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Comparative Study on the effect of Chemical Fertilizers, Bio-fertilizers and Arbuscular Mycorrhizal fungi on Maize Growth

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ABSTRACT: A comparative study to evaluate the effect of chemical, commercial bio-fertilizers, arbuscular mycorrhizal fungi (AMF) and their combinations on the soil properties and some physiological metabolites of maize (*Zea mays* L.) was carried out on a pot experiment under field conditions. Six species of the AMF were used for inoculation were isolated and identified from rhizospheric soil of cultivated maize. Application of half the recommended dose of chemical fertilizers (CF_{hd}) mixed with commercial bio-fertilizers (BF) to mycorrhizal plants led to the highest root colonization by hyphae (97.67%), vesicles (76.744%) and arbuscules (58.140 %). This reflected on the plant metabolism and significantly increased the root soluble protein (SP) and soluble sugars (SS). The highest content of total free amino acids in roots (TAA) and soluble sugars in shoots obtained under application of the full recommended dose of chemical fertilizers (CF_{fd}) to the mycorrhizal soil. Mycorrhizal soils amended with bio-fertilizers and CF_{hd} recorded the highest significant increase in soil PO_4^{-3} and SO_4^{-2}, and organic matter content (OM). Both the potential and available supply of the plant-nutrient content of the soil were increased by CF application, while only the available supply increased by inoculation by AMF and BF. In both roots and shoots of maize, CF_{hd}, AMF and BF exerted the highest effect on changes of contents of SS, SP and TAA, respectively. The bio-fertilizers has the highest magnitude of effect on changes of contents of shoot phosphate and root nitrate, while CF_{hd} has the highest magnitude of effect on changes of content of shoot nitrate and root phosphate.

Keywords: Bio-fertilizer, Chemical fertilizer, Arbuscular mycorrhizal fungi, Maize.

INTRODUCTION

Cost increases, food safety, quality and environmental protection are major concerns for many scientists dealing with agriculture production worldwide. Chemical fertilizers have a clear role in increasing crop productivity, but they can also negatively impact on soil and water quality (Chirinda *et al.,* 2010). In addition, they can cause soil acidification and fertility degradation, pollution of the soil, air and groundwater and generation of gases such as N_2O because of their inefficient utilization by crops. To reduce the risks of inorganic fertilizers, natural and organic fertilizers could be used as an alternative to chemical fertilizers (Kennedy *et al.*, 2004).

Bio-fertilizers derived from composting of organic substances (Aranda *et al.,* 2015) consist of living cells of dissimilar types of microorganisms (mostly bacteria, fungi, and cyanobacteria), which can biologically convert nutritionally essential elements from unobtainable to obtainable form (Bashan *et al.*, 2004). Application of bio-fertilizers have some benefits to soil include increasing the chemical, physical and biological soil properties, as well as supplying slow release nutrients to plants. Humification and mineralization of bio-fertilizer release safe and friendly nutrients to the plants and soil (Aranda *et al.*, 2015).

Amongst the fungi which used as bio-fertilizer are arbuscular mycorrhizal fungi (AMF). They belong to the phylum Glomeromycota and have mutualistic relations with roots of many plants (Smith and Read, 2010). AMF provide the host plant with mineral nutrients and water in exchange for photosynthates. AMF can reduce the restriction in plant growth caused by an insufficient nutrient supply or shortages of water supply around roots and it could be helpful for enhancing soil structure (Rillig *et al.,* 2015). AMF could be used as a bio-fertilizer by inoculation of AMF propagules into the target soil (Smith and Read, 2010). Some microorganisms, for example, *Azospirillum*, AMF, phosphate solubilizing bacteria and *Rhizobium* increase crop growth and productivity, stimulate water absorption, and suppress phytopathogenic microorganisms (Bashan *et al.,* 2004).

Root colonization by AMF is reduced only when both elements nitrogen and phosphorus are available in sufficient concentrations in soil. Mycorrhizal fungi and *Azospirillum* associations can decrease the uses of chemical fertilizers, by increasing the efficiency of nutrients uptake (Gemma *et al.*, 1997). Recently, it was reported that the application of a chemical fertilizer mixed with organic or bio-fertilizer considered as an effective method for controlling microbial pathogens (Tao *et al.*, 2015).

Maize (*Zea mays* L.) is one of the most widely important crop in Egypt. It has highly nutritional values for animals and humans (IITA, 2006) and can be easily colonized by AMF. The aim of the present study is to evaluate the effects of AMF on the growth of maize in the presence or absence of other chemical fertilizers and/or commercial bio-fertilizers (Nitrobine and Phosphorein) and the impact of these fertilizers on the soil properties.

MATERIALS AND METHODS

A. Preparation of mycorrhizal inoculum

The efficient strains of AM fungi were isolated from the rhizospheric soil samples of maize collected from old cultivated fields adjacent the Nile River or from recently reclaimed fields approximate to the western desert of Egypt. The AMF propagules were obtained from the soil by 'Wet Sieving and Decanting Method (Gerdemann and Nicolson, 1963). Identification of AMF according to (Schenck and Perez, 1990).

To culture mycorrhizal species, spore isolation method was followed, which later served for inoculation of experimental maize plants. The host plant were allowed to grow for up to 3 months (May, June, and July) under natural field condition to trap AMF fungi of interest (Walker and Vestberg, 1994). After removing the host plant from the soil, the roots with rhizospheric soil were used as inocula for the experiment.

Bio-fertilizers. Nitropin and phosphorein are commercially produced bio-fertilizers (BF), and were provided by the Agricultural Research Institute, Giza, Egypt. Nitropine contains two aerobic non-symbiotic, or free-living, nitrogen fixing bacteria *Azotobacter chroococcum* and *Azospirillum barasilense* carried on peat moss, vermiculite and plant charcoal. Phosphorein contains phosphate dissolving bacteria.

Chemical fertilizers. The mineral fertilizers were applied in the form of urea (46.5 % N), and calcium super phosphate (15% P_2O_5). Both fertilizers were combined as one treatment but with two levels: full recommended dose (CF_{fd}) and half recommended dose (CF_{hd}). The recommended doses for maize was calculated according to Abbas *et al.* (2006) on the rate of 300 Kg urea + 200 Kg calcium super phosphate/Feddan.

B. Pot Experiment

Each of thirty pots (25×21 cm size) was filled with 5 kg sterilized soil (1 sand: 2 clay). A layer of inoculum consisting of AM colonized root pieces and soil containing spores were spread over the pots, except three pots as a control. Maize caryopses (cultivar Hybrid corn 131) were surface sterilized with ethanol (70%) and NaOCl (5%), then washed several times with sterilized water. Caryopses were sown in each pot, 3 pots were separated as control and 27 pots were randomly grouped in triplicates to study the following treatments: full recommended "CF_{fd}" (6 g/pot) and half recommended "CF_{hd}" (3 g/pot) doses of chemical fertilizers; Nitropin and Phosphorin as bio-fertilizers "BF" (30 g/pot); a combination of CF_{hd} +BF; mycorrhizal inoculation alone "AMF"; or in combination as AMF+CF_{fd}, AMF+CF_{hd}, AMF+BF and AMF+CF_{hd}+BF. The experimental pots were maintained under natural field condition (from mid-July to the end of August/2016) and irrigated regularly to maintain the soil water level close to the field capacity which was estimated to be about 30%. After sprouting, five homogenous seedlings were left in each pot. Bio-fertilizers were added to the pots with a rate of 30g/pot and divided into three equal doses applied after 2, 3 and 4 weeks from cultivation.

Soil analysis. Rhizosphere soil extracts (1:5) were prepared, and in the clear extract, electric conductivity (EC) was determined using conductivity meter (LUTRON meter model # - YK 22 CT), and total soluble salts (TSS) was calculated according to the association of official analytical chemists (William, 1980). Electric pH-meter (LUTRON meter model # - pH 208c) was used to determine the soil reaction of the collected samples. Organic matter, calcium (Ca^{+2}), magnesium (Mg^{+2}), phosphate (PO_4^{-3}), sulphate (SO_4^{-2}) and nitrates (NO_3^-) were determined according to William (1980).

Plant analysis. After 45 days of cultivation, plants of each pot were harvested, divided into shoots and roots and the roots washed well with tap water. The shoot and root lengths (cm) per individual plant were determined. The fresh and dry mass were determined.

Mycorrhizal colonization assessment. The mycorrhizal colonization assessment in the roots were determined according to Phillips and Hayman (1970).

Estimation of some metabolites in maize. Soluble sugars in roots and shoots of maize plants were determined according to Dubois *et al.* (1956). The total free amino acids were determined by using ninhydrine reagent according to Lee and Takahashi (1966). The soluble proteins were determined according to Lowry *et al*. (1951).

C. Statistical analysis

Data were subjected to statistical analysis using SPSS (version 16). One-way ANOVA was performed followed by the post hoc Duncan's multiple-range test for comparison between means at *P*< 0.05. Factorial ANOVA was carried to achieve the effect of each fertilizer and their in-between interaction on different parameters estimated in plants, and partial eta square "η^2" was calculated as: $\eta^2 = SS_{between} / SS_{total}$. Correlation analysis (Pearson correlation) was performed to obtain the relation between some parameters.

RESULTS

Six morphotypes of arbuscular mycorrhizal fungi belonging to the order Diversisporales and Glomerales were isolated as the most dominant species from the old and reclaimed maize fields. The population consists of *Acaulospora gedanensis* Blaszk.; *Acaulospora koskei* Blaszk.; *Diversispora eburnea* (Kenn., Stutz & Morton) Walker & Schüßler comb. nov.; *Diversispora trimurales* (Koske & Halvorson) Walker & Schüßler comb. nov.; *Glomus badium* Oehl, Redecker & Sieverd.; *Pacispora robigina* Sieverd. & Oehl, (Fig. 1 A-F). These species used as mycorrhizal inoculum in the present work.

The segments of maize roots which infected by AMF were characterized by the presence of dense hyphae, vesicles and arbuscules. Generally, addition of fertilizers to mycorrhizal maize plants increased the percentage of arbuscular colonization (Figures 2&3). Application of AMF + CF_{hf} + BF to maize led to the highest hyphae colonization (98%), vesicles (77%) and arbuscules (58%). In contrast, the lowest hyphae (85%) and vesicles (47%) were recorded in mycorrhizal plants amended with CF_{fd} and bio-fertilizer, respectively. However, application of chemical fertilizers resulted in decreasing the vesicles colonization. Approximately, similar hyphal colonization (93%) were observed in mycorrhizal plants amended with CF_{hd} or bio-fertilizer.

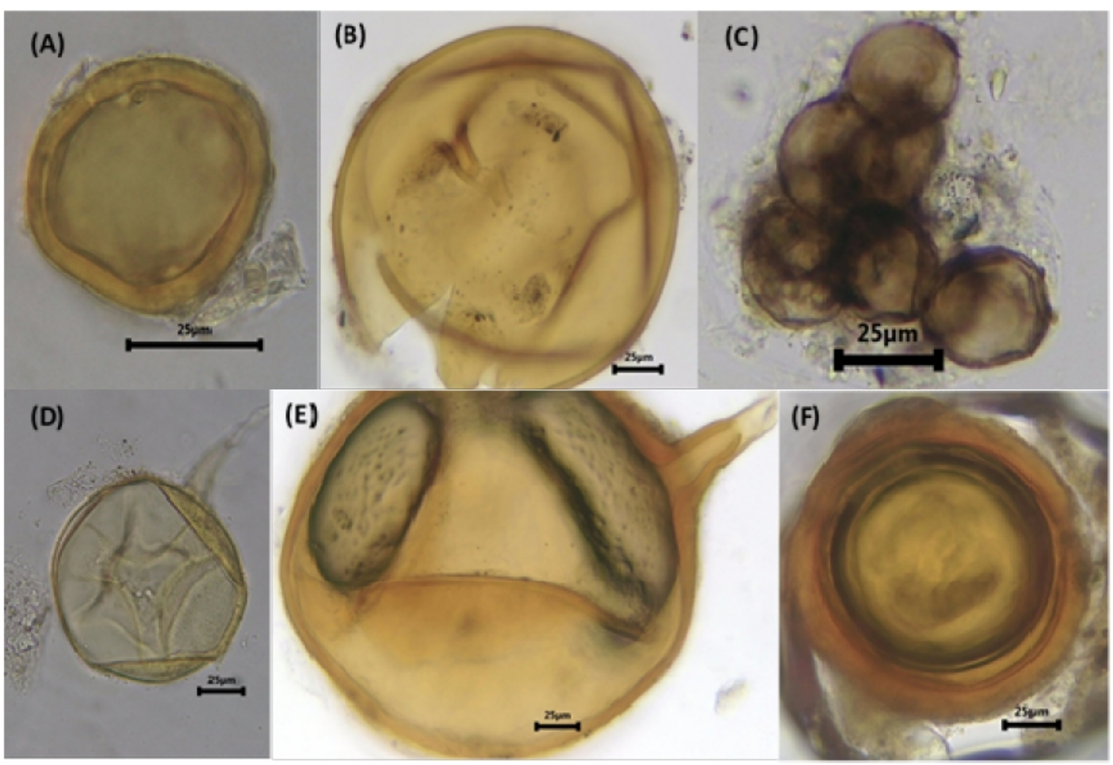

Fig. 1. Spores of (A) *Acaulospora gedanensis* Blaszk.; (B) *Acaulospora koskei* Blaszk; (C) *Glomus badium* Oehl, Redecker & Sieverd.; (D) *Diversispora eburnea* (Kenn., Stutz & Morton) Walker & Schüßler comb. nov.; (E) *Diversispora trimurales* (Koske & Halvorson) Walker & Schüßler comb. nov.; (F) *Pacispora robigina* Sieverd. & Oehl.

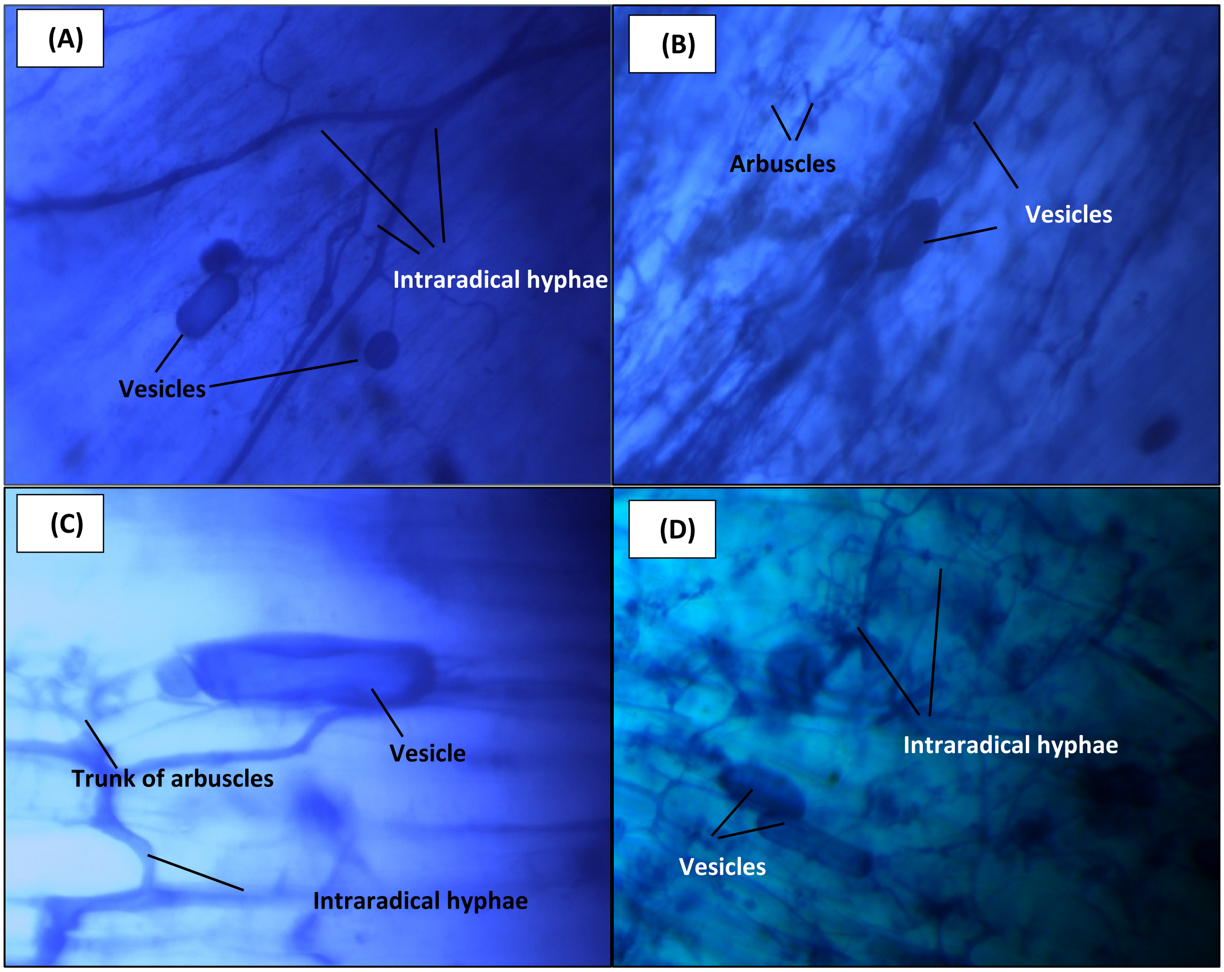

Fig. 2. Root colonization patterns of 45-old maize plants inoculated with mycorrhizal fungi. The intraradical hyphae and vesicles are shown in all panels **A-D**; arbuscles are shown in **B** and trunk of arbuscles are shown in **C**.

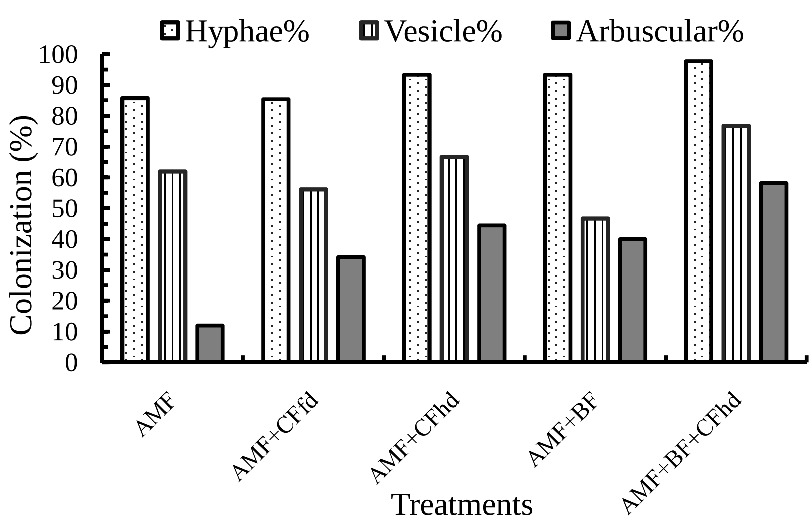

Fig. 3. Effect of chemical fertilizers (CF_{fd} for full recommended dose; CF_{hd} for half recommended dose) and bio-fertilizers (BF) on the percentage of maize mycorrhizal roots colonization.

A. Rhizospheric soil analysis

Results in Table 1 reveal that the pH of the used soil, before treatment, is neutral (7.04). After addition of different fertilizers, the pH values were still neutral or decreased to be slightly acidic and ranged between 6.47 and 6.88. When the chemical fertilizers are incorporated into the soil either alone or in combination with BF and/or AMF, the soil pH significantly decreased. Applying chemical fertilizers at the highest rate (CF_{fd}) resulted in a significant decrease of soil pH.

Applications of chemical fertilizers and/or bio-fertilizers to mycorrhizal or non-mycorrhizal soil significantly increased the electrical conductivity (EC), and concomitantly the soluble salts in the intrinsic soil solution. The maximum EC (2.74 $mScm^{-1}$) was observed in soil receiving CF_{hd}+BF, while the lowest EC (0.51 $mScm^{-1}$) was estimated in mycorrhizal soil; which was the only value that decreased significantly compared to control (Table 1). However, AMF additively increased the demand for soil nutrients and hence the soil EC and TSS decreased.

The results in the Table 1 indicate that amendment the soil with AMF has a great beneficial effect, leading to a high significant increase in its organic matter in comparison with non-inoculated soils. When the soil treated with AMF+CF_{hd}+BF, its content of organic matter approximately doubled. In contrast, it is found that addition of the only BF to the soil decreased significantly soil organic matter compared to control.

Results in Table 1 reveal that the highest concentration of available phosphate (0.03 mg g^{-1} DW) was estimated in the soil amended with AMF+CF_{hd}+BF. Also, the highest hyphae, vesicles and arbuscules colonization were found in this treated soil. Amendment the soil with CF at any level will normally increase the phosphate concentration in the soil. As it is shown from the data, application of BF either only or in combination with AMF suppressed, relative to control, the phosphate concentration in soil. The AMF proved a significant increase in the soil phosphate concentration compared to control soil. Our data demonstrated that soil SO_4^{-2} increased significantly in all treatments except in soil amended with AMF+BF.

Results in the Table 1 show that there is non-significant difference in the concentration of NO_3^- by application of CF_{fd} or CF_{hd} to either mycorrhizal or non-mycorrhizal soil. Meanwhile, the addition of CF_{fd} and/or CF_{hd} to mycorrhizal soil increased significantly nitrate concentration compared to mycorrhizal alone, non-mycorrhizal and control soils. Combination of AMF+BF suppressed nitrate concentration in the soil. Highest significant increase in the concentration of NO_3^- obtained when the non-mycorrhizal soil amended with CF_{hd}+BF or with only BF.

Results presented a significant difference in concentrations of both Ca^{+2} and Mg^{+2} in the soil of different treatments (Table 1). The highest significant increase of both divalent cations estimated in the soil amended with BF+CF_{hd}. There were non-significant differences of concentrations between mycorrhizal soil amended with CF_{fd} or with CF_{hd}. In contrast, a significant difference in soil Ca^{+2} recorded by amendment with only CF_{fd} or CF_{hd}. Addition of bio-fertilizers to mycorrhizal soil showed no effect compared to mycorrhizae alone. Meanwhile, the application of BF only resulted in a significant increase in Ca^{+2} concentration compared to mycorrhizae alone and control soils. High soil Mg^{+2} concentration was observed in mycorrhizal treatments compared to non-mycorrhizal treatments (Table 1). No significant difference of Mg^{+2} concentration observed between BF+AMF and HCF+BF+AMF treatments.

Table 1: Effect of AMF, bio-fertilizers (BF) and chemical fertilizers (full recommended dose, CF_{fd}; or half recommended dose, CF_{hd}) or their combinations on some physical and chemical properties of the soil before or after application of treatments by 45 days. E.C, electrical conductivity; TSS, total soluble salts; O.M, organic matter. All values are means± SD, n=3. Means of each parameter with different letters are significantly differ at P< 0.05 according to Duncan's test.

Parameters		Pre Exp.	Non inoculated with AMF					Inoculated with AMF				
			Control	CF_{fd}	CF_{hd}	BF	CF_{hd} +BF	Control	CF_{fd}	CF_{hd}	BF	CF_{hd} +BF
pH		6.68 ± 0.01^{h}	7.04 ± 0.01^{j}	6.47 ± 0.01^{a}	6.58 ± 0.01^{d}	6.63 ± 0.01^{f}	6.60 ± 0.00^{e}	6.51 ± 0.01^{b}	6.88 ± 0.01^{i}	6.53 ± 0.00^{c}	6.65 ± 0.01^{g}	6.57 ± 0.01^{d}
E.C (mS/cm)		0.99 ± 0.04^{d}	0.67 ± 0.00^{b}	0.79 ± 0.00^{c}	1.15 ± 0.00^{f}	1.15 ± 0.00^{f}	2.74 ± 0.01^{i}	0.51 ± 0.00^{a}	1.09 ± 0.01^{e}	1.40 ± 0.00^{h}	0.80 ± 0.01^{c}	1.35 ± 0.04^{g}
TSS%		0.32 ± 0.01^{d}	0.22 ± 0.00^{b}	0.25 ± 0.00^{c}	0.37 ± 0.00^{f}	0.37 ± 0.00^{f}	0.88 ± 0.00^{i}	0.16 ± 0.00^{a}	0.35 ± 0.00^{e}	0.44 ± 0.00^{h}	0.26 ± 0.00^{c}	0.43 ± 0.01^{g}
O.M%		0.61 ± 0.002^{a}	0.75 ± 0.00^{d}	0.96 ± 0.00^{f}	0.68 ± 0.00^{b}	0.73 ± 0.00^{c}	1.04 ± 0.00^{g}	0.80 ± 0.00^{e}	1.20 ± 0.00^{i}	1.11 ± 0.00^{h}	1.25 ± 0.00^{j}	1.43 ± 0.00^{k}
PO_4^{-3}	mg/g dry	0.017 ± 0.00^{a}	0.02 ± 0.000^{b}	0.022 ± 0.00^{d}	0.024 ± 0.00^{e}	0.018 ± 0.00^{a}	0.02 ± 0.00^{d}	0.021 ± 0.00^{c}	0.022 ± 0.00^{d}	0.02 ± 0.00^{b}	0.018 ± 0.00^{a}	0.03 ± 0.00^{f}
SO_4^{-2}		3.55 ± 0.29^{ab}	3.52 ± 0.11^{a}	3.64 ± 0.02^{ab}	3.91 ± 0.02^{ab}	3.68 ± 0.20^{ab}	3.81 ± 0.74^{ab}	3.56 ± 0.16^{ab}	3.81 ± 0.01^{ab}	4.00 ± 0.04^{ab}	3.53 ± 0.01^{a}	4.04 ± 0.02^{b}
NO_3^-		0.14 ± 0.01^{ab}	0.09 ± 0.00^{a}	0.16 ± 0.02^{bc}	0.14 ± 0.02^{ab}	0.24 ± 0.07^{d}	0.34 ± 0.05^{e}	0.11 ± 0.02^{ab}	0.21 ± 0.00^{cd}	0.19 ± 0.02^{cd}	0.09 ± 0.003^{a}	0.12 ± 0.027^{cd}
Ca^{+2}		1.31 ± 0.16^{c}	1.06 ± 0.12^{b}	0.83 ± 0.08^{a}	1.56 ± 0.21^{c}	1.45 ± 0.15^{c}	2.95 ± 0.20^{e}	0.68 ± 0.06^{a}	1.5 ± 0.09^{c}	1.43 ± 0.14^{c}	0.80 ± 0.10^{a}	1.81 ± 0.06^{d}
Mg^{+2}		1.79 ± 0.08^{a}	2.37 ± 0.08^{c}	2.02 ± 0.01^{b}	1.74 ± 0.24^{a}	2.14 ± 0.04^{b}	5.73 ± 0.12^{g}	2.56 ± 0.11^{c}	3.14 ± 0.11^{d}	3.54 ± 0.15^{e}	4.99 ± 0.20^{f}	5.11 ± 0.15^{f}

Table 2: Effect of AMF, bio-fertilizers (BF) and chemical fertilizers (full recommended dose, CF_{fd}; or half recommended dose, CF_{hd}) or their combinations on percentage of water content and dry mass of maize roots and shoots.

Treatments	WC%		Dry mass (g/Individual)		Root/Shoot Mass ratio	Plant DW/ind
	Root	Shoot	Root	Shoot		
Cont.	73.78 ± 0.10^{bc}	86.39 ± 0.85^{cd}	0.07 ± 0.01^{ab}	0.48 ± 0.04^{bc}	0.14 ± 0.01^{bcd}	0.54 ± 0.05^{bc}
CF_{fd}	72.03 ± 0.03^{bc}	84.12 ± 0.49^{a}	0.04 ± 0.00^{a}	0.87 ± 0.04^{ef}	0.05 ± 0.00^{a}	0.91 ± 0.04^{de}
CF_{hd}	44.92 ± 11.5^{a}	86.92 ± 0.29^{de}	0.08 ± 0.03^{ab}	0.95 ± 0.09^{f}	0.08 ± 0.03^{ab}	1.03 ± 0.12^{de}
BF	80.28 ± 0.14^{cd}	87.21 ± 0.10^{de}	0.13 ± 0.04^{bc}	0.78 ± 0.04^{ef}	0.17 ± 0.04^{cd}	0.92 ± 0.08^{de}
BF+CF_{hd}	72.61 ± 0.65^{bc}	89.35 ± 0.63^{g}	0.02 ± 0.01^{a}	0.14 ± 0.05^{a}	0.18 ± 0.04^{d}	0.16 ± 0.05^{a}
AMF	67.06 ± 0.10^{b}	85.34 ± 0.06^{abc}	0.17 ± 0.04^{c}	1.82 ± 0.01^{g}	0.09 ± 0.02^{ab}	1.99 ± 0.06^{f}
AMF+CF_{fd}	90.72 ± 0.64^{d}	87.96 ± 0.35^{ef}	0.03 ± 0.01^{a}	0.32 ± 0.03^{ab}	0.10 ± 0.02^{abc}	0.35 ± 0.04^{ab}
AMF+CF_{hd}	79.63 ± 0.48^{cd}	85.52 ± 0.21^{bc}	0.08 ± 0.00^{ab}	0.70 ± 0.04^{de}	0.12 ± 0.00^{abcd}	0.79 ± 0.04^{cd}
AMF+BF	78.00 ± 0.37^{bc}	84.63 ± 0.15^{ab}	0.09 ± 0.01^{ab}	0.95 ± 0.05^{f}	0.09 ± 0.01^{ab}	1.04 ± 0.07^{e}
AMF+BF+CF_{hd}	72.62 ± 0.79^{bc}	88.67 ± 0.31^{fg}	0.04 ± 0.01^{a}	0.58 ± 0.15^{cd}	0.08 ± 0.01^{ab}	0.62 ± 0.15^{c}

Means in each column with different letters are significantly differ at $P< 0.05$ according to Duncan's test.

B. Effects on plant growth

The water contents of maize roots (WC%) unchanged significantly by most treatments (Table 2). Interestingly, the significant increase in the water content detected in mycorrhizal plants amended with CF_{fd}. In contrast, non-mycorrhizal plants amended with CF_{fd} recorded a significant decrease in shoot water content. Also, water content increased significantly in the shoot of plants amended with BF+CF_{hd} and AMF+BF+CF_{hd}. Plants amended with AMF inoculation showed significant increase in roots and shoots dry weights, while a non-significant change in root dry weight detected in plants amended with other fertilizers (Table 2). Application of CF_{fd} or BF to the non-mycorrhizal plants resulted in a significant increase in plant dry weight compared to control plants. Plants amended with (AMF+CF_{fd}), (AMF+BF+CF_{hd}) showed no significant difference in dry weight. Application of (BF+CF_{hd}) to non mycorrhizal plants recorded a significant decrease in dry weight. The root: shoot mass ratio unchanged significantly by different treatments compared to control, except in plants treated with CF_{fd}. Results in Table 3 show that BF and CF_{hd} exerted the greatest magnitude effect on root and shoot dry weights, respectively.

C. Nitrates and phosphates in plants

Data illustrated in Figure 4 show a significant difference in nitrate concentration between mycorrhizal and non-mycorrhizal plants (roots and shoots) receiving CF_{fd} or CF_{hd}. In plants supplied with CF_{fd}, the concentration of NO_3 increased dramatically in their roots to about 27-fold of that in control plants, while, the highest concentration in shoots (66.91 mg g^{-1} DW) was estimated in plants treated with AMF+CF_{fd}. In addition, the concentration of NO_3 in roots of plants treated with AMF+CF_{fd} was significantly less than that in plants only treated with CF_{fd}.

Table 3: Eta-square (η^2) calculated for the effect of arbuscular mycorrhizal fungi (AMF), bio fertilizer (BF), chemical fertilizers (CF) and their interaction on maize different parameters. SP = soluble protein, SS = soluble sugars, TAA = total soluble amino acids, DW = dry weight.

Organ	Parameter	Treatments						
		AMF	BF	CF_{hd}	AMF*BF	AMF*CF_{hd}	BF*CF_{hd}	AMF*BF*CF_{hd}
Shoot	SP	0.467	0.118	0.003	0.172	0.032	0.004	0.079
	SS	0.068	0.053	0.000	0.044	0.019	0.143	0.389
	TAA	0.133	0.699	0.048	0.036	0.060	0.000	0.000
	DW/Ind.	0.010	0.181	0.515	0.132	0.019	0.032	0.000
	PO_4^{-3}	0.141	0.416	0.109	0.022	0.011	0.132	0.148
	NO_3^-	0.034	0.059	0.844	0.000	0.039	0.022	0.001
Root	SP	0.000	0.291	0.335	0.025	0.002	0.008	0.321
	SS	0.086	0.083	0.508	0.117	0.004	0.001	0.177
	TAA	0.009	0.498	0.167	0.066	0.032	0.038	0.158
	DW/ Ind.	0.003	0.208	0.042	0.166	0.000	0.125	0.042
	PO_4^{-3}	0.109	0.185	0.365	0.010	0.135	0.003	0.172
	NO_3^-	0.057	0.053	0.141	0.267	0.183	0.194	0.105
Individual DW		0.008	0.192	0.472	0.144	0.016	0.040	0.002

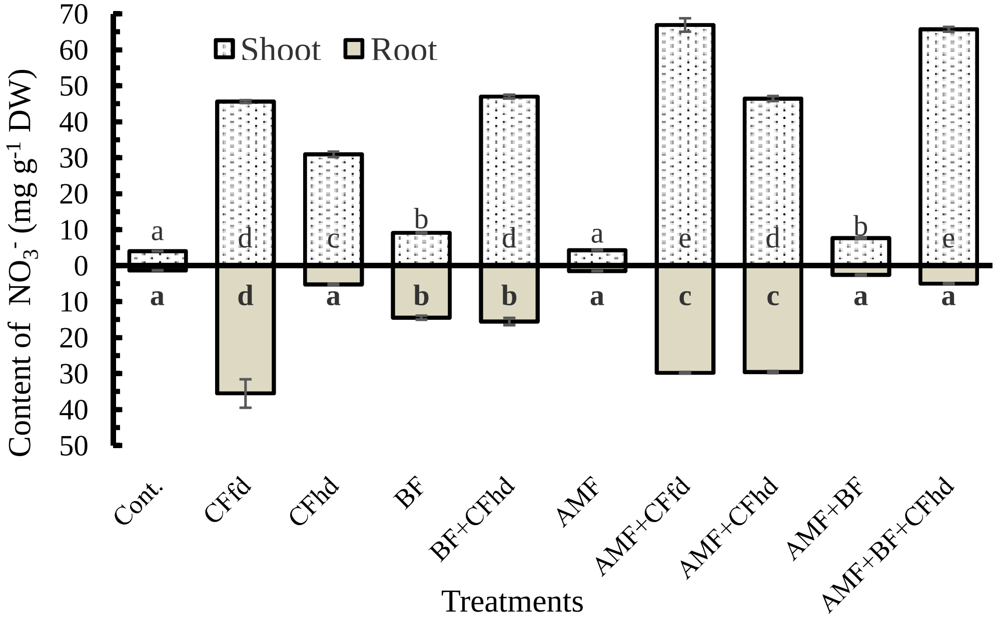

Fig. 4. Effect of chemical fertilizers (CF_{fd} for full recommended dose; CF_{hd} for half recommended dose) and bio-fertilizers (BF) on concentrations of nitrate in mycorrhizal and non-mycorrhizal maize roots and shoots. The data are means ±SE, n = 3. Means for shoots or roots with different letters are significantly differ at $p < 0.05$ according to Duncan's test.

It is also found that application of BF to the soil increased significantly nitrate concentration in root and shoots of maize, while application of AMF only resulted in non-significant change. As shown in Table 3, the interaction between BF*AMF and CF_{hd} have the greatest magnitude of effect on changes of nitrate content in maize roots and shoots, respectively.

The data illustrated in Fig. 5, all treatments significantly increased the concentration of PO_4^{3-} either in roots or shoots of maize. The highest concentration of PO_4^{3-}in the roots (4.57 mg g^{-1} DW) was estimated in case of application of CF_{hd}, while the highest content of PO_4^{3-} in the shoots (3.911 mg g^{-1} DW) was obtained when the plant treated with AMF+BF+CF_{hd}.

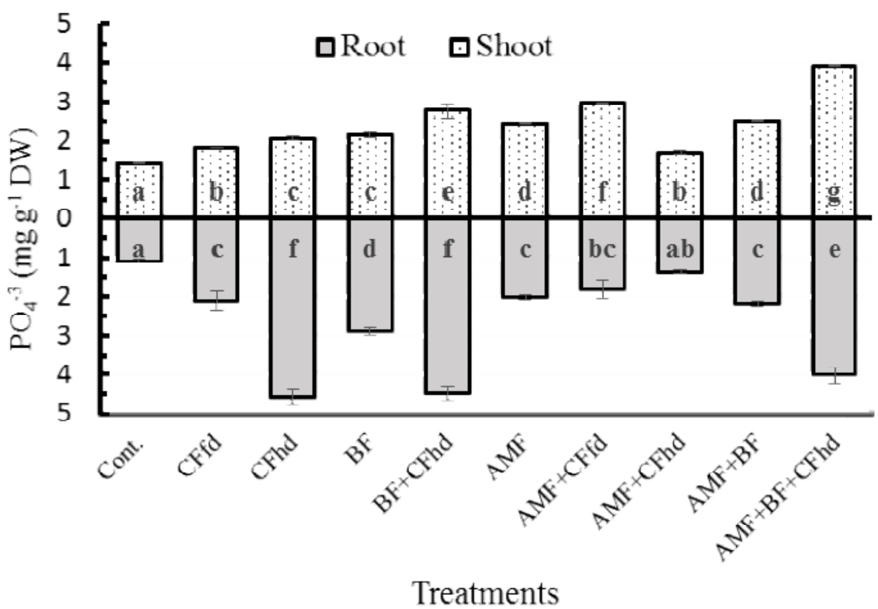

Fig. 5. Effect of chemical fertilizers (CF_{fd} and CF_{hd}) and bio-fertilizers (BF) on concentrations of phosphate in mycorrhizal and non-mycorrhizal plants (roots and shoots). Statistics as in Figure 4.

The results also proved that mycorrhizal plants showed a significant increase in the concentration of phosphate plants (roots and shoots) in comparison with control plants. On the other hand, the addition of P increased the P concentration in the plants of the non mycorrhizal plants. The CF_{hd} and BF have exerted the highest magnitude of effect on the changes of phosphate concentrations in roots and shoots, respectively (Table 3). Table 4 shows the r-values of correlation analyses between corresponding parameters in roots and shoots of maize with that in the soil. There is a strong or significant correlation between the concentration of PO_4^{-3} in the plant and that in the soil. The concentration of nitrate in maize weakly correlated with that in the soil. Root soluble protein significantly correlated with shoot nitrate, while root nitrate negatively correlated with root and shoot soluble sugars.

Table 4: r-values of correlation analyses between different parameters in soil, roots or shoots of maize.

Parameters		Root					Shoot				
		NO_3^-	**PO_4^{-3}**	**SP**	**SS**	**TAA**	**NO_3^-**	**PO_4^{-3}**	**SP**	**SS**	**TAA**
Soil	NO_3^-	0.094	0.507	0.457	0.418	0.437	0.537	0.346	0.656*	0.226	0.565
	PO_4^{-3}	-0.108	0.587	0.655*	0.796**	0.403	0.640*	0.643*	-0.033	-0.077	0.216
Root	NO_3^-		-0.309	0.1	-0.038	-0.128	0.336	-0.384	-0.043	-0.306	-0.360
	PO_4^{-3}			0.614	0.817**	0.354	0.294	0.507	0.324	-0.102	0.629
	SP				0.843**	0.844**	0.738*	0.695*	0.477	0.004	0.573
	SS					0.629	0.644*	0.51	0.353	0.005	0.56
	TAA						0.656*	.667*	0.566	0.367	0.675*
Shoot	NO_3^-							0.536	0.278	0.26	0.327
	PO_4^{-3}								0.201	0.219	0.595
	SP									0.37	0.547
	SS										0.542

*: Significant at $P< 0.05$; **: Significant at $P< 0.01$. SP= soluble protein, SS=soluble sugars, TAA= total soluble amino acids.

D. Some metabolites in maize

It is found that both of the root and shoot soluble proteins increased significantly by treatment with AMF+BF+CF_{hd} (Fig. 6). The content of soluble proteins in roots or shoots unchanged significantly by CF_{fd} or CF_{hd}. On the other hand, soluble proteins in roots and shoots of mycorrhizal plants amended with CF_{fd} increased significantly compared to those amended with CF_{hd}.

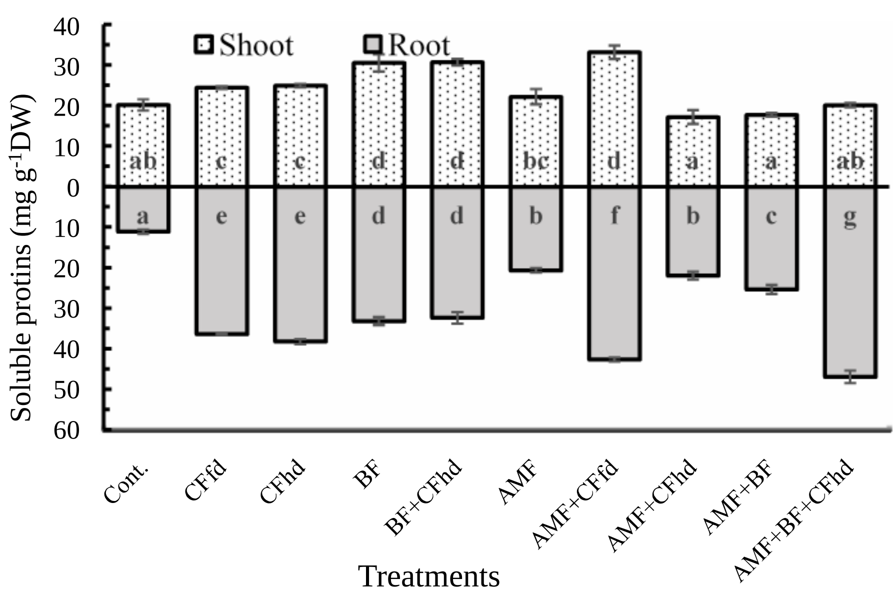

Fig. 6. Effect of chemical fertilizers (CF_{fd} and CF_{hd}) and bio-fertilizers (BF) on concentrations of soluble protein in mycorrhizal and non-mycorrhizal plants (roots and shoots). Statistics as in Figure 4.

Treatment of mycorrhizal plants with BF, CF_{hd} or their increased significantly the soluble protein content compared to mycorrhizal or control plants. Results shown in Table 3 indicate that interaction between AMF*BF*CF_{hd} and AMF have the greatest magnitude of effect on changes of soluble protein contents in root and shoot, respectively. Application of BF and BF+CF_{hd} resulted in significant increases in contents of free amino acids in maize shoots and roots (Fig. 7). Interestingly, it was detected that free amino acids in roots and shoots of maize treated with the only BF increased significantly compared to those treated by CF. The content of amino acids in root of maize treated with AMF+BF+CF_{hd} increased significantly compared to those receiving BF+Cf_{hd} or only BF. The changes in amino acid concentrations in both shoots and roots greatly affected by BF.

It is observed that CF_{hd} or AMF+CF_{fd} significantly increased the content of soluble sugars in maize roots (Fig. 8).

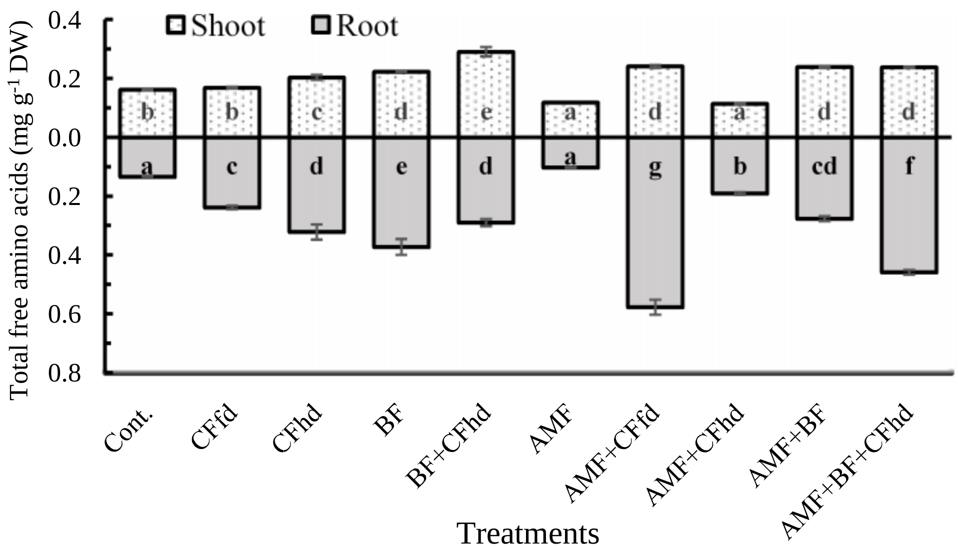

Fig. 7. Effect of chemical fertilizers (CF_{fd} and CF_{hd}) and bio-fertilizers (BF) on concentrations of free amino acids in mycorrhizal and non-mycorrhizal plants (roots and shoots). Statistics as in Figure 4.

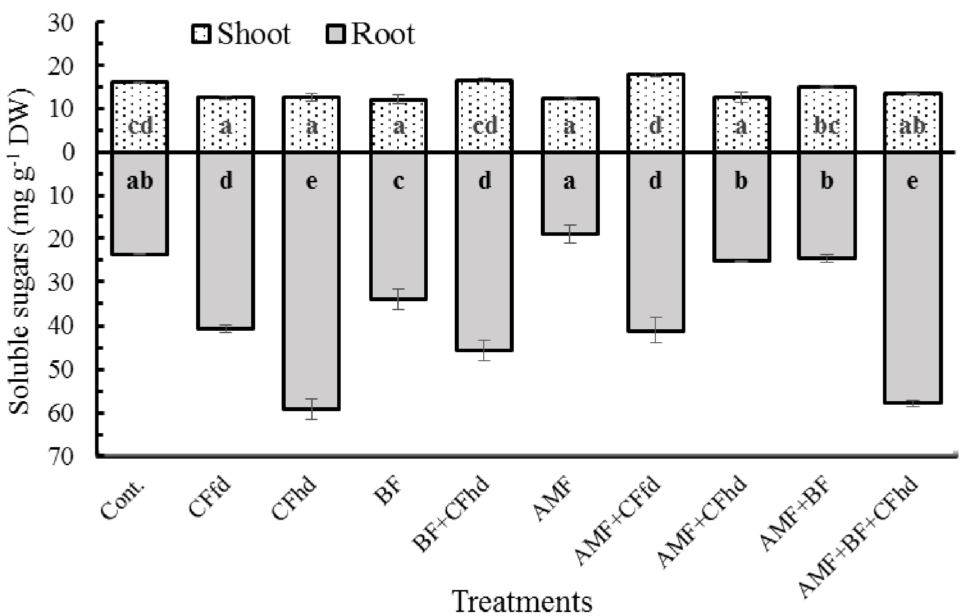

Fig. 8. Effect of chemical fertilizers (CF_{fd} and CF_{hd}) and bio-fertilizers (BF) on concentrations of soluble sugars in mycorrhizal and non-mycorrhizal plants (roots and shoots). Statistics as in Figure 4.

The soluble sugars in the plant shoot either unchanged or decreased significantly by all treatments. AMF significantly decreased the plant soluble sugars. Also, in plants treated with AMF+BF+CF_{hd} the soluble sugars significantly increased in their roots but decreased in shoots. Results in Table 3 show that CF_{hd} and interaction between AMF*BF*CF_{hd} have the greatest magnitude effect of on changes of soluble sugars in shoot and root, respectively.

DISCUSSION

In this work, it has been shown that AMF with beneficial bacteria present in the bio-fertilizer and low chemical fertilization level increased root colonization, and this will magnify the beneficial effect of AMF. Recently, Frey-Klett *et al*. (2007) presented clear evidence that some mycorrhiza helper bacteria promote the functioning of the mycorrhizal symbiosis. For example, *Azospirillum brasilense* interact positively with *Gigaspora margarita* and introduced increasing in percentage of mycorrhizal *Pennisetum americanum* root colonization.

Soil pH is the deciding factor for the availability of essential plant nutrients. Results agree with previous studies which found that addition of different fertilizers such as NPK (applied as urea), and superphosphate (Qin *et al.*, 2015) phosphogypsum, cow manure and microbial inoculation (*Azotobacter chroococcum*, and phosphate-solubilizing bacteria) decreased the soil pH (Al-Enazy *et al.,* 2017). Organic matter in bio-fertilizers also induces acidity which did not counterbalance as there are no cations were added (Roy and Kashem, 2014). Harleen Kau*r et al.* (2017) explained the decrease in pH due to the extracellular secretions made by microbes.

The EC of soil is directly related to the ions present in it. Data agree with Harleen Kaur *et al.* (2017) who found also that electrical conductivity (EC) increased with the addition of inorganic and bio fertilizers. The increasing in EC values could be explained by the addition of chemical fertilizers and induction of nutrient availability by the action of bio-fertilizers and AMF (Peng *et al.*, 2013). Khan *et al.* (2000) reported that the AMF increase the surface area of the root greater than that of the plant root system. Thus, the AMF hyphae can explore a larger volume of soil compared to the root system of the plant. Subsequently, it released the nutrients and improve water uptake by plants.

The results of this study documented that microbial inoculum improved the soil organic matter content (Wu *et al.*, 2005). The organic matter and humus are capable of retaining relatively large quantities of water thus increasing the water holding capacity of the soil and concomitantly improve the plant growth. In addition, organic matter and humus, like clay, have higher cation exchange capacity and will increase considerably the cation holding capacity of the soil. Decreasing in soil organic matter in case of treatment with only BF may attributed to that BF-microorganisms, which are mainly bacteria, consume a considerable amount of organic matter, e.g. carbohydrates and proteins, to provide their energy for maintenance and growing demand. This also indicates that the rate of biological activity of BF-microorganisms is higher than the rate of addition of organic matter in the BF. (Natsheh and Mousa, 2014) revealed that the application of BF combined with or without CF to the soil is considered as a good management practices in any agricultural production system to improve soil fertility.

It is obvious that the plant-mycorrhizae association is hampered in either high or low extremely fertile soil. The low fertilization level of chemical fertilizers showed an increase of AMF, which indicated that plants could be more dependent on mycorrhizal symbiosis than chemical fertilizers (Wu *et al.*, 2005). Our results disagree with the results of (Wu *et al.*, 2005) who found that available P in soil was significantly increased with the inoculation of AM fungi in combination with rhizobacteria. This decrease in soil phosphate concentration could be explained by the consumption of phosphate by microorganisms for building its cell components (Rodríguez and Fraga, 1999). On the other hand, we agree with (Wu *et al.*, 2005) who found that available P in soil was significantly increased with the inoculation of AM fungi alone.

AMF colonization has mainly been attributed to the enhanced uptake by AM of relatively immobile soil ions such as phosphorus (P), calcium (Ca), magnesium (Mg) and sulfur (S) (Liu *et al.,* 2007).

Salama (2011) revealed that the inoculation of Arable soil with bio-fertilizers enhances the nitrogen content. The bio-fertilizer, therefore, may have a potential to decrease the input cost of agricultural production, and be applied to the revegetation of low commercial value sites, such as metal tailings ponds (Carlot *et al.,* 2002).

The increasing in cation availability in soil may be due to the beneficial effects of BF and AMF in supplying and availability of plant nutrients, improving the cation exchange capacity that enables them to retain nutrients in the root zone and supporting microbial activities (Sarker *et al.,* 2012). (Peng *et al.,* 2013) revealed that the addition of BF to soil planted with maize enhanced the different cations and anions in the soil.

This study reflects the role of AMF in improving the plant water status by acting as an extension of the plant roots.

This positive effect was associated with the mycorrhiza contribution in the uptake of host mineral nutrient especially immobile soil nutrients. Previous studies such as (Yousry *et al.*, 1978) which found that inoculation of pea (*Pisum sativum*) plants with bio-fertilizer increased plant dry matter by 10.9%. (El-Khateeb *et al.,* 2010) which proved that bio fertilizers significantly increased the fresh and dry weights of roots. Results disagrees with the study of (Mohammad *et al.,* 2003) which proved that both the application of P fertilizers and the soil inoculation with a mixture of AMF increased the dry weight. Also, results disagrees with the study of Jnawali *et al.* (2015) which proved that combined application of bio-fertilizer with 50% of chemical fertilizers (N and P) has a positive role for safflower growth in comparison with chemical fertilizers alone.

Nitrogen is a vital macronutrient for plants, necessary for the biosynthesis of many basic cellular components, such as DNA, RNA, and proteins (Allen and Shachar-Hill, 2009). Researchers showed that 75% of the nitrogen in a young maize leaf originated from the extra mycelium (Tanaka and Yano, 2005). The results indicated that increasing nitrates concentration in the plant, as it was expected, is mainly due to the application of chemical fertilizers, but AMF seems to have a paramount role in regulating NO_3 translocation between the roots and shoots of the plant. There are some evidence that nutrients such as NO_3, NH_4 and PO_4 can be absorbed, whine available, freely at all locations of the root surface of maize (Sharp *et al.,* 1998). Results proved that translocation of PO_4^{3-} from the roots to the shoots is magnified by mycorrhizal association. Early calculations by Sanders and Tinker (1971) showed that the mycorrhizal association-roots can transport PO_4^{3-} at a rate more than 4-fold of that a non-mycorrhizal root. In agreement with the study of Mohammad *et al.* (2003) which revealed that phosphorus concentration in the plants was higher in the mycorrhizal plant compared to the non-mycorrhizal ones when P was not added. The significant positive correlation between the content of amino acids in roots and NO_3 in shoots indicates to the rapid incorporation of NO_3 into amino acids in the shoots and transportation of amino acids into the roots.

CONCLUSION

This study indicated that while CF increase both of the potential and available supply, the AMF and BF increase the available supply of the plant-nutrients content of the soil. The maize growth and metabolism are determined by the status of the chemical changes taking place in the soil and plant, and not completely by the potential plant-nutrient content of the soil. Inoculation of maize with AMF and BF have beneficial effect on supplying the plant with continuously available essential nutrients by which reflected on the plant growth and metabolism. This will reduce the needs for chemical fertilizers, and the kind and low quantity of CF have to be use will be to give the most satisfactory yields.

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