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Comparative impact of Arbuscular Mycorrhizal fungi from Moroccan soils and commercial inoculum on potato yield and nutrient composition

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Abstract

Potato (*Solanum tuberosum* L.) is a widely grown crop in Morocco. Mycorrhiza fertilization of potatoes needs to be monitored in order to observe their effects on the growth of potatoes. The aim of this study was therefore to test the effect of fungal strains on potato yield and tuber quality. The inoculation treatments included a commercial inoculum (T2), a natural inoculum (T3), represented by leek roots fragments, obtained after trapping by this host plant of AMF spores naturally present in the soil of the region of Guercif-Morocco, and a control treatment consisting of a sterile substrate. Our results showed significant differences in the frequency and intensity of mycorrhization between the T3 inoculum and the T2 inoculum, while control plants showed no AMF infection. Compared to the T1 control (non-inoculated plants), the gains in number of tubers/plant was 24% and 39% higher, whereas gains in yield was 34% and 48% higher for potato plants inoculated with T2 and T3 inocula, respectively. Furthermore, chemical analyses showed the important role of controlled mycorrhization in the accumulation of mineral elements; N, P, K, Zn, Cu and Fe in potato tubers. The T3 inoculum obtained from the soil of Guercif-Morocco allowed to obtain the best value of dry matter (16.82 g/100 g fM) and the best contents in Zn; K and P and the tubers.

Keywords: Potato; endomycorrhiza; mineral composition of tubers.
Abbreviations: AMF_Arbuscular mycorrhizal fungi; fM_Fresh matter; N_Nitrogen; K_Potassium; P_Phosphorus; Zn_Zinc; Cu_ Copper; Fe_Iron; Ca_Calcium.

Introduction

Potato (Solanum tuberosum L., Solanales Solanaceae) is the most important tuber in the world. The potato crop is largely grown due to its valuable marketing (AL-Shmary and AL-Taey, 2020). It is widely grown in more than 125 countries and consumed daily by more than one billion people (FAO, 2015).
Potato (Solanum tuberosum) occupies an important place in the staple diet of people in developing countries. Since its introduction to Morocco in the 19th century, the potato has become an increasingly important part of the Moroccan diet. Its cultivation occupies an area of up to 60,000 hectare (ha) per year, equivalent to 23% of the total market garden area resulting in a production of 1.5 million tons annually. From a nutritional point of view, it is one of the most nutritious tuber plants with a high energy content (Hassan, 2003). However, this crop does not always yield as expected since it is one of the most demanding crops in terms of chemical fertilizer inputs compared to other vegetable crops. Its nitrogen (N), phosphate (P) and potassium (K) requirements are 100%, 100% and 33% higher than other vegetable crops, respectively (Khoshnevisan et al., 2013). However, these chemical fertilizers have some adverse effects on the environment and human health (Kumar and Keshar, 2017). For this reason, it is necessary to find safer alternatives to these chemical fertilizers to improve soil fertility and plant growth. According to many researchers, a shift towards a more productive agriculture that is less dependent on chemical phosphate inputs cannot be achieved without better management of biological interactions in agrosystems (Plenchette et al., 2005). Mycorrhizae, symbiotic associations between soil fungi and plant roots, are an example of such agrosystems. These fungi form symbiotic associations with almost 80% of plant species, including the majority of staple crops (Cobb et al. 2021; Thirkell et al. 2022).
The beneficial effects of arbuscular mycorrhizal symbiosis on plant growth and health are well documented (Begum et al., 2019). It increases their resistance and/or tolerance to numerous abiotic and biotic stresses (Sánchez-Romera et al., 2016; Ravnskov et al., 2020). In fact, arbuscular mycorrhizal fungi (AMF) have been shown to improve soil fertility, promote increased phosphorus (P) uptake by plants (Püschel et al., 2021), as well as the uptake of other essential nutrients such as N, S, Cu and Zn) (Bhandari and Garg 2017; Ruytinx et al. 2020).
The use of AMF has been the subject of previous studies (Wu et al., 2013; Ettlili et al., 2022; Boussageon et al., 2023) that showed the effectiveness of these microorganisms on improvement of potato growth.
Although mycorrhizal fungi are ubiquitous in natural soils, cultural practices most often disrupt their populations and diversity (Jansa et al., 2003). Under these conditions, numerous experiments conducted for decades have shown that the addition of spores or propagules of these fungi to

the soil results in faster plant development and increased yields.
It is in this context that the present study was initiated and its objective is to evaluate the effectiveness, in the short term, of a commercial product/inoculum that is composed by the genus *Glomus*, and of a natural inoculum constituted by fragments of leek roots having trapped AMF spores naturally present in the soil of the region of Guercif-Morocco on the growth, the yield and the nutritious quality of potato.

Results

Mycorrhizal colonization of plant roots

Compared to the control (non-inoculated plants; T1), the mycorrhization rates of potato was 100% and 78% for the natural inoculum from Guercif-Maroc soil and the commercial inoculum, respectively. No presence of mycorrhizal fungi was observed in the roots of non-inoculated control plants.
The impact of the application of the different mycorrhizal inocula was reflected in different frequencies of mycorrhizal colonization (48% for plants inoculated with the commercial inoculum (T2) and 84% for plants inoculated with naturel inoculum (T3)). The best frequency was recorded for the T3 inoculum and was significantly different from the T2 treatment (Figure 1).
The colonization intensity of potato plant roots was significantly higher in T3 (NI) than T2 (CI) (52% in T3 vs. 24% in T2) (Figure 1). These results suggest the presence of more active AMF species in the inoculum from the Guercif-Morocco soil (T3) than in the commercial inoculum (T2).

Richness and diversity of AMF

The density of AMF spores extracted from the potato culture substrate inoculated with T3 inoculum at the end of the experiment was lower (201spores/100g substrate) than that found in the Guercif -Morocco soil (3860/100g soil) that was used for the preparation of the T3 inoculum (Chafai et al.,2022).
The morphological study of the spores revealed the presence of seven different morphotypes represented by the following species: *Funneliformis constructum*; *Rhizophagus clarus (Glomus clarum)*; *Clomus macrocarpum*; *Rhizophagus intararadices*; *Glomus deserticola*; *Funneliformis mosseae* and *Claroideoglomus etunicatum (Glomus etunicatum)* (Table 1). These species belonged to the family Glomaceae, and the order Glomerales.
Rhizophagus intraradices and *Funneliformis mosseae (Glomus mossea)* were the most frequent species from the T3 inoculated culture substrate (Figure 3).

Effect of mycorrhizae on yield

Compared to non-inoculated control plants, Mycorrhizal inoculation resulted in an increase of the potato's total yield, which was 34% higher with the T2 inoculum and 48% higher with the T3 inoculum.
The best yield (244g tubers/plant) was recorded in plants inoculated with natural inoculum isolated from the Guercif-Morocco soil (T3). These results show a significant difference between the two inocula (T1 and T3) (Figure 4).
The number of tubers per plant were improved by mycorrhization (Figure 5). T3 significantly increased the number of tubers while T2 were not significantly higher than the control. Plants mycorrhized by T2 and T3 increased their number of tubers per plant by about 24% and 39%,

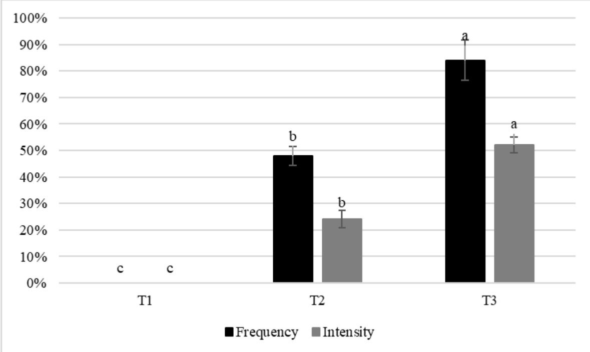

Figure 1. Frequency and intensity of mycorrhization of potato roots. (T1: plant control; T2: Plants inoculated with commercial inoculum; T3: Plants inoculated with natural inoculum). Each of the mycorrhization parameters (frequency and intensity) was treated statistically independently of the other. Treatments sharing the same letter are not significantly different ($p < 0.05$).

respectively, compared to non-mycorrhized control plants (T1).

Tuber nutrient uptake

Inoculation with commercial inoculum (T2) and natural inoculum (T3) significantly increased the potato tuber biomass by approximately 2.3% and 2.9% for T2 and T3, respectively (Table 2).
Mycorrhization significantly improved the N content of the tubers. In T2, The N content of the tubers was almost 10 times that of the control.
The results presented in Table 2 show that inoculation of potato plants with the different inocula (T2 and T2) had a positive influence on the content of Ca; Zn; Cu; P, Fe and K in the tubers.
Compared to the non-treated control, treatment with T2 resulted in a significant increase of the Ca content of the potato tubers, while the inoculation with T3 resulted in a significant decrease in the Ca content.
The highest Zn content was recorded for T3 (0.76%) with a significant difference from T2 (0.586%) and T1 (0.497%) (Table 2).
The highest Cu content was found for T2 followed by T3 and T1 with a significant difference.
T2 and T3 had higher Fe content than the control (T1) with a significant statistical difference.
The highest K content (77.199%) was recorded for T3 compared to T2 and T1 (71.934% and 69.221%, respectively). The inoculated plants showed a significant difference from the non-inoculated plants (T1) (Table 3).
Mycorrhization increased the P content of the inoculated plants compared to the control plants where the highest value was observed in the T3 treatment.
The S and Si contents are higher in the control than in the inoculated plants.

Discussion

While the roots of the potato plants were well mycorrhized by both the commercial inoculum T2 and the natural inoculum T3 (isolated from the Guercif -Morocco soil), No mycorrhization was observed in the non-inoculated T1 control plants in our experimental set-up, indicating the absence of endogenous fungi in our growing medium. The contribution of the mycorrhizal inocula resulted in the formation of different structures common to endomycorrhizae (Béreau and Garbaye, 1994).

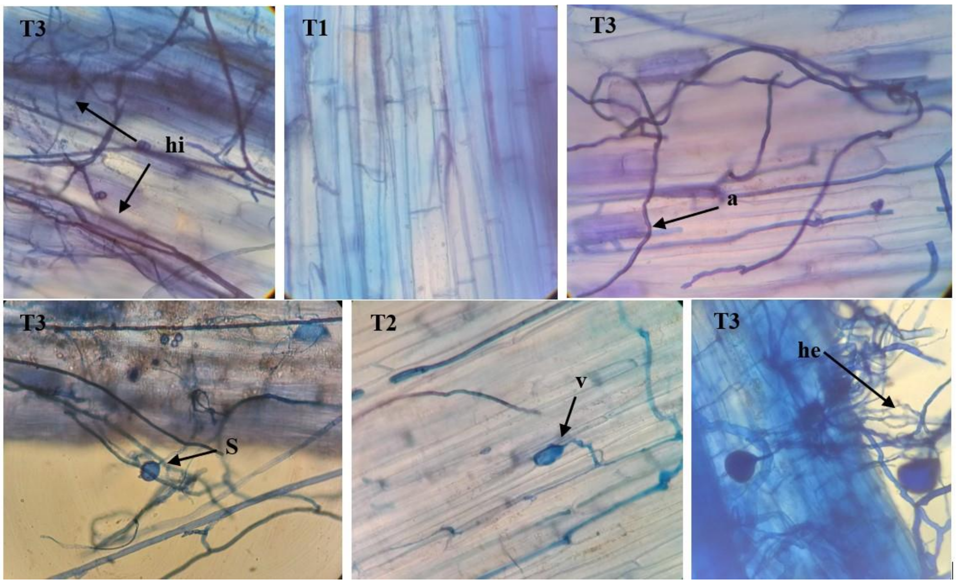

Figure 2. Structures of endomycorrhizal fungi observed in root samples of potato plants inoculated with commercial inoculum (T2) and natural inoculum (T3) and non-inoculated plants (T1): arbuscules (a), vesicles (v), spores (s), and extra and intracellular hyphae (he), (hi) (G×400).

Table 1. Characteristics of all AMF isolated from the culture substrate inoculated with T3 of inoculum.

Species	form	Color	Size of the spore (µm)	Wall size (µm)	Length of the hypha (µm)	Surface of the spore
Funneliformis constructum	globular	Almost black	110	1	-	smooth
Rhizophagus Clarus	globular	Brown	96	1		smooth
Glomus macrocarpum	globular	Yellow-brown	104	1.1	-	smooth
Rhizophagus intararadices	globular	Hyaline	96	1.5	99	smooth
Glomus deserticola	globular	Reddish brown	40	1.3	-	smooth
Funneliformis mosseae	globular	Yellow	90	1.2	-	smooth
Claroideoglomus etunicatum	globular	Orange	100	2	-	smooth

The addition of AMF inoculum to the substrate positively affected the yield parameters of the potato crop. Indeed, the number of tubers and yield of inoculated plants were much higher than those of non-inoculated plants. In fact, we showed that the number of tubers/potato plant increased by 24% and 39% for plants inoculated with T2 and T3 respectively. In addition, the yield of plants inoculated with T2 and T3 were increased by 34% and 48% respectively. Our results corroborate that a recent study by Lombardo et al. (2020), who showed an increase in marketable tuber yield by 25% and in tuber/plant number by 21% for inoculated plants.
Tohidi-Moghaddam et al. (2004) reported that by solubilizing phosphates, the microorganisms would increase available phosphorus in the soil, which could increase the number of tubers per plant. This suggest that the parameters for estimating the degree of root colonization vary among treatments, and optimization of symbiotic interaction may depend on the selection of appropriate AMF isolates for specific cultivars. In fact, the mycorrhization frequency (F) and mycorrhizal intensity (M) values obtained with the T3 inoculum (84% and 52%, respectively), were higher than those obtained with the T2 inoculum (48% and 24%, respectively). This suggests that our inoculum isolated from Guercif-Morocco soil (T3) has a higher mycorrhizogenic potential when compared to commercial inoculum (T2). These interesting results obtained with the T3 inoculum could be the result of the development of a larger network of hyphae in the rhizosphere of inoculated plants, which was also shown in previous studies (Zhong-Qun et al. (2007) and Tahat et al. (2008).
The assessment of the biodiversity of mycorrhizal fungi in the rhizosphere of potato plants that received T3 inoculum showed the presence of seven morphotypes. The majority of these species belong to the genus *Glomus* which generally dominates AMF communities in Moroccan agricultural soils (Sghir et al., 2014; Chliyeh et al., 2014; Talbi et al., 2015; Selmaoui et al., 2017). The other species identified in the Guercif-Maroc soil, which was the source of the T3 inoculum (Chafai et al., 2022) have disappeared, or have stopped their activity. Several factors can affect the development of AMF communities in the soil. For example, early colonization of host plant roots by one AMF species may prevent further colonization by other fungi. This phenomenon is known as self-regulation (Hepper et al., 1988; Person et al., 1993; Vierheilig et al., 2000; Vierheilig, 2004).
Rhizophagus intraradices and *Funneliformis* mosseae are the most frequent AMF fungal species in the substrate of our potato crop. A similar result was obtained by Bharadwaj et al. (2007), who showed, that when using a potato trap crop, *Rhizophagus intraradices* and *Funneliformis mosseae* are the most common fungi in the host plant roots and the crop soil.
The diversity of AMF species in the potato growing medium that received the T3 inoculum, was found to be effective on the growth and yield of the host plant under our growing conditions. Ortas and Ustuner (2014), also showed that the richness and interaction of AMF species positively impacted plant growth. Functional complementarity between species

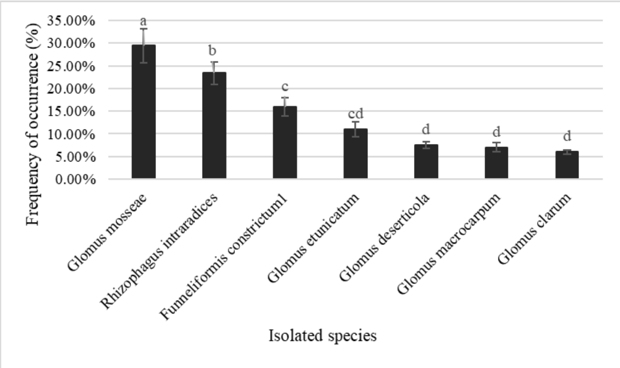

Figure 3. Frequency of occurrence of AMF species in potato crop substrate inoculated with naturel inoculum (T3) at the end of the experiment. (*Glomus mosseae= Funneliformis mosseae*; *Glomus etunicatum=Claroideoglomus etunicatum*; *Glomus clarum= Rhizophagus Clarus*). Treatments sharing the same letter are not significantly different (p< *0.05).*

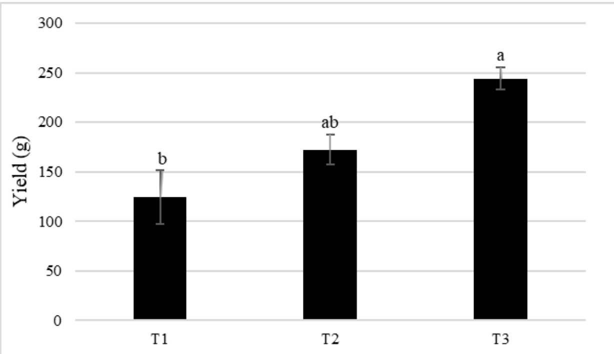

Figure 4: Potato crop yields. T1: Control-non-mycorrhized plants; T2: Plants inoculated with commercial inoculum; T3: Plants inoculated with natural inoculum. Treatments sharing the same letter are not significantly different (p < 0.05).

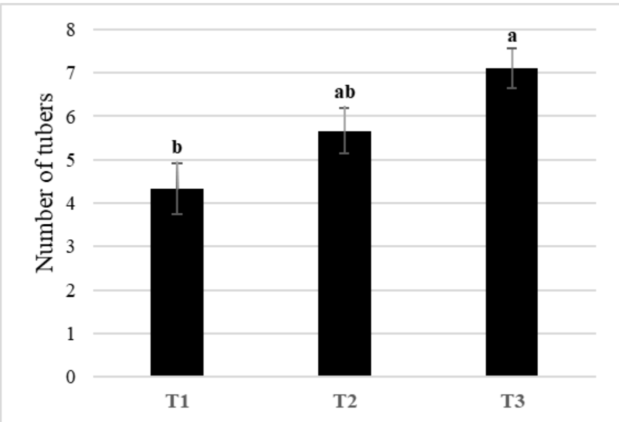

Figure 5. Number of potato tubers (T1: Control; T2: Plants inoculated with commercial inoculum; T3: Plants inoculated with natural inoculum. Treatments sharing the same letter are not significantly different (p < 0.05).

in the AM community colonizing a single root system has been suggested by Jansa et al., 2008; Koide, 2000.

Similar to previous studies by Vosatka and Gryndker (2000) and Ndiaye et al. (2005), our study showed that potato plants mycorrhized by T2 and T3 had a higher dry biomass production compared to the non-mycorrhized control.

The addition of AMF T2 and T3 to the growing medium, significantly modified the content of potato tubers for many mineral elements such as Zn; Cu; Fe; P, N and K. As already shown by different previous studies that highlight the importance of AMF in improving the absorption of mineral elements (nitrogen, phosphorus, potassium and microelements) with low mobility in the soil by tomato, (Hart et al.2015; Sánchez-Bel et al.2018; Higo et al.2020). Nitrogen was the mineral element with the highest content in inoculated plants compared to uninoculated plants, while S and Si were not affected by mycorrhization and the values recorded in uninoculated plants exceeded those in mycorrhized plants. Our work clearly showed that the T3 inoculum resulted in an increase in P, K, Zn and Fe content of potato tubers compared to the T2 commercial inoculum.

Our results confirmed the important role of adding AMF to the crop soil. This makes these microorganisms an eco-sustainable tool to increase yield and increase the levels of macro and micro mineral elements in potato tubers.

Materials and methods

Producing natural inoculum

A composite endomycorrhizal inoculum was prepared from the soil of Guercif-Maroc (33°58'45''N, 3°15'41''W, Altitude: 688 m). AMF trapping was performed with leek plants as host plants. After 16 weeks of culture, the roots of the seedlings were excised, rinsed with distilled water and cut into 1-2 mm fragments. These root fragments were used as endomycorrhizal inoculum (T3). The inoculum consisted of 31 endomycorrhizal fungal species, identified morphologically by Chafai et al (2022).

Plant cultivation and treatment

A pot experiment with potato was carried out under semi-controlled conditions in order to evaluate the effectiveness of inoculation of potato plants with two types of endomycorrhizal fungi inocula and to compare the effects of these inocula with those of non-inoculated control plants.

Potato seed tubers were sterilized with 10% H_2O_2 for 10 min to remove surface fungi, and then rinsed thoroughly with water.

The inoculation was carried out at the time of sowing, on sterilized substrate, according to the following experimental protocol:

T1: non-inoculated control plants.

T2: Plants inoculated with a commercial liquid multi-species inoculum (genus *Glomus*).

T3: The plants inoculated with a natural inoculum, in the form of fragments of mycorrhized leek roots, obtained by trapping AMF spores naturally present in the soil of the Guercif-Morocco region.

Each treatment had three replicates, which were placed in a completely randomized design.

No fertilizers were applied during the entire growing cycle. However, three treatments against downy mildew were applied.

Experimental materials

The variety of potato used is "Spunta". The substrate used for the pot experiment was a mixture of sand and peat (v/v) and it was autoclaved for 20 min at 120 ˚C. Sand was considered an inert substrate and a peat had a super fine structure; pH 6.1; salinity 1.2 g L-1; N 210 mg L-1; P,O 120 mg L-1; K,O 260 mg L-1; NPK balance 1-0.6-1.2.

The cylindrique pots used for the experiment had a volume of 7L with 20 cm inner diameter and 24 cm in height. After pouring the substrate into each pot, the height of the soil was 20 cm.

Table 2. Potato tuber dry matter (g/100gMf), nitrogen and mineral element content (%/ g dry matter).

Traitement	dry matter (g/100g FM)	N (mg/gMs)	Mineral element in %							
			Ca%	Zn%	Cu%	Fe%	K%	S%	P%	Si%
T1	16.34^{b}	$26,75^{c}$	13.958^{b}	0.497^{c}	0.922^{c}	1.069^{b}	69.221^{c}	6.785^{a}	2.516^{b}	4.972^{a}
T2	16.72^{a}	216.87^{a}	14.517^{a}	0.586^{b}	1.39^{a}	1.731^{a}	71.934^{b}	5.053^{b}	2.676^{ab}	2.111^{b}
T3	16.82^{a}	117.5^{b}	9.971^{c}	0.761^{a}	1.155^{b}	1.759^{a}	77.199^{a}	4.38^{b}	3.013^{a}	1.761^{b}

T1: Non-inoculated plants; T2: plants inoculated with commercial inoculum; T3: Plants inoculated with natural inoculum.
Two values in the same column followed by the same letter are not significantly different at the 5% level.

Evaluation of root colonization

Potato root samples were taken at the end of the crop and the assessment of mycorrhization parameters was performed by observing randomly selected root fragments of about 1 cm (Trouvelot and kough, 1986; Amir and renard, 2003). The frequency (F %) and mycorrhizal intensity (M%) of root MACs were measured by assigning a mycorrhizal index ranging from 0 to 5 (Derkowska et al., 2008).

- Mycorrhization frequency (F): extent of infection of the root system

$$F\% = \left(\frac{N - No}{N}\right) * 100$$

N = number of observed fragments and No = number of fragments without any trace of mycorrhization.

- Colonization intensity (M): It expresses the portion of the colonized cortex compared to the whole root system.

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

N: number of observed fragments

Where n5, n4,...nl are the number of fragments noted 5, 4,..., 1 respectively; class 5: more than 91%, class 4: from 51 to 90%, class 3: from 11 to 50%, class 2: less than 10%, class 1: trace and class 0: no mycorrhization.

Extraction and identification of spores from the culture medium (T3)

Extraction of spores of endomycorrhizal fungi from the substrate was performed according to the method of Gerdemann and Nicolson (1963). The number of spores in 100 g of substrate was estimated by direct counting under a binocular loupe. Spores were then observed between slide and coverslip under a light microscope (x40) and classified according to color, shape, wall structure, and hyphal attachments to identify their genus (Ferrer and Herrora 1981; Schenck and Smith 1982; Walker and Mize 1982; Hall 1984; Schenck and Perez 1987; Morton and Benny 1990; and information available in various databases (INVAM 2017)).

Isolated spores were quantified by direct counting under a binocular loupe to estimate the number in 100 g of substrate (spore densities). Three replicates were performed.

The frequency of appearance of the species (% A.F.S) designates the percentage of a morphotype compared to the totality of the spores.

$$A.F.S = \left(\frac{Ns}{Nt}\right) * 100$$

Ns : Number of isolated spores of the species x.

Nt : Total number of spores.

Dry weight of tubers

The dry matter of the tubers was determined by steaming by taking the fresh weight of the departure and proceeding to the drying in the study at 80°C until constant weight.

Nitrogen content of tubers

The determination of nitrogen was carried out by the Kjeldhal method, which consists of the mineralization of organic matter under heat with concentrated sulfuric acid (H_2SO_4) in the presence of catalysts ($CuSO_4$ and K_2SO_4).

The diammonium sulfate is then displaced from its soda salt, distilled by steam distillation and collected in a known amount of excess hydrochloric acid. The unreacted hydrochloric acid is measured back with sodium hydroxide.

Analysis of mineral elements

The analysis of mineral elements was carried out by X-ray fluorescence spectrometry (EDX 7000 Shimadzu); the chemical elements present in the samples are represented by their mass concentration.

Statistical analysis

Data presented as means of 3 replicates ± standard deviation were analyzed by one-factor ANOVA test using SPSS 21.0 software for Windows.

Conclusion

We conclude that arbuscular mycorrhizal fungi can be used as a promising approach for high quality and quantity of potato production. Using commercial inoculum and natural inoculum isolated from Guercif-Morocco soil; we observed an increase in yield and biomass, in addition to the improvement of tuber mineral content. Furthermore, the results obtained with the natural inoculum (T3) exceeded those recorded for the commercial inoculum. These results shows the potential of this natural inoculum and the need for the development and improvement of such preparation and to test it on other crops.

Acknowledgments

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