.67 [h]	9.67 [c]	108.00 [d]	544.0 [f]	135.0 [g]
	500	93.33 [d]	35.67 [d]	9.67 [c]	116.00 [c]	805.0 [d]	230.0 [c]
	600	87.33 [e]	24.17 [g]	6.67 [d]	73.00 [f]	492.0 [h]	145.0 [f]
				2nd Season			
AMF inoculum	**0.0**	71.00 [g]	22.33 [d]	6.33 [e]	60.33 [g]	542.0 [g]	134.0 [h]
	300	86.00 [e]	34.33 [c]	8.33 [c]	120.30 [c]	700.0 [e]	185.0 [e]
	400	101.00 [b]	50.33 [a]	11.33 [a]	140.30 [b]	1002.0 [b]	301.0 [a]
	500	113.00 [a]	50.33 [a]	9.33 [b]	155.30 [a]	1076.0 [a]	285.0 [b]
	600	99.00 [c]	42.33 [b]	8.33 [c]	70.33 [f]	848.0 [d]	230.0 [d]
Native AMF	**0.0**	55.33 [j]	20.67 [f]	4.67 [g]	58.00 [h]	442.0 [j]	120.0 [i]
	300	60.33 [i]	19.67 [g]	6.67 [e]	61.00 [g]	483.0 [i]	135.0 [h]
	400	69.33 [h]	21.67 [e]	7.67 [d]	99.00 [e]	567.0 [f]	150.0 [g]
	500	90.33 [d]	34.67 [c]	8.67 [c]	107.00 [d]	850.0 [c]	241.0 [c]
	600	85.33 [f]	21.67 [e]	5.67 [f]	71.00 [f]	540.0 [h]	153.0 [f]

[†]AMF Inoculum, propagated AMF isolated from different plants by trap culture and inoculated to broad bean; Native AMF, AMF exist in soil as native AMF, no inoculation. Means followed by the same letter (s) per column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

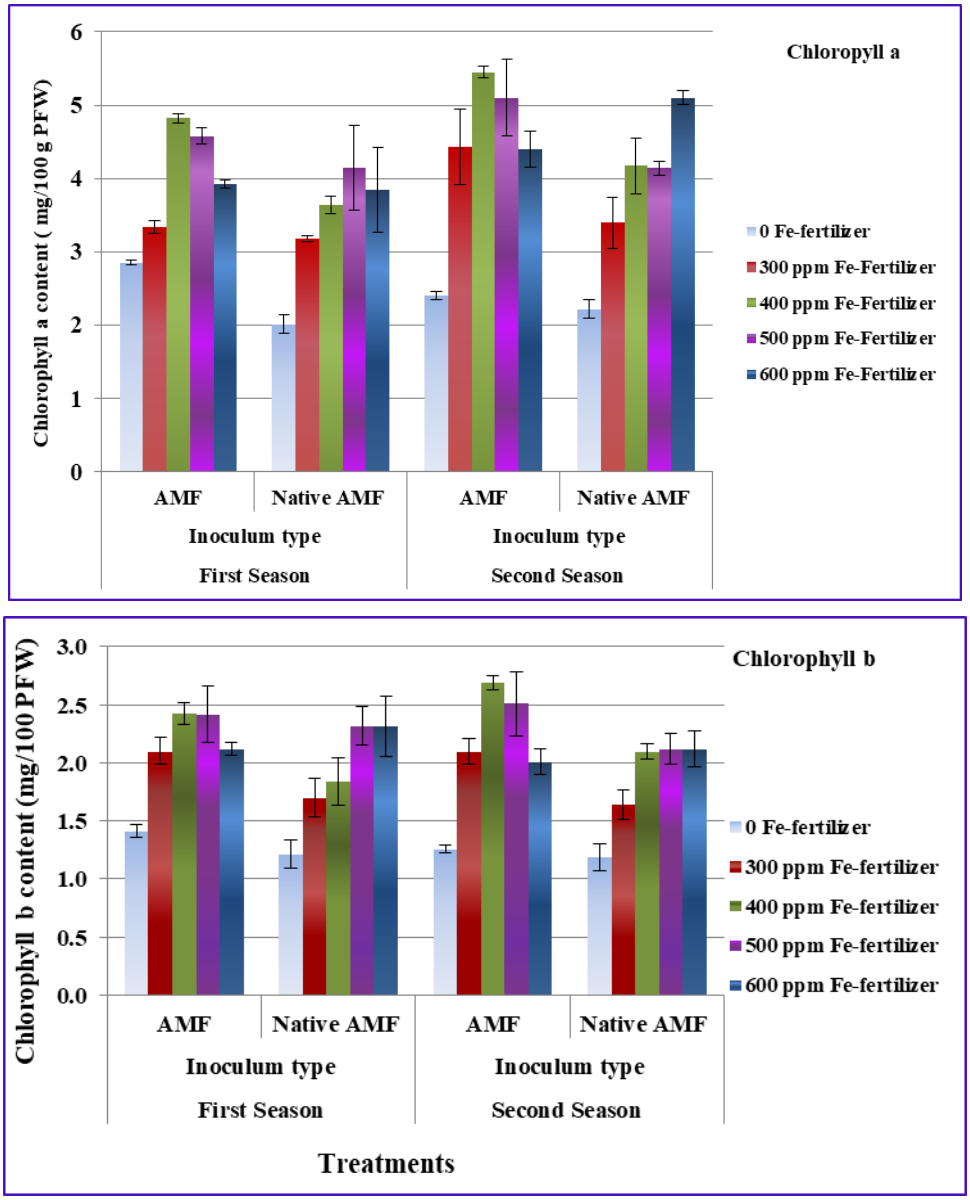
hand, native AMF recorded the lowest value of chlorophyll content except for fertilization with foliar-Fe at 600 ppm which recorded 5.1 mg/100 g FW (Fig. 1).

Data in Fig. 2 show that application of foliar-Fe at 500 ppm was the superior treatment on Leaf chlorophyll a and b content in first season, where it recorded the highest Chl a and b content. While, in the 2nd season Fe at 400 ppm recorded the highest value. On the other hand, the lowest values for Leaf chlorophyll a and b content were detected in season with control treatment, no AMF inoculation or foliar-Fe application.

Application of inoculation broad bean plants with AMF and spray with foliar Fe at a concentration of 400 or 500 ppm showed the promising increased photosynthetic pigments content in both seasons without significant differences as shown in Figs. 1 and 2. Meanwhile, synergistic effects of control treatment (native AMF with 0.0 foliar Fe) had the lowest values in both seasons.

Effect of AMF inoculum, foliar-Fe concentrations and their interaction on *Vicia faba* yield and its components

Table 7 shows that inoculated AMF resulted in increasing all broad bean yields and its components in both seasons of the study, while, the least values were gained by the treatment of native AMF in both seasons. The increase in yield and its components recorded in treatment of inoculation with AMF might be owed



Figures (1) and (2): Effect of AMF inoculum, foliar-Fe fertilizer concentrations and their interaction on chlorophyll a and b content, respectively, of *Vicia faba* plant at 90 days of sawing. Data are represented in mean ±SE.

directly to the increment in nitrogen supply reflecting an increase in plant growth as shown in Table 7.

Obviously, Table 7 showed that the applying foliar-Fe on broad bean plants at 500 ppm significantly increased pod length, number of pods /plant, average pod weight (g) and yield (ton/faddan) in the 1st season. However, the highest values of number of green seeds per pod and weight of green seeds/pod were obtained with 600 ppm of Fe application. In the second season, foliar Fe at 500 ppm reflected the highest values of weight for green seeds / pod, number of pod / plant, average pod weight and total yield/faddan. However, the highest value for pod length recorded with application of 400 ppm of Fe and for number of green seeds/pods with using of 300, 500 and 600 ppm of Fe as well.

Meanwhile, the increments in broad bean yield and its components resulted from spraying with Fe at 500 ppm might be owed to the increase in plant growth as shown in table 7. The increments in yield and its components might be also owed to the increment in active nodules formation, increasing in photosynthetic pigments, dry weight of plant and therefore increasing in yield.

Inoculation broad bean plants with AMF and spray with foliar-Fe at a concentration of 400 ppm showed the promising treatment in both seasons as shown in table 7. The same previous interaction treatment increased all the components of yield; viz, pod length, number of green seeds/pod, number of pods/ plant, and yield (tons/feddan) in both seasons as well as weight of green seeds/pod in the 2nd one. In addition, inoculated AMF with foliar Fe at a concentration of 600 ppm interaction treatment achieved the highest number of green seeds/pod and weight green seeds/pod in the 1st season. Meanwhile, interaction between inoculated AMF with foliar Fe at a concentration of 500 ppm increased average pod weight in the 2nd season. It could be concluded that the best interaction treatments for yield and its components followed the order at Fe concentration of 400 ppm, 600 ppm and 500 ppm for the application of inoculated AMF with foliar-Fe.

DISUSSION

This study included two studied factors: two mycorrhizal treatments (inoculum AMF and native AMF) and five foliar Fe concentrations (0.0, 300, 400, 500 and 600 ppm) and their interactions. Isolated AMF spores have been identified with the help of keys suggested by (Silva *et al*., 2005; Amutha and Shamini 2016; INVAM 2020). Our findings agree with many researchers who found that genus *Glomus* is a predominantly distributed genus in the soil all over the world (Abdulla and Al-Khaliel, 2010; Mansour, 2010 and Yaseen *et al*., 2016). Obtained results are in a good line with the findings of Nafady *et al*., (2018). The author reported that five morphotypes of AMF were belonging to three families (Aculosopraceae, Glomeraceae, and Gigasporaceae) were recovered from soil samples under study. AMF species were *Acaulospora bireticulata*, *Acaulospora leavis*, *Glomus caesaris*, *Glomus clarum* and *Gigaspora margarita*. The spore density found in this study was like that found by Teixeira *et al*., (2017) who showed that a total of 59 AMF species was distributed in 12 genera and seven families of Glomeromycota. Most of the species found (42%) belong to the genus *Acaulospora* (13 species). Fernandes *et al*., (2016) found that a total of 42 AMF species were detected in four land uses. They reported that *Acaulospora* had the largest number of species (18), followed by *Glomus* (6) and *Gigaspora* (5), *Gigaspora margarita* and *Gigaspora decipiens* were present in all areas.

The inoculum potential of AMF in soil as spores have been increased by growing maize (*Zea mays* L.) as a recommended trap plant for a period of 4 months to increase spore density. These results were in harmony with those found by (Watson and Milner, 1996; Liu and Wang 2003; Yaseen *et al*., (2016). In addition, it is well known that trap cultures, using some crops such as *maize*, onion, grass and sorghum plants grown in soil diluted with sterile sand are most used to isolate AMF and the number of spores increased gradually from time such as increased from vegetative stage to fruiting stage. The trap culturing method usually results in the isolation of more species than other methods. Spore counts and total root colonization (%) in the soils under investigation showed great variability with inoculum AMF and native AMF at different concentrations of foliar Fe application. These results are in a line with (Rachid *et al*., 2013; de Araujo Pereira *et al*., 2017; Nafady *et al*., 2018 and Pereira *et al*., 2019). The authors found that dual inoculation of AMF significantly increased spore density and mycorrhizal colonization compared with single inoculation with AMF in faba bean plant. These results are in a line with Juntahum *et al*. (2020) who observed the number of spore changes with time, and the spore density is highest after 8 months. In the unfertilized and non-inoculated controls, the spore density was the lowest. In this respect, Sathiyadash *et al*. (2017) showed that AM fungal hyphal growth and root colonization are suppressed by high levels of micronutrients (Cu, Zn, Mn, and Fe) in soil. It is worthy to mention that the effect of inoculum with AMF and foliar Fe concentrations and their interaction on nodules formation of *Rhizobium* strain on broad bean roots are in agreement with many researchers (Dubova *et al*., 2015 and Pereira *et al*., 2019). The authors showed that applying *Rhizobium laguerreae* with or without AMF in legume crops significantly increased number of nodules/plant.

In addition, El-Tantawy and Nawar (2013) observed that inoculation of faba bean plants with or without Fe as foliar spray at 500 ppm and inoculation with Rhizobium reflected significant interaction effect on number of active nodules/plant. The promotive effect of Fe on faba bean nodulation might be attributed to that Fe contributes in many of enzymatic activities, such as catalase, peroxidase, and nitrate reductase. Fe metabolism is of importance in nodules since this metal

Table (7): Effect of Inoculum AMF, foliar iron (Fe) concentrations and their interaction on yield and its component of *Vicia faba* plant.

Treatment†		Marketable yield and its components					
		pod length (cm)	Number of green seeds/pod	weight of green seeds/	Number of pods/ plant	Average Pod weight (g)	Yield (Ton./ faddan)
Inoculation AMF verses Native AMF		**1st Season**					
AMF Inoculum		17.80 a	4.27 a	12.83 a	12.30 a	14.69 a	5.17 a
Native AMF		15.48 b	3.57 b	10.82 b	10.04 b	12.15 b	3.59 b
		2nd Season					
AMF Inoculum		16.40 a	4.27 a	12.68 a	13.20 a	14.51 a	5.50 a
Native AMF		14.70 b	3.57 b	10.82 b	10.91 b	12.47 b	3.95 b
Doses of foliar-Fe fertilizers (ppm)		**1st Season**					
0.0		13.40 e	3.51 c	7.14 e	8.75 d	10.63 d	2.67 e
300		16.10 d	4.01 b	11.36 d	8.60 e	12.65 c	3.12 d
400		17.40 c	4.01 b	13.49 b	12.00 c	14.11 b	5.00 c
500		18.40 a	3.51 c	11.86 c	13.60 a	15.59 a	5.97 a
600		17.90 b	4.51 a	15.27 a	12.90 b	14.11 b	5.12 b
		2nd Season					
0.0		10.90 d	2.02 c	6.11 e	8.99 e	11.09 e	2.83 e
300		15.85 b	4.52 a	13.07 b	9.99 d	12.11 d	3.43 d
400		18.25 a	4.01 b	12.44 d	12.77 c	13.99 c	5.20 c
500		18.05 a	4.52 a	14.08 a	15.00 a	15.74 a	6.64 a
600		14.70 c	4.52 a	13.05 c	13.50 b	14.53 b	5.52 b
Effect of their interaction							
AMF	**Foliar-Fe conc. (ppm)**	**1st Season**					
	0.0	15.00 d	4.07 b	7.64 i	10.50 f	12.00 g	3.55 g
	300	15.00 d	4.07 b	11.40 f	10.00 g	14.21 e	3.99 f
AMF Inoculum	**400**	21.00 a	5.07 a	16.43 b	15.00 a	17.00 a	7.18 a
	500	19.00 b	3.07 d	12.11 d	14.00 b	16.00 b	6.30 b
	600	19.00 b	5.07 a	16.56 a	12.00 e	14.25 d	4.81 e
	0.0	11.80 e	2.97 e	6.65 j	6.99 j	9.26 j	1.81 j
	300	17.20 c	3.97 c	11.33 g	7.21 i	11.09 i	2.24 i
Native AMF	**400**	13.80 d	2.97 e	10.56 h	8.99 h	11.22 h	2.83 h
	500	17.80 bc	3.97 c	11.61 e	13.21 d	15.18 c	5.64 c
	600	16.80 c	3.97 c	13.98 c	13.80 c	13.97 f	5.42 d
		2nd Season					
	0.0	11.00 i	2.07 f	6.60 h	10.00 g	12.21 g	3.44 g
	300	14.90 f	4.07 d	11.87 f	11.00 f	13.00 f	4.02 f
AMF Inoculum	**400**	22.20 a	6.07 a	18.43 a	16.00 a	16.00 b	7.20 a
	500	18.30 b	5.07 b	15.43 b	15.00 b	16.50 a	6.97 b
	600	15.60 e	4.07 d	11.09 g	14.00 d	14.85 d	5.85 d
	0.0	10.80 i	1.98 g	5.62 j	7.99 j	9.97 j	2.23 j
	300	16.80 d	4.97 c	14.28 d	8.99 i	11.22 i	2.83 i
Native AMF	**400**	14.30 g	1.97 g	6.45 i	9.54 h	11.97 h	3.20 h
	500	17.80 c	3.97 e	12.73 e	15.00 c	14.97 c	6.31 c
	600	13.80 h	4.97 c	15.01 c	13.00 e	14.21 e	5.19 e

†AMF Inoculum, propagated AMF isolated from different plants by trap culture and inoculated to broad bean; Native AMF, AMF exist in soil as native AMF, no inoculation. Means followed by the same (s) per column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test

is a constituent of key proteins such as nitrogenase and leghemoglobin (Moreau *et al*., 1995). The increments in plant growth parameters due to application of inoculum with AMF might be attributed to the enhancing of nutrients uptake, increment in nitrogen fixation, phosphate solubilization and synthesis of phytohormones as reported by previous research work (Al-Karaki 2017; Sathiyadash *et al*. 2017; Pereira *et al*., 2019). These results might be due to Fe roles in contributing to other nutrients in increasing plant growth. According to Clark and Zeto (1996), levels of nutrients are low in alkaline soil (like the soil in the present study), mycorrhizae hyphae enhance Fe uptake. In this connection, Kobrace *et al*., (2011) reported that growth, photosynthesis pigments, nodulation and dry matter production were increased by adding Fe. In addition, these results are in accordance with the findings of (El-Tantawy and Nawar 2013 and Houimli *et al*., 2015).The increment of plant growth due to the combination of inoculum with AMF and foliar Fe application at 500 ppm might be owed to the vital roles of AMF on releasing phosphates for plants, nitrogen fixation, alleviating soil stress, improving soil structure through a network hypha as reported by (Abdel Latef and Chaoxing 2011; Porcel *et al*., 2012 and Abd-Alla *et al*., 2014).

Additionally, the vital roles of Fe on nodulation and consequently achievement in nitrogen fixation, its roles in heam and non heam enzymes, as a component of photosynthetic pigments as well as in photo assimilation process that was reflected on broad bean plant growth. Furthermore, the obtained results are coinciding with those reported by many researchers (Oliveira *et al*., 2017; Fouda and Abd-Elhamied, 2017; Nafady *et al*., 2018; Pereira *et al*., 2019). Finally, Abd-Alla *et al*., (2014) found increases in dry matter accumulation of shoots and roots of faba bean which inoculated with AMF and *R. leguminosarum* in alkaline soil. In this direction, Khatun (2020) indicated that AMF inoculation significantly increases all the growth parameters such as height of the plants, length of the roots, fresh weight of shoot, roots, tubers of Coleus plants than the control. In addition, Elkhatib *et al.*, (2008) found that mycorrhizal inoculation plants gave higher yield fed^{-1} than those inoculated with phosphorein. Also, the increments in yield and its components might be owed to the increment in active nodules formation, increasing in photosynthetic pigments, dry weight of plant and therefore increasing in yield. Srivastava and Gupta (1996) reported that Fe present in heam containing enzymes and in non heam compounds; i.e., peroxidase, catalase, cytochrome oxidase, ferodoxin and nitrate reductase. Nour (2004) found that spraying pea plants with Fe increased number of active nodules/plant, nitrogenase activity, seeds weight /pod, yield/plant and shelling out (%). Also, El-Tantawy and Nawar (2013) found that faba bean plants sprayed with Fe scored significantly the highest total yield (kg/faddan) compared to control treatment. According to our results the effect of interaction between inoculated AMF and foliar Fe concentrations agree with those reported by (Veselaj *et al*., 2018 and Pereira *et al*., 2019) who showed that the inoculation with AMF improved productivity parameters such as number of pods and weight of pods and seeds, so that increased yield significantly. Also, this result found by Juntahum *et al*., (2020) who showed an increased productivity in Sugarcane plant by inoculation AMF alone while, the highest plant biomass and productivity were observed in the AMF $^{+}$ 50% P treatment.

CONCLUSION

AMF in an agricultural ecosystem is required for good management of the beneficial symbiosis. At the end of this study, it brings to light the importance of inoculation of *Vicia faba* plants with AMF and application of foliar-Fe which generally increased all measured parameters (AMF spore counts, root colonization %, nodule formation, improved the overall plant biomass, chlorophyll content and plant yield as compared with control plants without any treatment). The proposed combined approach of the current work emphasized the utilization of foliar-Fe, at dose 400-500 ppm, and inoculation of legumes with AMF as an alternative step towards the establishment of cost effective and eco-friendly preparation of sustainable biofertilizer. The combination between inoculum AMF and foliar-Fe could increase the efficiency and commercialization of environmental friendly biofertilizer application in agriculture cropping systems.

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التأثير المشترك للتلقيح بفطريات الميكوريزا الداخلية والتسميد الورقي بالحديد على نمو وإنتاجية نبات الفول تحت ظروف شمال سيناء

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الملخص العربي

أُجريت هذه الدراسة في المزرعة التجريبية لكلية العلوم الزراعية البيئية، جامعة العريش، محافظة شمال سيناء، جمهورية مصر العربية، خلال المواسمين الشتويين 2017/2016 و 2018/2017.، تم دراسة الآثار المشتركة بالحقن بفطريات الميكوريزا الداخلية والتسميد الورقي بعنصر الحديد على الصفات التالية: عدد الجراثيم، والاستعمار الفطري لجذور النبات، وتكوين العقد الجذرية البكتيرية وكذلك النمو الخضري، والمحصول لنبات الفول المزروع في تربة رملية طمييه. وصممت تجارب هذا البحث لدراسة تأثير كلا من: ا.المعاملة بالفطريات الميكوريزا الداخلية (تلقيح التربة بالميكوريزا الداخلية بعد زيادة عددها عن طريق نبات الذرة، وبدون تلقيح بالميكوريزا والاكتفاء بالموجود منها في التربة بشكل طبيعي دون زيادة عددها)، ب: والمعاملة بالتسميد الورقي لخمس تركيزات من عنصر الحديد (،0.0 و 300، و 400، و 500، و600 جزء في المليون). ج: كذلك دراسة التاثير المشترك لهذين العاملان. وكشفت النتائج التي تم الحصول عليها أن الجمع بين التلقيح بالميكوريزا الداخلية والتسميد بالرش الورقي للحديد بتركيز 500 و 400 جزء في المليون أدي إلي زيادة عدد جراثيم فطريات الميكوريزاالداخلية، وكذلك واستعمارها لجذور نبات الفول. كذلك أدى الجمع بين التلقيح بفطريات الميكوريزاالداخلية والتسميد بالرش الورقي للحديد بتركيز 600 جزء في المليون إلي زيادة عدد العقد الجذرية البكتيرية النشطة/النباتات. كما أشارت النتائج إلى أن الجمع بين التلقيح بالميكوريزا الداخلية والتسميد بالرش الورقي للحديد بتركيز 500 و400 جزء في المليون إلي تسجيل أعلي قيم النمو للنبات مثل: طول الساق، وعدد الفروع / النبات، والوزن الجاف الكلي/ النبات، ومحتوى أصباغ الكلورفيل المتكونة نتيجة عملية البناء الضوئي في الموسمين مدة الدراسة. علاوة على ذلك سجلت نفس المعاملة أعلى القيم لانتاج النبات متمثلة في عدد القرون، والمحصول عاما، بإستثناء وزن البذور الخضراء/ قرن في الموسم الأول، ومتوسط وزن القرن (جم) في الموسم الثاني.