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ARTICLE

Effect of phospho-compost and phosphate laundered sludge combined or not with endomycorrhizal inoculum on the growth and yield of tomato plants under greenhouse conditions

EL Gabardi Soumaya[1], Mouden Najoua[1], Chliyeh Mohamed[1], Selmaoui Karima[1], Ouazzani Touhami Amina[1], Filali-Maltouf Abdelkarim[2], Ibnsouda Koraichi Saad[3], Amir Soumia[4], Benkirane Rachid[1], EL Modafar Cherkaoui[5], Douira Allal[1]*

[1]Laboratoire des Productions Végétales, Animales et Agro-industrie, Equipe de Botanique, Biotechnologie et Protection des Plantes, Faculté des Sciences, Université-Ibn Tofail- Kénitra-Maroc
[2]Laboratoire de Microbiologie et Biologie Moléculaire, Faculté des Sciences, Université Mohammed V, Rabat, Maroc
[3]Laboratoire de Biotechnologie Microbienne, Faculté des Sciences et Techniques, Université Sidi Mohamed Ben Abdellah, Fès, Maroc
[4]Laboratoire Polyvalent en Recherche et Développement, Université Sultan Moulay Slimane, Beni Mellal, Maroc
[5]Laboratoire d'Agrobiotechnologie et Bioingénierie Molécularie, Faculté des Sciences et Techniques, Université Cadi Ayyad, Marrakech, Morocco

ABSTRACT The study aims to evaluate the effect of endomycorrhizal inoculum (arbuscular mycorrhizal fungi), phospho-compost and phosphate sludge in single (M, PC, PS) or dual combinations (PC+M, PS+M, PS+PC) compared to agricultural and Mamora soils (A and S) on the growth, flowering, and yield of tomato plants. Among the studied treatments, the substrates containing 5% of phospho-compost combined with endomycorrhizal inoculum (PC+M) gave the most positive effect followed by phospho-compost (PC) and endomycorrhizal inoculum (M). In response to PC+M substrate, tomato plant height, the number of leaves and flowers attained 90 cm, 30, and 25, respectively. In substrates PC and M, tomato plants showed a height of 85 and 75 cm, leaves number of 30 and 19 leave/plant and number of flowers of 21, and 19 flower/plant. An optimal yield with (12 fruits/plant) was recorded in tomato plants grown on the substrate amended with bio-inoculant (AMF) and phospho-compost at a rate of 5%. In terms of qualitative parameters, the highest fresh and dry weight of aerial plant parts and root system were recorded in tomato plants grown in culture substrate incorporating 10 g of endomycorrhizal inoculum and 5% of phospho-compost reaching respectively103.4 g, 34 g 90.1 g, 28.9 g as compared to 87, 51, 23 and 24.1 g noted by tomato plants on the substrate with phospho-compost (5%) (PC). The highest mycorrhization parameters (frequency (F), intensity of mycorrhization (M), average arbuscular content (A), average vesicular content (V), average intraradicular spore content (S)) were found in the roots of tomato plants growing on substrates amended with 5% phospho-compost plus 10 g of endomycorrhizal inoculum, with percentages of 100% F, 61% M, 40.67% A, 18.36% V, and 56.9% S.

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***Corresponding author**
E-mail: douiraallal@hotmail.com

Introduction

Composting has always been one of the most effective strategies for organic waste recycling (Santos et al. 2011) and is also a beneficial practice to improve soil structure (Celik et al. 2004) and its restoration (Scotti et al. 2016). Generally, soil with compost amendments had a positive effect on crop lands fertility and productivity (Celik et al. 2004; Pérez-Piqueres et al. 2006). Indeed, compost, a product of the composting process, is considered an important source of nutrients for cultivated plants (Duong et al. 2012) by enhancing plant yields and protecting them from numerous soil-borne pathogens (Pane et al. 2013).

Composting phosphate laundered sludge to produce phospho-compost biofertilizer is an important way to be explored to valorize these sludges, which still contains a significant amount of phosphorus, stored at phosphate mine sites (Hakkou et al. 2009; Ouakibi et al. 2013). In this sense, very satisfactory results were noted in bean plants: culture substrates containing 2.5 and 7.5% of phospho-compost showed a significant impact on growth and yield parameters (El Gabardi et al. 2019b).

The efficiency of phosphate sludges, as an essential

component of phospho-compost, is due to its richness of infectious propagules of arbuscular mycorrhizal fungi (El Gabardi et al. 2019c, d). These microorganisms have the potential to solubilize the phosphate and can convert insoluble phosphate into soluble forms available to plants (Pradhan and Sukla 2006; Sharma et al. 2007), thus increasing the tolerance of plants to soil pathogens and abiotic stresses (Smith and Read 2008). They are also like better microorganisms capable of improving the yield and quality of crops (Hijri 2016).

Other studies have shown that incorporation of compost into the soil represents a reservoir of nutrients to plants (Scotti et al. 2016; Yang et al. 2017) which may be useful and beneficial for the formation and functioning of arbuscular mycorrhizal fungi. Several studies have reported that compost addition improved AM root colonization, spore production, and development of AM extraradical hyphae (Labidi et al. 2007; Valarini et al. 2009; Tanwar et al. 2013; Cavagnaro 2015). Also, some research has reported that AM fungi can directly take advantage of organic matter (Hodge et al. 2001; Govindarajulu et al. 2005; Jin et al. 2005) which may promote plant growth and enhance carbon allocation to soil fungi (Lee et al. 2004; Donn et al. 2014).

The aim of this work was to investigate *in vivo* the effect of phospho-compost, phosphate laundered sludge alone, and in combination with endomycorrhizal inocula on the growth and yield of tomato plants.

Materials and Methods

Plant material

Tomato seeds belonging to the Campbell 33 variety were superficially disinfected with sodium hypochlorite diluted to 1% for 10 min, rinsed thoroughly with sterile distilled water, dried and set to pre-germinate in plastic seedling trays, filled with wet peat, and covered with a 20-micron thick plastic film for two days. After the various treatments, the planting was carried out when the seedlings reached 2 true well-spreading leaves (Woo et al. 1996) in 17 cm × 14 cm plastic pots and perforated at the base.

Mycorrhizal inoculum

A composite endomycorrhizal inoculum was prepared from phosphate sludge and rhizospheric soils of plant species grown there and it was used to inoculate mycotrophic species (e.g., maize, leek, barley, sorghum). The inoculum contained multiple endomycorrhizal species, 31 of which were identified morphologically (El Gabardi et al. 2019b): *Acaulospora scrobiculata, Acaulospora laevis, Acaulospora colossica, Acaulospora gedanensis, Acaulospora morrowiae, Acaulospora excavate, Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Entrophospora schenckii, Entrophospora kentinensis, Entrophospora infrequens, Glomus intraradices, Glomus deserticola, Glomus etunicatum, Glomus caesaris, Glomus aggregatum, Glomus aureum, Glomus macrocarpum, Glomus mossae, Glomus claroideum, Glomus globiferum, Glomus fasciculatum, Glomus versiforme, Glomus pansihalos, Glomus manihoti, Glomus verruculosum, Glomus* sp., *Scutellospora nigra, Scutellospora biornata*, and *Scutellespora castenea*. Barley (*Hordeum vulgare* L.) was used as a host plant to multiply the composite mycorrhizal inoculum. Barley seeds were disinfected with 5% sodium hypochlorite for 2 min, then germinated in plastic pots filled with a mixture of sterile sand and endomycorrhizal inoculum. After 4 weeks of culture, the barley roots were excised, rinsed 3 times with distilled water, and cut into 1 - 2-mm long fragments. These root fragments were used as the endomycorrhizal inoculum.

Culture substrate

Two culture substrates were used: Mamora soil (S) and agricultural soil (A). Mamora soil is a loose structured very sandy soil (91.1% sand) with a slightly basic pH of 7.5. It is characterized by a low cation exchange capacity (7 meq/100 g), a very low salinity, and an organic matter content of not more than 0.7%. This soil was deficient in total phosphorus (0.239%), total potassium 0.15 (meq/100 g) and total nitrogen (0.05%). The soil was autoclaved three times for one hour at 121 °C.

The phospho-compost incorporated into the substrate 'soil of Mamora' was obtained after composting mining waste from phosphate laundered sludge and household waste (30% of phosphate sludge and 70% of household waste) by the Polyvalent Laboratory in Research and Development, Sultan Moulay Slimane University (Beni-Mellal). The dose of phospho-compost used in this study was 5%.

Experiments in tomato was conducted in randomized block design with 8 treatment combinations (with ten repetitions per treatment and each pot containing only one plant), viz,

S: 100% low phosphorus soil (Mamora soil)
A: 100% agricultural soil
NPK: 100% S amended after 20 days, following transplantation, by NPK (14/28/14) at a rate of 50 mg/kg of soil.
PC+S: phospho-compost (5%) + S (95%)
S+PS: phosphate sludge (5%) + S (95%)
S+M: S (100%), inoculated with 10 g of AMF (M).
PC+S+M: phospho-compost (5%) + S (95%), inoculated with 10 g of AMF (M).
S+S+PS: S (95%) + phosphate sludge (5%), inoculated with 10 g of AMF (M).

Tomato seedlings inoculation technique

Tomato plants were transferred separately to plastic pots filled with sterile Mamora sand. The inoculation of the plants consisted of placing near each root system approximately 10 g of inoculum used in the form of the infected barley roots, containing propagules of endomycorrhizal fungi. Plastic pots were filled with sterile substrate and they were placed in a plastic greenhouse. Watering was done with tap water every two days.

Experimental design

The experimental protocol was designed in random blocks with ten repetitions per treatment. Each pot contained only one plant.

Extraction and identification of spores from growing media in pots

Extraction of endomycorrhizal fungi spores from the substrate was performed according to the method of Gerdemann and Nicolson (1963). The number of spores in 100 g of soil was estimated by binocular direct counting (magnification × 10).They were then mounted on glass microscope slideswith polyvinyl alcohol lacto-glycerol (PVLG), observed under a microscope (× 40) and classified according to their color, size, hypha of attachment and their consistency in order to identify their genus (Ferrer and Herrera 1981; Schenck and Smith 1981; Walker and Mize 1982; Hall 1984; Schenck and Perez 1987; Morton and Benny 1990; Dalpé et al. 1992).

Root staining for the evaluation of AMF root colonization

The roots of the tomato plants which develop on the different substrates were colored, according to the technique of brightening and coloring of the roots of Phillips and Haymann (1970), to observe the different structures of endomycorrhizal fungi. The roots were washed with tap water and the finer roots were cut into fragments approximately 1 cm long. These fragments were bleached with a solution of potassium hydroxide: KOH (10%) for 45 min at 90 °C in the water bath. And then serially whitened them for 5 min by adding four drops of 33% H_2O_2. Then fragments were rinsed with distilled water and stained with a solution of cresyl blue for 15 min at 90 °C in water bath. They were finally rinsed with distilled water and observed using a microscope. The mycorrhizal roots proportion was identified for each sample.

Evaluation of the mycorrhization rate

The mycorrhization parameters were evaluated by the overall assessment of 30 fragments and described as Trouvelot et al. (1986), and Amir and Renard (2003). Root fragments were observed at the magnifications of 100 and 400. The arbuscules and vesicles of AMF frequency and levels were measured by assigning an index of mycorrhization of 0-5 (0: None; 1: trace; 2: less than 10%; 3: 11 to 50%; 4: 51 to 90%; 5: more than 91%).

Frequency of mycorrhization (F%)

The frequency of mycorrhization (F%) was calculated by the percentage of colonization of the host plant roots in arbuscular fungi.

$$F\% = 100 \times (N - N_0)/ N$$

N: the number of observed root fragments; N_0: the number of non-mycorrhizal fragments

Intensity of mycorrhization (M%)

Intensity of AM infection in the root cortex was determined by the five-class classification system following the method described by Trouvelot et al. (1986). This measurement is based on the infection (M%) in each root segment using values from 0 to 5. Numbers indicate the proportion of root cortex colonized by the fungus (0 = without colonization; 1 = colonization trace; 2 = less than 10%; 3 = from 11 to 50%; 4 = from 51 to 90%; and 5 = more than 90% of the volume of root segment occupied by the fungus). M% was estimated by the following equation:

$$M\% = (95n5 + 70n4 + 30n3 + 5n2 + n1) / N$$

where n5, n4, n3, n2, and n1 are the number of fragments in the respective categories 5, 4, 3, 2, and 1 (Alarcón and Cuenca 2005).

Arbuscular content (A%)

$$A\% = (100\ mA3 + 50\ mA2 + 10\ mA1)/100$$

where, mA3, mA2, mA1 are the percentages (%), respectively, assigned to the notes A3, A2, A1, with, mA3 = (95 n5A3 + 70 n4A3 + 30 n3A3 + 5 n2A3 + n1A3)/N. The same for A1 and A2.

In this formula, n5A3 represents the number of fragments noted 5 with A3; n4A3 is the number of fragments noted 4 with A3; A0: no arbuscules; A1: some (10%) arbuscules; A2: moderately abundant (50%) arbuscules; A3: very abundant (100%) arbuscules.

Vesicle content (V%)

$$V\% = (100\ mV3 + 50\ mV2 + 10\ mV1)/100;$$

Where, mV3, mV2, mV1 are the percentages (%), respectively, assigned to the notes V3, V2, V1, with, mV3 = (95 n5V3 + 70 n4A3 + 30 n3A3 + 5 n2A3 + n1A3) /N, The

Table 1. Averaged quantitative parameters (plant height, number of leaves, flowers, and fruits) of tomato plants as a function of different soil substrates.

Treatment	Plant height (cm)	Number of leaves	Number of flowers	Number of fruits
A	50^{e}	10^{d}	13^{c}	4^{d}
S	55^{e}	11^{d}	11^{d}	4^{d}
S+NPK	65^{d}	15^{c}	10^{d}	5^{d}
S+PS	57^{e}	14^{c}	12^{cd}	4^{d}
S+PS+M	60^{d}	12^{d}	13^{c}	6^{cd}
S+M	75^{c}	18^{b}	19^{b}	7^{c}
S+PC	85^{b}	19^{b}	21^{b}	9^{b}
S+PC+M	90^{a}	30^{a}	25^{a}	12^{a}

Two values in the same column followed by the same letter do not differ significantly at the level 5%.
A: 100% agricultural soil; S: 100 % soil; S+NPK: soil+NPK; S+PS: soil + 5% phosphate sludges; S+M: soil + 10 g of endomyrrhizal inoculum; S+PS+M: soil + 5% sludges + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum.

same for V1 and V2;
In this formula, n5V3 represents the number of fragments noted 5 with V3; n4V3 is the number of fragments noted 4 with V3;
V0: no vesicles; V1: some (10%) vesicles; V2: moderately abundant (50%) vesicles; V3: very abundant (100%) vesicles.

Quantitative parameters

The growth parameters, the length of the aerial part, the number of leaves, the weights of the aerial and root parts of the plants which develop on the different used substrates, were noted at the end of the experiment.

Qualitative parameters

The biomass of the aerial part and the fruits fresh weight were measured using a precision balance on the same day while the root biomass was measured after one night so that the rinsing water does not distort the results. The dry weights of the aerial and root parts were determined after drying at 80 °C for 72 h.

Statistical analysis

The data processing focused on the analysis of variance and the PPDS test at the 5% threshold.

Results

The obtained results showed a significant effect of compost amendment when combined with endomycorrhizal inoculums on the growth of tomato plants which exhibited a higher value of different parameters. In the culture substrate containing compost amendement at a rate of 5% and 10 g of the endomycorrhizal inoculums, the average plant height reached 90 cm, with 30 leaves, 25 flowers, and 12 fruits per plant. These outcomes differed significantly from those recorded in tomato plant grown in substrate amended with phospho-compost at a rate of 5% alone showing an average height of 85 cm, with 19 leaves, 21 flowers, and only 9 fruits per plant. While in the presence of the third treatment composed by endomycorrhizal inoculums, the average height of plants was 75 cm with a mean number of 18 leaves, 19 flowers, and 7 fruits per plant.

At the opposite, lowest values were observed in plants growing on agricultural soil and Mamora soil where the average plant height was 55 cm and 50 cm, with 11 and 10 leaves, 13 and 11 flowers, and 4 fruits per plant, respectively (Table 1; Fig. 1 and 2). The data regarding plant growth characteristics including fresh weight, dry weight of both aerial and root parts represented in Table 2, showed significant differences among the tested treatments.

The better result was observed in tomato plants grown

Figure 1. Tomato plant development on the different culture substrates tested. S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+M: 10 g of endomycorrhizal inoculum; S+PS: soil + 5% phosphate sludges; S: 100 % soil.

Table 2. Effect of different culture substrates on the averaged growth and yield parameters of tomato plants.

Culture substrates	FWA (g)	DWA (g)	FWR (g)	DWR (g)	WF (g)
A	20.9^{e}	10.2^{f}	12^{f}	4.7^{e}	32.5^{e}
S	14.4^{f}	4.5^{d}	10.2^{f}	2.1^{f}	27.3^{f}
S+NPK	43.6^{c}	16.7^{c}	24^{e}	7.4^{d}	36.4^{e}
S+PS	34.5^{d}	14.9^{d}	14^{f}	5.1^{e}	29.6^{f}
S+M	45^{c}	12^{e}	42^{c}	13.8^{c}	50.7^{c}
S+PS+M	37.7^{d}	9,9^{f}	32^{d}	11.1^{c}	43.6^{d}
S+PC	87^{b}	23^{b}	51^{b}	24.1^{b}	63.6^{b}
S+PC+M	103.4^{a}	34^{a}	90.1^{a}	28.9^{a}	70.7^{a}

Two values in the same column followed by the same letter do not differ significantly at the level 5%.
FWA: fresh weight of aerial part; DWA: dry weight of aerial part; FWR: fresh weight of root part; DWR: dry weight of root; WF: weight of fruits.
A: 100% agricultural soil; S: 100 % soil; S+NPK: soil+NPK; S+PS: soil + 5% phosphate sludges; S+M: soil + 10 g of endomyrrhizal inoculum; S+PS+M: soil + 5% phosphate sludges + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum.

in substrate supplemented with compost amendment and 10 g of endomycorrhizal inoculum with 104 g and 34 g for fresh weight and dry weight of aerial parts respectively exceeding those noted for the fresh weight (90.1 g) and dry weight (28.9 g) of root parts. Whilst, in the Mamora soil and agricultural soil, the fresh/dry weight of the aerial part was the lowest in the order of 14.4 g / 4.5 g, 20.9 g / 10.2 g, respectively, compared with the respective values 10.2 g / 2.1 g and 12 g / 4.7 g for the root parts.

The same trends were observed in the weight of tomatoes which was higher in plant developed on substrate amended with phospho-compost at rate of 5% and 10 g of endomycorrhizal inoculum (70.7 g) followed by those noted in substrate supplemented with a rate 5% of phospho-compost (63.6 g) and the substrate that only contains endomycorrhizal inoculum giving a weight of 50.7 g. Whereas, the lowest fruit weight was noted in tomato plants from agricultural soil and Mamora soil with 32.5 g and 27.3 g, respectively (Table 2, Fig. 2 and 3).

Microscopic observations of tomato roots stained with trypan blue after having been taken off from the studied culture substrates revealed that all of them were mycorrhized (Table 3). They had all structures of AMF (arbuscules, vesicles, spores, intra and extra-radicular hyphae (Fig. 4).

The maximal mycorrhizal frequency (100%) was noted in roots of tomato plants grown in three substrates. The frequency of mycorrhization is maximum (100%) in the roots of tomato plants growing on the substrate amended with phospho-compost (5%) + 10 g of endomycorrhizal inoculum, as well as in those of plants in the substrates amended with 10 g of endomycorrhizal inoculum alone and with 5% of mud plus 10 g of endomycorrhizal inoculum. In addition, the substrates amended with 5% phospho-compost alone, 5% mud alone and agricultural soil, presented mycorrhization frequencies of 96%, 89% and 73%, respectively. However, no significant difference was observed between the different treatments at the 5% threshold (Table 3).

The intensity of mycorrhization of the roots of tomato plants is medium (61%) at the level of the substrate amended with 5% of phospho-compost + 10 g of endomycorrhizal inoculum and low at the level of the substrates amended with 10 g of endomycorrhizal inoculum alone, phospho-compost alone, 5% mud plus 10 g of endomycorrhizal inoculum, 5% mud alone and agricultural soil, the

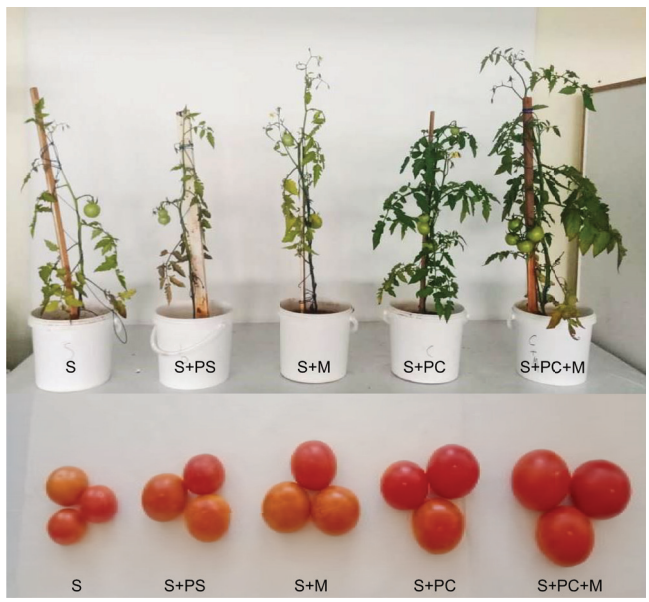

Figure 2. Tomato plant development as regarding plant height (A) and fruit caliber produced by tomato plants (B) grown on different culture substrates. S: 100 % soil; S+PS: soil + 5% phosphate sludges; S+M: soil + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum.

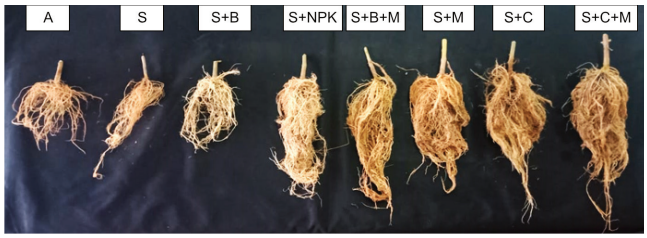

Figure 3. Root system growth of tomato plants in the response of different soil substrates. A: 100% agricultural soil; S: 100 % soil; S+PS: soil + 5% phosphate sludges; S+NPK: soil+NPK; S+PS+M: soil + 5% phosphate sludges + 10 g of endomycorrhizal inoculum; S+M: soil + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum.

percentages of which are 38%, 35%, 27%, 19% and 12%, respectively.

The average arbuscular content was higher in tomato plants grown on the soil which was amended with endomycorrhizal fungi (10 g) and phospho-compost (5%) or only with the endomycorrhizal fungi alone registering respectively percentages of 40.67% and 39.23%. The lowest rates of colonization were found on those grown on soil adding with sludge (5%) and 10 g of endomycorrhizal inoculum (29.56%), 5 g of sludge (16%), phospho-compost-amended soil (15.81%) as compared to the agricultural soil (10%).

As stated above, combining the endomycorrhizal inoculum (10 g) and phospho-compost (5%) in culture substrate of tomato plants was more favorable showing an average content in vesicles of 18.36%, followed by those recorded in substrates containing 10 g of endomycorrhizal inoculum alone (11.23%), 5% of phospho-compost (10.14%), and 5% of mud added with endomycorrhizal inoculum (8.45%).

The highest intra-radicular spore content of tomato plants (56.9%) was noted in the roots of plants grown on the amended substrate with 5% phospho-compost + 10 g endomycorrhizal inoculum reaching (56.9%) while in the substrate supplemented with 10 g endomycorrhizal inoculum alone, the content was 33.21% (Table 3). By the contrary, the lowest content (13.2%) was recorded in the root cortex of tomato plants grown on the substrate containing 5% of the phosphate laundered sludge.

The extracted spores had a lower density in all tested substrates; 14 and 10 spores / 100 g soil were found in the rhizosphere of plants grown in substrate incorporating 5% of phospho-compost with 10 g of endomycorrhizal fungi and that containing endomycorrhizal inoculum alone, respectively. The lowest spore numbers, 3 and of 2 spores / 100 g soil, were detected in the rhizosphere of plants developed on substrate enriched with 5% phosphate laundered sludge alone and on agricultural soil, respectively.

The morphological study of spores revealed the presence of 12 different morphotypes represented by the following species *Acaulospora sporocarpia, Acaulospora delicata, Rhizophagus clarus, Acaulospora* sp., *Rhizophagus aggregatus, Funneliformis smoseae, Rhizophagus intraradices, Glomus* sp., *Funneliformis constrictum, Gigaspora gigantia, Diversispora trimurales, Acaulospora foveate* (Fig. 5). These species belonged to the three families (*Glomaceae, Acaulosporaceae* and *Gigasporaceae*), 2 orders (Glomerales and Diversisporales).

Discussion

The supply of 5% phospho-compost to the cultivation substrate combined with an endomycorrhizal inoculum had positively affected all growth and yield parameters of tomato plants. Indeed, plant height, their leaves, flowers, and fruits number were far greater than those noted in plants grown in substrates without supplements. In this context, recent study results had revealed that phospho-compost at a rate 2.5% and 5% increased both growth

Table 3. Parameters of root mycorrhization of tomato plants grown on different soil substrates.

Culture substrates	F%	M%	A%	V%	S%
A	73^{b}	12^{f}	10^{e}	-	-
S	-	-	-	-	-
S+PS	89^{ab}	19^{e}	16^{d}	-	13,2^{d}
S+M	100^{a}	38^{b}	39.23 ^{b}	11.23^{b}	33,21^{b}
S+PS+M	100^{a}	27 ^{d}	29,56^{c}	8.45^{c}	29,91^{c}
S+PC	96^{a}	35^{c}	15.81^{d}	10.14^{b}	31,45^{bc}
S+PC+M	100^{a}	61^{a}	44.67^{a}	18.36^{a}	56,9^{a}

Two values in the same column followed by the same letter do not differ significantly at the level 5%.
A: 100% agricultural soil; S: 100 % soil; S+PS: soil + 5% phosphate sludges; S+M: soil + 10 g of endomyrrhizal inoculum; S+PS+M: soil + 5% phosphate sludges + 10 g of endomycorrhizal inoculum; S+PC: soil + 5% phospho-compost; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum.

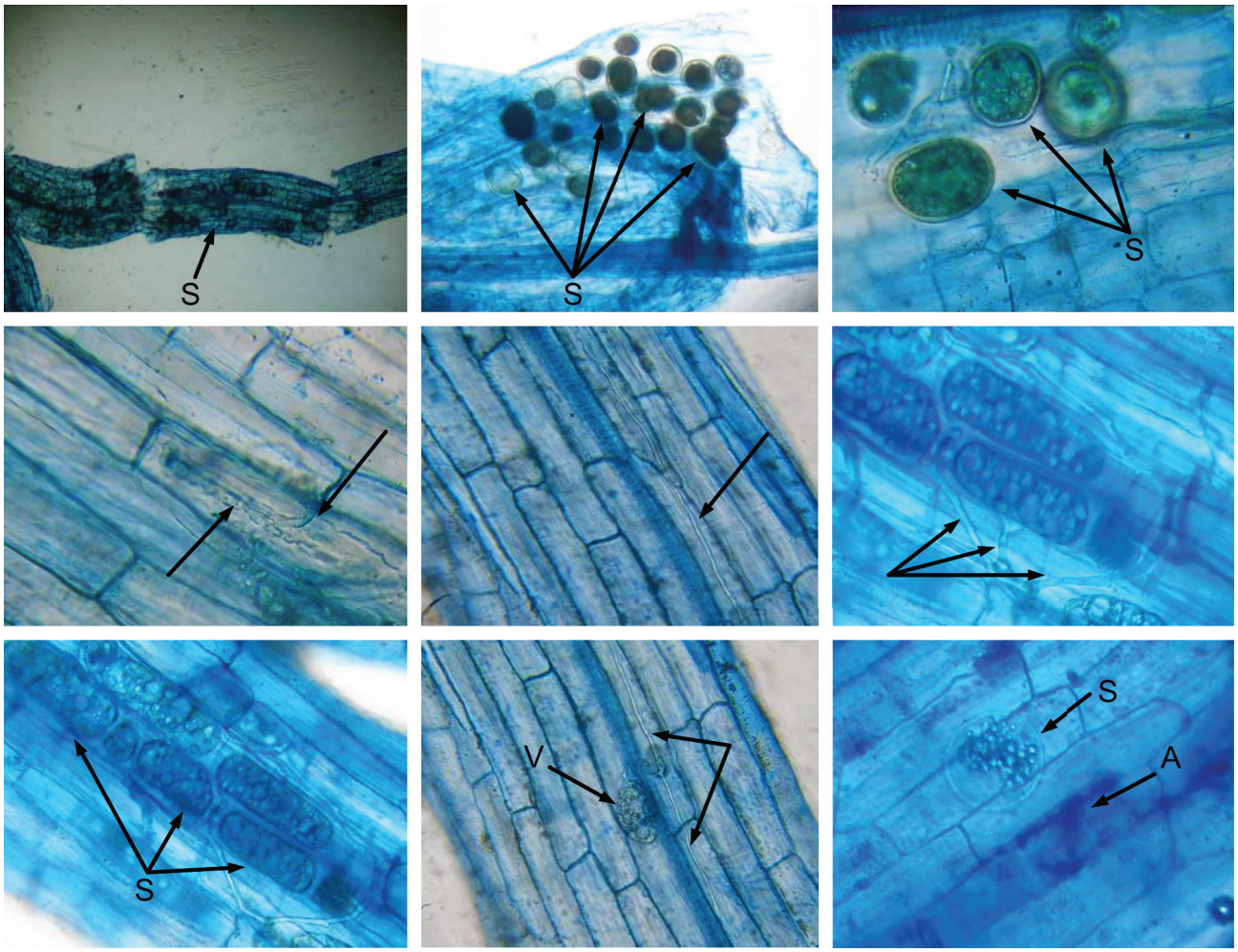

Figure 4. Endomycorrhizal fungi structures observed in root samples of tomato plants grown in the tested culture substrates: arbuscules (a), vesicles (v), spores (s), and intracellular hyphae (hi) (G×400).

parameters and yield of bean plants (El Gabardi et al. 2019e).

In accordance with our findings, many other studies have shown that the application of organic manure in combination with arbuscular mycorrhizal fungi (AMF) enhanced crop yield under greenhouse conditions (Gapasin and Ronayre 2003; Diongzon and Gapasin 2000; Serfoji et al. 2010) and in the field (Germani and Plenchette 2005). Akhter et al. (2015) reported a significant effect of compost and AMF complex on the growth of tomato (*Solanum lycopersicum* L.) plants reflected by an improvement of root and shoot dry weight of tomato plants as well as AMF colonization of the roots. Similarly, El Kinany et al. (2019), have demonstrated the beneficial effect of compost application and inoculation with a commercial strain of the arbuscular mycorrhizal fungi (AMF), *Glomus iranicum*, on the growth of micropropagated date palm

Table 4. Spores number counted in the rhizosphere of tomato plants growing on different soil substrates.

Culture substrates	Number of spores / 100 g of soil
S	-
A	2^{d}
S+NPK	-
S+PS	3^{d}
S+PS+M	5^{c}
S+PC	6^{c}
S+M	10^{b}
S+PC+M	14^{a}

Two values in the same column followed by the same letter do not differ significantly at the 5% threshold.
S: 100 % soil; A: 100% agricultural soil; S+NPK: soil+NPK; S+PC: soil + 5% phospho-compost; S+PS: soil + 5% phosphate sludges; Soil+M: 10 g of endomycorrhizal inoculum; S+PC+M: soil + 5% phospho-compost + 10 g of endomycorrhizal inoculum; S+PS+M: soil + 5% phosphate sludges + 10 g of endomycorrhizal inoculum.

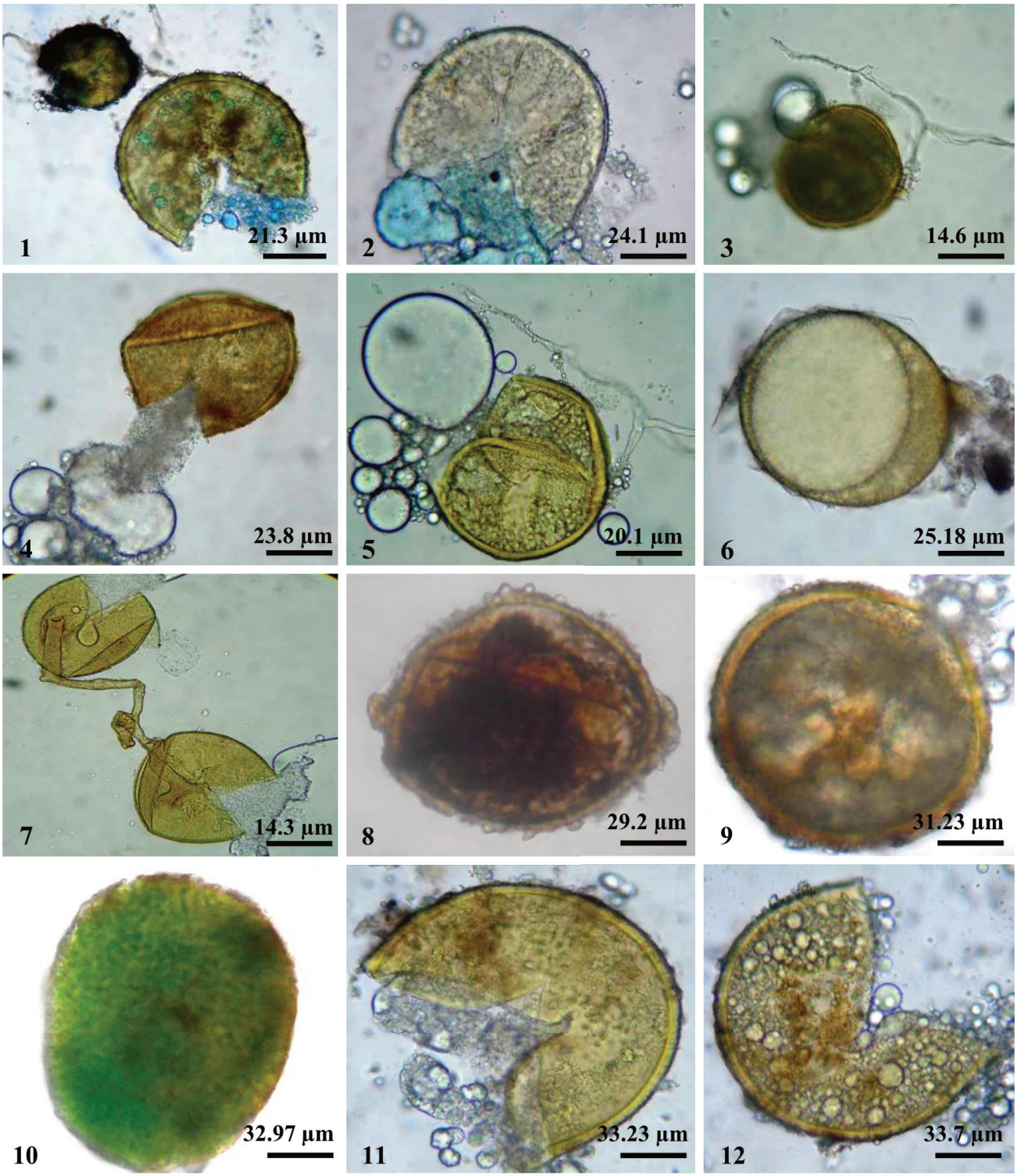

Figure 5. Spores of endomycorrhizal fungal species isolated from the rhizosphere of tomato plants grown on different culture substrates. 1: *Acaulospora sporocarpia*; 2: *Acaulospora delicata*; 3: *Rhizophagus clarum*; 4: *Acaulospora* sp.; 5: *Rhizophagus aggregatus*; 6: *Funneliformis moseae*; 7: *Rhizophagus intraradices*; 8: *Glomus* sp; 9: *Funneliformis constrictum*; 10: *Gigaspora gigantea*; 11: *Diversispora trimurales*; 12: *Acaulospora foveata*.

plantlets. These authors have signed the important contribution of AMF inoculation to micronutrient uptakes like iron and zinc. Also, the mixture of compost and AMF had contributed to the uptake of another micro-element such as Bor (El Kinany et al. 2019).

The incorporation of only 5% of the phospho-compost or 10 g mycorrhizal roots by mycorrhizal fungi to the substrate also has a positive effect on growth and yield parameters (90 cm, 12 fruits/plant). De Bertoldi et al. (1983) and Pommel and Lasserre (1982) noted that soil amendment with composts is an agricultural practice that can correct plant nutrient deficiencies, ensure adequate nutrition, help plants to tolerate stressful situations, maintain optimal soil fertility and improve crop quality. Vanai (1995) reported that the growth of maize (*Zea mays* L.) was enhanced after the incorporation of urban compost into crop plots. The study conducted on tomato cultivation has revealed that the production and profitability were optimal with the addition of compost rate of 30 t/ha (Kitabala et al. 2016). Mouria et al. (2010) found that solid waste compost and its extracts increased plant growth parameters and yields of tomato crops. Charland et al. (2001) indicated that a good compost, even when applied at a low rate, ensured yield improvement of cultivated plants with respect to those planted in unfertilized soils. Lee et al. (2004) have demonstrated that different concentrations of commercial compost promote lettuce (*Lactuca sativa*) growth. In addition, under soil stress conditions, it has been found that two commercial composts incorporated into the soil at a 4% rate of application tended to the growth, nodulation and nitrogen fixationof soybean plants (*Glycine max* L.) (Lawson et al. 1995). El Kadiri Boutchich (2016) claimed the efficiency of composts based on sewage sludge, and wastewater alone or mixed with organic substrates on the growth of wheat shoot and roots.

In agreement with our results, Salvioli et al. (2008) suggested that AMF application provides numerous benefits on both the quantity and quality of tomato productivity. Those of El Amerany et al. (2019) reported that tomato growth parameters significantly improved in mycorrhized plants compared to non-mycorrhized plants. Jochems-Tanguay (2014) reported that inoculation with endomycorrhizal fungi increased average crop yields by 0.079 t/ha (2.4%) for soybeans and 3.3 t/ha (8.7%) for potatoes. According to (Koomen et al. 1987), the use of mixed (multiple) inoculums was equally or more effective than inoculum with only a single species in promoting plant growth in a low-phosphate soil at pH 4.8 and pH 6.8.

Cosme et al. (2018) showed that the roots of more than 71% of vascular plant species, such as tomato, can get in symbiosis with AMF. Nevertheless, its ultimate effect on plant growth may vary upon fungal species involved in the association (Koomen et al. 1987; Duponnois et al. 2013; Chen et al. 2017).

Regarding the impact of different tested substrates on the mycorrhizal statuts of tomato plants, it revealed that combination of 5% of phospho-compost and 10 g of endomycorrhizal inoculum in substrate had the greatest benefits as evidenced by increased rates of root colonization and mycorrhizal intensity, arbuscular and vesicular contents and densities of spores encountered in rhizospheric soil of tomato plants.

Thereby, our findings indicated that AM fungal spore density, AM root colonization and extraradicular hyphae density were improved by this substrate. These results were consistent with previous studies which suggest that compost addition most often had a positive effect on AM growth, and sporulation (Labidi et al. 2007; Tanwar et al. 2013; Cavagnaro 2015). According to Gryndler et al. (2009), this beneficial effect can be attributed to the richness of compost with humic acid capable to stimulate arbuscular mycorrhizal hyphal growth and sporulation. For Yang et al. (2017), N, P-rich compost can also stimulate them. Indeed, AM fungi will grow more when the soil available phosphorus concentration is sufficient for AM growth (Treseder and Allen 2002). According to Yang et al. (2018), compost provides a sustained release of P and thus maintained a moderate level of soil available phosphorus. Valarini et al. (2009) have reported significant increases in root colonization of wheat and bean as the dose of compost added to culture substrate increases. However, Copetta et al. (2011) observed that AM root colonization in *S. lycopersicum* decreased along with increasing compost addition gradient. In view of these inconsistent results, AM fungi responses to compost may be influenced by compost type (Copetta et al. 2011; Cavagnaro, 2014), plant species (Copetta et al. 2011; Cavagnaro 2014), and dosage of compost (Copetta et al. 2011).

In the same manner, the spore density of AMF was differently affected by studied substrates where the most efficient containing 5% of phospho-compost amendment and 10g of endomycorrhizal inoculum which hosted 14 spores/100 g of soil followed by that incorporating 10 g of endomycorrhizal inoculum singly hosting 10 spores/100 g of soil. Variables densities were reported in sludge soil of different sites (El Gabardi et al. 2019d), and in rhizospheric soil of bean as a function of different compost doses addition (El Gabardi et al. 2019) or of three crops bean, wheat, and grass land receiving compost amendment (Valerini et al. 2009).

Morphological identification of AMF spores collected from different growing substrates showed the presence of 12 species, and the predominance of the genus *Glomus* which was also encountered in the rhizospheric zone of Citrus (Artib et al. 2016), Carob tree (Talbi et al. 2015), oleaster (Sghir et al. 2013), date palm tree (Sghir et al.

2014), beans (El Gabardi et al. 2019e), phosphate laundered sludge (El Gabardi et al. 2019b), and from soil sites adjacent to Khouribga phosphate mine (El Gabardi et al. 2019a).

Conclusion

The combination of phospho-compost produced from phosphate-laundered sludge and endomycorrhizal inoculum in culture substrate has significantly contributed to plant growth improvement. This association seems to confer a synergetic effect increasing both plant growth and root colonization compared to the single application of each amendment. The concomitant presence of these biofertilizers has probably facilitated more exchanges between the plants and the growing area and will have to offer potential protection of the roots against soil borne pathogens.

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