

Arbuscular mycorrhizal isolate and phosphogypsum effects on growth and nutrients acquisition of cotton (*Gossypium hirsutum* L.)

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Key words: Arbuscular mycorrhizal fungi, *Gossypium hirsutum*, indigenous, phosphogypsum.

Abstract: Cotton was grown in pots with added phosphogypsum (PG) to evaluate the effect of indigenous arbuscular mycorrhizal fungi (AMF) and phosphogypsum on cotton growth and acquisition of phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn). AMF isolate was a mixture of *Glomus intraradices*, *Glomus viscosum*, and *Glomus mosseae* previously isolated from a cotton field. Shoot dry biomass was enhanced significantly by both indigenous AMF and PG. Shoot dry biomass and seed cotton yields were enhanced by the AMF and PG combination and even more when PG in compost was added to mycorrhizal plants. P content in AMF with PG and in AMF with PG/compost treated plants was, respectively, 209.3 and 278.7%, significantly higher than control. Acquisition of K, Ca, and micronutrients was significantly enhanced by the combination of AMF and PG. The treatment of AMF with PG/compost induced the highest contents in Mn, Fe, Cu and Zn which were found to be, respectively, 287, 201, 192.8, and 171% higher compared to control. Results indicate that cotton growth responded to indigenous AMF in soils amended with PG. Combination of AMF with PG added in compost can ensure satisfactory benefits for cotton growth in low input, sustainable cropping systems.

1. Introduction

Acquisition of mineral nutrients is important to plant growth and productivity. The ability of plants to acquire nutrients may be associated with root colonization with arbuscular mycorrhizal fungi (AMF) (Clark and Zeto, 1996). AMF are obligatory biotrophic symbionts occurring in nearly all natural and agricultural soils and commonly colonize roots of many plant species (Smith and Read, 1997). Acquisition of mineral nutrients by plants with AMF depends on factors such as soil pH, soil nutrient deficiencies, AMF isolate, and plant species (Sylvia and Williams, 1992). Previous studies showed a positive response of cotton to AMF (Liu *et al.*, 1994; DeFeng *et al.*, 1998; Ibrahim, 2010). In subsistence agriculture systems, it is important to use indigenous AMF that are ecotypically adapted to the site (Davies *et al.*, 2005). Native AMF can grow and function better in soils from which

they are isolated, e.g. agricultural systems (Calvente *et al.*, 2004).

Phosphogypsum (PG) is the main by-product of the industrial production of phosphoric acid by treatment of rock phosphate with sulfuric acid. Calcium sulfate is the dominant component in PG. PG contains the radioactive materials ^{226}Ra and ^{210}Po , phosphorus, silicon, Fe, Cu, and F⁻ (Al-Masri *et al.*, 2004). Studies suggest that PG can be used in the improvement of soil structure, plant growth and agricultural production (Alcordo and Rechcigl, 1993), enhancing seedling emergence (Vyshpolsky *et al.*, 2010), and increasing available S and P (Al-Oudat *et al.*, 1998). The use of PG as a fertilizer in agriculture has been practiced in many parts of the world (Enamorado *et al.*, 2009) without constituting environmental hazards to soil and crop tissue (Al-Oudat *et al.*, 2011).

Application of PG (a poorly soluble source of P) to soil may become available to plants by solubilization from AMF (Al-Karaki and Al-Omouh, 2002). Solubilization of PG might insure a continuous supply of P without inhibiting root AMF colonization (Cui *et al.*, 2014). In addition, enhanced acquisition of nutri-

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Received for publication 21 April 2016

Accepted for publication 1 July 2016

ents by AMF plants in combination with PG has been reported (Al-Karaki and Al-Omouh, 2002; Bai *et al.*, 2011).

Compost increases soil organic carbon (El Mrabet *et al.*, 2014) and could increase the release of micronutrients in the soil, making them more available to plants (Habashy *et al.*, 2008; Jan *et al.*, 2014). On the other hand, addition of PG during manure composting decreased the amount of ammonia lost by volatilization (Prochnow *et al.*, 1995). Therefore, addition of compost and the introduction of mycorrhizal technology may become an effective way of applying PG to soils with P deficiency.

The objective of this research was to determine the effects of indigenous AMF, in combination with Syrian PG alone or integrated in compost, on growth and mineral nutrients acquisition of a Syrian cotton variety.

2. Materials and Methods

The experiment was conducted in pots during the summer season (May-September 2013) at Der-Alhajar research Station, located southeast of Damascus, Syria (33°21' N, 36°28' E) at 617 m above sea level. The area is located within an arid region in which the total annual precipitation is 120 mm. Sandy clay loam soil was air dried, sieved to pass a 3 mm screen, and pasteurized at 5 kGy of gamma ray (GR) with ^{60}Co source using a gamma irradiator (ROBO, Russa).

Phosphogypsum (PG) was previously collected from the area near the phosphoric acid factory in Homs (180 km N of Damascus). A total of 20 composite samples of PG were obtained from the levels corresponding to pile ages of approximately 1, 3-6 and 7-12 years, from the top, middle and bottom layers, respectively (each sample weighed 1 kg). PG samples were ground, homogenized, and sieving through a 0.5 mm sieve. Before planting, soil was mixed with PG alone (at a rate of 30 g kg⁻¹ dry soil) and PG integrated in compost (PG/compost mixture was prepared to be applied at a rate of 30 g of compost plus 30 g of PG per kg dry soil). Compost was pasteurized at 120°C for 20 min in the autoclave. Apart from PG, no chemical fertilizers were added during the experiment.

Mycorrhizal inoculum was a mixture of *Glomus intraradices* (Schenck & Smith), *Glomus viscosum*

(Nicolson), and *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. AMF inoculum was previously isolated (Ibrahim, 2010) from a cotton field at Der-Alhajar Research Station and multiplied in pot cultures using onion (*Allium cepa*) as a host. The inoculum consisted of fragments of onion root and spores mixed with soil.

Half of the pots were inoculated with AMF inoculum (100 g per pot) at the time of sowing. Non-mycorrhizal pots were prepared by mixing the same amount of sterilized AMF inoculums.

The treatments were: T1, no AMF inoculation without PG; T2, AMF inoculation without PG; T3, no AMF inoculation with PG; T4, AMF inoculation with PG; T5, no AMF inoculation with PG/compost; and T6, AMF inoculation with PG/compost.

Seeds of cotton (*Gossypium hirsutum* cv. Aleppo 33/1) were sterilized in 20% NaClO for 1 min and subsequently rinsed with sterilized water. They were then sown five per pot and placed to grow under natural conditions. Ten days after emergence, seedlings were thinned to one per pot; the roots of discarded plants were left in the soil to avoid removing the AMF inoculum. Plants were watered with tap water as needed. Growth parameters, such as plant height and fresh weight, were measured at the physiological maturity stage. The total shoot dry weights were measured after oven drying to constant weight at 70°C. Boll weight and number of mature bolls per plant at the first handpicking were recorded. The seed cotton yield and percent lint of each plant was determined at one handpicking for all treatments.

The vegetative portion of the plants was ground to a fine powder (0.5 mm). Nitrogen was determined using the Kjeldahl method and phosphorus was determined colorimetrically using a spectrophotometer (Thermo Spectronic, UK), while determination of Ca, K, Fe, Zn, Cu, Mn was performed by x-ray fluorescence (XRF). Root samples were rinsed free of soil, cut into 1 cm fragments, thoroughly mixed, cleared with KOH and stained with acid fuchsin in lactoglycerol. Percent root colonization and percent root length colonized by AMF were determined microscopically using a gridline intercept method (Giovannetti and Mosse, 1980).

The experimental design was randomized complete blocks with four replications. Data were subjected to analysis of variance by the SAS program (SAS Institut Inc, 2004) and means were compared using the Least Significant Difference (LSD) test at a probability level of $P \leq 0.05$.

3. Results

Addition of PG to the soil significantly decreased pH (Table 1). AMF root colonization was between 22.8 and 30.8% regardless of PG addition; the percent was higher for plants grown without added PG than added PG alone. Adding PG in compost increased the percent of AMF root colonization of cotton plants (Table 1). The percentage of mycorrhizal root length was 66.3, 54.5 and 73.3% in the AMF plants, AMF plus PG, and AMF with PG/compost treated plants, respectively (Table 1). No AMF root colonization was noted for plants grown without AMF.

Table 1 - Experimental soil pH, mycorrhizal root colonization, and some growth parameters of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	AMF colonization (%)	Mycorrhizal root length (%)	Soil pH	Plant height (cm)	Fresh biomass (g plant ⁻¹)	Dry biomass (g plant ⁻¹)
Control	0	0	8.1 a	38.3 e	141.72 c	44.40 e
AMF	24.60 b	66.30 b	8.1 a	55.0 d	256.30 ab	70.38 cd
PG	0	0	7.5 b	58.0 cd	240.86 b	65.79 d
AMF+PG	22.80 bc	54.50 bc	7.5 b	62.0 bc	295.87 a	78.05 b
PG/compost	0	0	7.2 c	64.3 b	255.43 ab	72.97 bc
AMF+PG/compost	30.80 a	73.30 a	7.1 c	73.8 a	316.52 a	96.64 a

Mean values within columns followed by different letters are significantly different at $P < 0.05$.

Significant differences between mycorrhizal and nonmycorrhizal plants were noted for shoot dry biomass regardless of PG addition (Table 1). Shoot dry biomass was significantly enhanced by indigenous AMF and when the mixture of PG/compost was added to soil. Application of PG to soil significantly increased shoot dry biomass for both mycorrhizal and nonmycorrhizal plants. Shoot fresh weight significantly increased in AMF inoculated and PG treated plants in comparison to the control (Table 1). Plant height at harvest was between 38.3 and 73.8 cm, and it was significantly enhanced by AMF inoculation and PG addition compared to control. The maximum plant height was observed for mycorrhizal cotton plants grown with PG/compost mixture.

The growth response of cotton plants to AMF, PG, and AMF plus PG treatments increased by 59.6, 49.4, and 76.3% over control, respectively (Table 2). In addition, the growth response of cotton to the combination of AMF with PG/compost was higher by 118.9% over control (Table 2).

Yield components of cotton under different treatments are shown in Table 2. The number of bolls per

Table 2 - Growth response and some yield components of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	Growth response (%)	Boll number (per plant)	Boll weight (g)	Seed cotton yield (g plant ⁻¹)	Lint (%)
Control	0	5.3 c	4.9 c	18.4 d	34.3 f
AMF	59.6 bc	7.8 b	5.1 b	32.9 c	37.9 e
PG	49.4 c	7.3 b	5.2 b	29.8 c	42.3 d
AMF+PG	76.3 b	8.3 b	5.3 b	36.9 b	42.9 c
PG/compost	64.7 bc	8.0 b	5.5 b	36.5 b	46.4 b
AMF+PG/compost	118.9 a	9.5 a	6.1 a	50.8 a	47.3 a

Mean values within columns followed by different letters are significantly different at $P < 0.05$. Values are mean ($N = 4$).

Growth response (%) = $(DW_{AMF} - DW_{control}) / DW_{control} \times 100$.

Lint (%) = $(\text{lint weight} / \text{seed cotton weight}) \times 100$.

Seed cotton = seed + lint.

plant was significantly increased by both AMF and PG in comparison with control. The plants showed the highest number of bolls when PG/compost mixture was added to AMF plants (T6). The increase in boll number led to an increase in seed cotton yield, which was improved by both AMF and PG. Seed cotton yield varied between 50.83 g plant⁻¹ (4574 kg ha⁻¹ on the basis of a density of nine plants m⁻²) and 18.38 g plant⁻¹ (1654 kg ha⁻¹). The highest seed yield of cotton was observed in AMF plants with added PG/compost. Boll weight was generally higher in AMF plants and PG treated plants than control and it was significantly higher with the AMF plus PG/compost treatment compared to other treatments. Percent lint varied between 47.3 and 34.3%, and AMF inoculation increased it significantly. In addition, lint percentage was significantly increased by PG and this increase was clearly noted when PG was contained in compost.

N and P concentrations in the vegetative portions of plants were significantly affected by AMF inoculation (Table 3, Fig. 1). The concentrations of N and P

Table 3 - Concentrations of K, Ca, and micronutrients in vegetative portion of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	K (mg g ⁻¹ DM)	Ca (mg g ⁻¹ DM)	Mn (μg g ⁻¹ DM)	Fe (μg g ⁻¹ DM)	Cu (μg g ⁻¹ DM)	Zn (μg g ⁻¹ DM)
Control	22.28 c	30.42 e	85.05 c	593.8 d	3.63 d	15.23 d
AMF	28.57 b	33.44 d	127.75 b	676.3 c	4.62 c	18.78 b
PG	23.19 c	34.65 d	104.75 bc	688.3 c	3.78 d	16.28 cd
AMF+PG	29.44 b	40.28 b	154.75 a	797.5 b	4.69 c	20.58 a
PG/compost	29.61 b	36.72 c	116.25 b	766.0 b	5.12 bc	16.68 c
AMF+PG/compost	32.67 a	43.61 a	172.75 a	930.8 a	5.59 a	21.88 a

Mean values within columns followed by different letters are significantly different at $P < 0.05$.

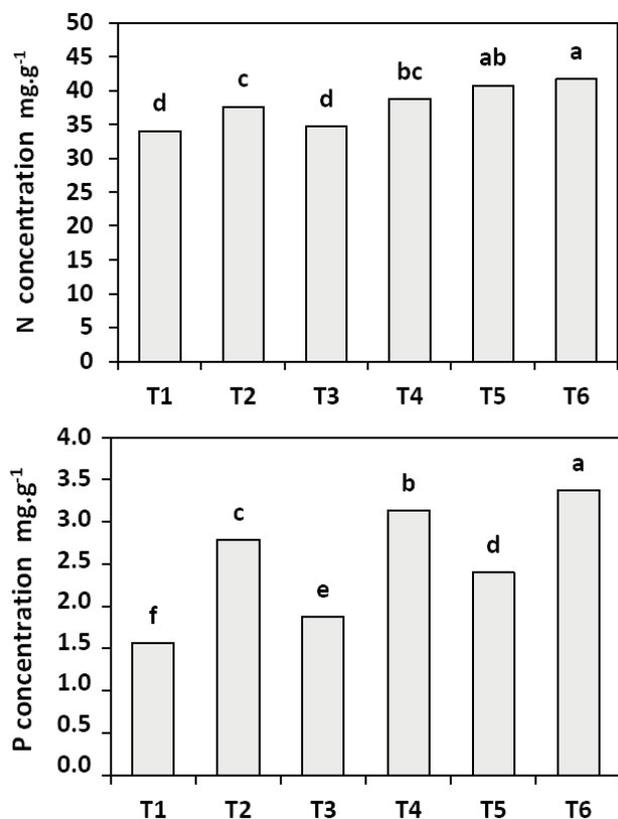


Fig. 1 - Concentrations of N and P in vegetative portion of cotton plants grown at different treatments (T1= no AMF without PG; T2= AMF without PG; T3= no AMF with PG; T4= AMF with PG; T5= no AMF with PG/compost; and T6= AMF with PG/compost).

were generally higher for mycorrhizal than for non-mycorrhizal plants. PG significantly increased N concentration only when combined with compost for nonmycorrhizal (T5) and mycorrhizal plants (T6), while it significantly increased P concentration in nonmycorrhizal and mycorrhizal plants.

The role of the combination of AMF and PG in improving plant P content was noted (Table 4). Data indicate that maximum plant P contents of 107.8 and 131.9 mg plant⁻¹ were found in AMF plus PG and AMF with PG/compost treatments, respectively, which was significantly higher (P≤0.05) by 209.3 and 278.7%, respectively, over control (Table 5).

The concentrations of K and Ca were significantly higher in the vegetative portion of mycorrhizal compared to nonmycorrhizal plants regardless of PG (Table 3). PG significantly increased Ca concentration in nonmycorrhizal and mycorrhizal plants; added alone it had no significant effect on K concentration in either group. The concentrations of both elements increased significantly when PG/compost mixture was added to mycorrhizal plants.

Higher concentrations of Mn, Fe, Cu, and Zn were noted for mycorrhizal than for nonmycorrhizal plants (Table 3) and PG significantly increased Fe concentration. Mn and Zn concentrations were increased by PG only in AMF plants while PG had no significant effect

Table 4 - Contents of nutrients in cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	Nutrient content (mg plant ⁻¹)							
	N	P	K	Ca	Mn	Fe	Cu	Zn
Control	708.0 e	35.2 e	465.3 e	634.0 e	1.77 d	12.5 d	0.08 d	0.32 e
AMF	1165.7 c	86.5 c	884.8 c	1036.8 c	3.97 c	21.0 c	0.14 c	0.58 c
PG	890.4 d	45.2 d	595.0 d	887.5 d	2.69 d	17.6 c	0.10 d	0.42 d
AMF+PG	1331.8 b	107.8 b	1015.0 b	1388.5 b	5.35 b	27.5 b	0.16 c	0.71 b
PG/compost	1402.7 b	82.3 c	1013.7 b	1262.0 b	4.02 c	26.5 b	0.18 bc	0.57 c
AMF+PG/compost	1627.8 a	131.9 a	1276.5 a	1705.1 a	6.76 a	36.4 a	0.22 a	0.85 a

Mean values within columns followed by different letters are significantly different at P<0.05.

Table 5 - Percentage change in nutrient contents (NC) due to PG amendment and AMF inoculation of cotton plants

Treatment	Nutrient content change (%)							
	N	P	K	Ca	Mn	Fe	Cu	Zn
AMF	65.4 c	148.5 c	91.8 b	65.2 c	128.6 c	73.3 bc	90.6 b	85.0 c
PG	25.9 d	30.0 d	28.2 c	40.6 c	56.2 d	44.4 cd	29.3 c	31.8 d
AMF+PG	89.1 bc	209.3 b	119.1 b	120.9 b	207.3 b	126.3 b	115.6 b	123.9 b
PG/compost	97.7 b	135.6 c	118.5 b	100.0 b	129.2 c	115.3 b	87.4 b	80.4 c
AMF+ PG/compost	130.9 a	278.7 a	177.5 a	171.2 a	287.1 a	201.1 a	192.8 a	171.0 a

Data in the same column followed by the same letter are not significantly different (P<0.05). Nutrient Content (NC) change=(NCAMF - NCnonAMF)x100/NCnonAMF.

on Zn and Mn concentration in nonmycorrhizal plants. Also, PG had no significant effect on Cu concentration in either AMF or non-AMF plants. The highest concentrations of Mn, Fe, Cu, and Zn were observed at AMF plus PG/compost treated plants compared to other treatments.

Plant contents of K, Ca, Mn, Fe, Cu, and Zn were significantly higher for mycorrhizal than for nonmycorrhizal plants (Table 4). The data revealed that maximum plant uptake of K, Ca, Mn, Fe, Cu, and Zn was found in the treatment of indigenous AMF with PG/compost, which was significantly ($P \leq 0.05$) higher by 177.5, 171.2, 287.1, 201.1, 192.8, and 171%, respectively, over control (Table 5).

4. Discussion and Conclusions

The soil P concentration in this study was low (3 mg kg⁻¹), and this nutrient normally has to be added to this soil to provide sufficient P for plant growth. Addition of poorly soluble forms of P, such as phosphogypsum, to soil had no negative effect on AMF root colonization, as was reported by Cui *et al.* (2014). This may be because AMF root colonization often depends on given amounts of soluble P in the soil at the time of root colonization (Stribley *et al.*, 1980). In particular, greater root infection was found with AMF inoculation plus PG/compost treatment and it was possibly due to the improvement of the rooting zone environment which stimulated better root proliferation (Nagahashi *et al.*, 1996; Van der Heijden and Kuyper, 2001).

Soil pH affects the availability of nutrients and how the nutrients react with each other. Application of PG to soil lowered soil pH, a result which is in agreement with literature reports of previous studies (Al-Karaki and Al-Omouh, 2002; Lee *et al.*, 2009). The lower soil pH caused by PG might be attributed to the release of phosphoric acid and sulfuric acid contained in PG.

The increase in cotton plant biomass by PG corroborates reports by Zhang *et al.* (2014) who showed that amendment of PG significantly increased shoot biomass in tobacco, regardless of AMF inoculation. According to Quintero *et al.* (2014), increased dry matter of tomato by PG can be ascribed, at least in part, to an increase in water use efficiency. Greater fresh biomass and plant height of inoculated cotton compared to control was noted in this study, which is in accordance with other earlier studies on cotton

(Afek *et al.*, 1991; DeFeng *et al.*, 1998).

Enhanced cotton growth with the combination of AMF and PG agrees with previous studies conducted with different plant species such as wheat (Al-Karaki and Al-Omouh, 2002), maize (Bai *et al.*, 2011), tomato (Cui *et al.*, 2014), and shallot (Gu *et al.*, 2012). Improved growth of AMF and PG treated plants may have been due to improved soil P availability. The trend noted for N concentration and biomass of AMF plus PG/compost treated plants might be due to the compost releasing its nitrogen gradually to the soil/crop to produce a greater number of leaves.

Strong mycorrhizal effects on cotton were also observed when looking at the nutrients uptake. Higher K and Ca in mycorrhizal than in nonmycorrhizal cotton is supported by Liu *et al.* (2002) who reported that AMF enhanced acquisition of the nutrients that move mainly by mass flow. PG/compost had a positive effect on Ca and K concentration in mycorrhizal cotton which could be due to an improvement in soil organic matter and exchangeable Ca and K by compost (Adeleye *et al.*, 2010). El Mrabet *et al.* (2014) also showed that bio-compost improved soil K-extractable. Increasing P uptake of plants due to AMF inoculation has been widely reported (Deguchi *et al.* 2007; Sharif *et al.*, 2009; Ibrahim, 2010). In our study, increased P concentration and uptake by PG addition to mycorrhizal cotton is supported by Zhang *et al.* (2015) who reported that PG amendment significantly increased the concentration and absorption of P in mycorrhizal and nonmycorrhizal tobacco plants. Also, Gu *et al.* (2012) found that P concentration in shallot was increased by increasing PG, and the combination of PG and AMF colonization can improve P uptake by shallot to different degrees. Our results show that indigenous AMF increased the concentrations of Cu, Zn, Fe, and Mn in cotton; similar results were obtained in cotton inoculated with different species of AMF (Liu *et al.*, 1994; Ibrahim, 2010). Our result regarding enhanced acquisition of P and micronutrients in AMF cotton grown with PG is supported by Al-Karaki and Al-Omouh (2002) in their work on mycorrhizal wheat grown with PG.

The extension of AMF hyphae, beyond the root zone, provides P and other nutrients to plants during growth stages. The ability of the hyphae to extend the root system should be especially beneficial in the case of cotton because its roots have a low density per unit soil volume (McMichael, 1990). In this case,

AMF likely contributed P (and other mineral nutrients) from soil and PG particles with which roots would not make contact. High absorption of Zn, Cu, and Fe may be due to greater P uptake by AMF plants (Clark and Zeto, 1996; Davies *et al.*, 2005). On the other hand, the positive response of cotton to AMF inoculation for nutrient concentration could be due to the effectiveness of the AMF isolate in improving soil properties and nutrient availability.

Previous reports showed that improvement of plant growth and nutrients acquisition by the combination of PG and AMF depends on compatibility between plant species, the rate of PG added, and the AMF isolate. Bai *et al.* (2011) reported that shoot growth of PG treated maize strain (40 g kg⁻¹) was significantly enhanced when inoculated with *Diversispora spurcum*, but was significantly inhibited when inoculated with *Glomus aggregatum*. Gu *et al.* (2012) reported that the treatment of PG40 addition with *Glomus mosseae* inoculation had a significant effect in improving shallot biomass and P, S uptake. According to Zhang *et al.* (2014), the combination of PG40 and *G. aggregatum* inoculation had the most desirable effects on tobacco growth.

Under the conditions in this study, added PG/compost enhanced P and nutrient concentrations of mycorrhizal plants. Previous reports have shown that PG application induced changes in soil chemical properties (decreased soil pH and enhanced E_c, available P, SO₄, exchangeable K, Ca and Mg) (Al-Oudat *et al.*, 1998; Lee *et al.*, 2009). In addition, organic fertilizers and AMF inoculation could improve soil physico-chemical properties (Warnock *et al.*, 2007). AMF colonization enhances soil aggregation by exuding the glycoprotein, glomalin, from extraradical hyphae (Wright and Upadhyaya, 1998). The improved soil structure enhances air and water percolation, improves root system access to soil water and nutrients, and improves crop production (Celik *et al.*, 2004). Therefore, the increase in nutrients noted in AMF and PG/compost treatments could be due to improved soil structure and to increased release of nutrients in the soil, which become more available to the plant (Habashy *et al.*, 2008; Jan *et al.*, 2014).

Enhanced acquisition of nutrients and plant growth by AMF and PG was reflected by increased yield and yield components of cotton. Previous studies showed that PG increased grain yield of barley, wheat, and cotton (Al-Oudat *et al.*, 2011). Cui *et al.* (2014) reported that tomato yield of AMF or AMF plus PG seedlings were significantly higher than those

of the non-mycorrhizal seedlings. Al-Karaki and Al-Omouh (2002) reported that grain yield of wheat was enhanced by PG, and even more so when roots were colonized with AMF.

Cotton inoculation with indigenous arbuscular mycorrhizal fungi (AMF) and soil amendment with phosphogypsum (PG) enhanced nutrients acquisition from soil and improved growth and yield of cotton. However, mycorrhizal plants grown with PG and compost mixture had greater growth and yield than plants grown with PG alone. Therefore, the combination of AMF with PG added in compost can ensure satisfactory benefits for cotton growth and yield in low input, sustainable cropping systems.

Acknowledgements

The author would like to thank the Atomic Energy Commission of Syria for encouragement and technical support.

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