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Research Article

Fumigation Effect on the Mycorrhizal Status, the Mycorrhizal Diversity and the Roots System Development of Strawberry in Morocco

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Abstract

This study was undertaken to evaluate the fumigation effect on the mycorrhization status of strawberry roots, the diversity of indigenous arbuscular mycorrhizal fungi in the rhizosphere of strawberry and the roots system development of strawberry. Two parcels were taken randomly in the perimeter of Loukkos with fumigated one. For each parcel, samples of strawberry's rhizosphere soil were taken regularly all over the cycle of culture. The mycorrhization parameters (mycorrhizal frequency, mycorrhizal intensity, arbuscular content, vesicular content and spores number) were calculated using Phillips and Hayman technique according to the scale of Trouvelot. An identification of spores was made according to the key International culture collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). The frequency of the apparition of genera and species of spores at the sites were calculated. The roots system development was evaluated with the visual analogical scale (VAS: 0-100 mm). All the parameters were compared between the two parcels. Results showed that Mycorrhizal frequency, the number of spores and the roots system development between fumigated soil and non-fumigated one were respectively ((99.3 ±2.8 vs. 100); (69 ±12.3 vs. 137.5 ±47.8); (23.8 ±9.6 vs. 63.1 ±21.6)). Six genera with nine species were identified and three undefined. Glomus genera were the dominant. In multivariate analysis, the roots system development was dependant to the fumigation status (yes/no), the vesicular content and the mycorrhization frequency. The roots system development was dependant to the fumigation status (yes/no) and spores number. As conclusion, this study suggests that the fumigation has a significant effect on the mycorrhization colonisation of roots, diversity of AMF and roots system development of strawberry.

Keywords

Mycorrhizal Fungi, Strawberry, Diversity, Fumigation, Soil, Roots, Morocco

1. Introduction

Strawberry is a worldwide culture; it is cultivated in a great number of countries on an interesting area. Morocco is among countries where strawberry is a valued culture. It is cultivated in two perimeters: Loukkos and Gharb [1].

Perimeter of Loukkos is situated on the Northwest of Morocco, it is known for its Mediterranean climate favourable to a wide range of cultures and mainly the strawberry plant [1, 2].

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Its mode of culture required the use of a big quantity of fertilizers and phytosanitary products which echoes negatively on the health of the environment and the Man [3]. Researches showed the relation between chemicals and several troubles on Man and environment. These used products threaten the development of every biological agent who can have a beneficial role for the plant [3, 4].

Plant protection products have been associated with a wide range of risks to human health, ranging from short-term effects such as headaches and nausea to chronic effects such as cancers, reproductive harm and endocrine disruption [3].

Chronic health effects can occur years after even minimal exposure to pesticides in the environment, or result from pesticide residues we ingest in our food and water [5].

Today, there are strict and severe regulations on the use of chemical pesticides, in addition to political pressure to remove the most dangerous chemicals from the market. However, the spread of plant diseases in natural ecosystems can prevent the implementation of successful applications. Therefore, some pest control research has focused efforts on developing alternatives to synthetic chemicals to control plant pests and diseases. Among these alternatives are those called biological control [6].

Arbuscular mycorrhizal fungi (AMF) are essential components of terrestrial ecosystems, forming mutually beneficial (mutualistic) symbioses with the roots of approximately 80% of vascular plants and often increasing phosphate (P) uptake and growth. Mycorrhizal fungi can dominate P supply to plants, independent of growth responses [7].

Although various epiphytes and endophytes can contribute to biological control, the ubiquity of mycorrhizae deserves special attention. Mycorrhizae form early in the development of plant species and represent root colonists that help plants absorb nutrients (especially P and micronutrients). During colonization, AMF can prevent root infections by reducing access sites and stimulating host defense. AMF was found to reduce the incidence of root-knot nematode [8].

This study wants to demonstrate the fumigation effect on the mycorrhization status, diversity of arbuscular mycorrhizal fungi (AMF) and on roots system development.

2. Materials

Two strawberry's parcels were chosen in a random way with one fumigated and for each we took samples of soil. Characteristics of each parcel are demonstrated on the table 1.

Table 1. *Soils characteristics.*

Parcels	Variety	Culture type	Age	Treatment
P_1	Festival	Under greenhouse	First year	Without fumigant
P_2	Festival	Under greenhouse	First year	With fumigant

3. Methods

Soil and root sampling: Soil samples were taken from the rhizosphere of the strawberry yields in two parcels of the Loukkos perimeter. In each site, soil was sampled randomly. All samples were taken from the area near the root and a composite sample of soil was prepared at each site. Very fine roots were taken at the same time with the soil.

3.1. Determination of Mycorrhization Parameters

The mycorrhizal frequency and intensity were quantified using Philips and Hayman technique [9] as modified by Koske and Gemma [10]. The roots were carefully washed with tap water, cut into segments of 1-2 centimeter (cm) in length, and submerged in a solution of 10% potassium hydroxide (KOH) for 20 min at 100 ℃. They were then washed again in tap water. To bleach those with excess pigment roots were submerged in hydrogen peroxide (H2O2 10%). After this, root segments were placed in a beaker containing 100 milliliter (ml) of distilled water and 0.05 (g) of Cresyl blue, transferred to a 90 ℃ water bath and incubated for 15 minutes.

The arbuscules and vesicles contents of AMF inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 (Koske and Gemma) [8, 9]. Stained root fragments per root sample were mounted on a microscope slide; five observation fields for each of 1.5 (cm) root pieces were examined and tallied for percent of root colonized under 40 × magnifications with the optical microscope.

1. The mycorrhizal frequency (M.F.) reflects the importance of the host root system infection expressed in percentage (%) was calculated using the following formula:

$$M.F.\ \% = 100 \times (N - n0) / N$$

N: Number of observed fragments,
n0: Number of non-mycorrhizal fragments.
2. The mycorrhizal intensity (M.I.) expressed in percentage

(%) was determined as follows:

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

It expresses the proportion of colonized cortex compared to the whole root system. The numbers n5, n4, n3, n2, and n1 denote the number of recorded fragments 5, 4, 3, 2 and 1 estimating the proportion of root colonized by mycorrhizae according to the scale of Trouvelot [11]:

n: Number of fragments assigned with the index 0, 1, 2, 3, 4 or 5.

n1: traces, n2: few than 10%, n3: from 11 to 50 %, n4: from 51 to 90% and n5: more than 90 %.

3. The Arbuscular content (A.C.) was the proportion of the root cortex containing arbuscular, expressed in percentage (%).

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

mA3, mA2, mA1 are the percentages of mycorrhizal arbuscular respectively assigned to the notes A3, A2, A1, with, mA3 = (95n5A3 + 70 n4 A3 + 30 n3 A3 + 5 n2 A3 + n1A3) / N.

The same for A1 and A2.

n5A3 represents the number of fragments marked 5 with A3; n4A3 marked the number of fragments 4 with A3; etc…

A1: some arbuscules (10%), A2: moderately abundant arbuscular (50%), A3: very abundant arbuscular: (100%).

4. The Vesicular content (V.C.) was the proportion of the root cortex containing vesicles, expressed in percentage (%).

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

mV3, mV2, mV1 are the percentages respectively assigned notes V3, V2, V1, with V3;

mV3 = (95 n5V3 + 70 n4V3 + 30 n3V3 + 5 n2V3 + n1V3) / N. The same for V1 and V2. n5V3 represents the number of fragments marked 5 with V3; n4V3 marked the number of fragments 4 with V3; etc...

V1: some vesicles (10%), V2: moderately abundant vesicles (50%); V3: abundant vesicles: (100%).

Mycorrhizal frequency, mycorrhizal intensity, arbuscular and vesicular content were compared between the samples in the three sites.

3.2. Determination of the Endomycorrhizal Spores Population

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson [12]. In a 1 liter (L) beaker, 100 (g) of each soil was submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through a sieve of 315 microns mesh size. The same soil sample was again submerged, stirred, and the wet sieving is repeated 3 times. Deposition in the used sieve contained the maximum of spores; it was recovered with 6 (ml) distilled water and transferred to centrifuge tubes. After 5 minutes of the first centrifugation at 2000 rotation per minute (RPM), debris and the supernatant were discarded and the pellet was suspended in a solution of 4 ml of 50% sucrose.

After agitation, a second centrifugation was performed for 1 minute at 2000 RPM and a 3th one was realized for 1 minute at 3000 RPM. Spores contained in the supernatant were passed through the sieve and the pellet was discarded. Spores in the sieve were rinsed with distilled water to remove the sucrose, and then disinfected with a solution of streptomycin. The spores were then recovered with 5 (ml) distilled water in an Erlenmeyer flask. At the end, endomycorrhizal spores were quantified to estimate their number in 100 (g) of soil.

Total number of spores isolated from the rhizosphere of fumigated and non fumigated strawberry parcels was calculated and compared between both soils.

Appearance frequency of spores (A.F.S) expressed in percentage (%) designates the percentage of a morphotype relative to other species.

$$A.F.S\% = ns / nT \times 100$$

ns: Isolated spores number of the species X.

nT: Total spores number.

Appearance frequency of genera (A.F.G) expressed in percentage (%) designates the percentage of a total spore's species of one genus relative to species belonging to all genera.

$$A.F.G\% = nG / nT \times 100$$

nG: Number of spores of the genus X.

The appearance frequency of genera and species were compared between the two parcels.

3.3. Identification of AM Fungi

Identification was done by using the the key International culture collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). The following morphological characters of AMF spores were taken into consideration for identification of VAM spore structure, i.e., hyphal characters, vesicles, auxillary cells, subtending hyphae, spore germination, spore position, germinating shields, sporocarp, spore wall, ornamentation.

3.4. Evaluation of Roots System Development

Roots system development was evaluated with the visual analogical scale (VAS (0 mediocre) – (100 very well)). It was compared between the two parcels.

4. Statistical Analysis

The Statistical Package for Social Sciences software (SPSS, version 15, Chicago. Inc) was used for data processing and data analysis. Descriptive statistics included range, mean, mediane and standard deviation for interval variables; frequency and percentage for categorical variables. Comparisons between data of the two parcels were carried out by independent samples Student's t-test for interval variables and the $\chi 2$ test for categorical variables. Correlations were calculated with Spearman rank R. The remaining factors ($P<0.05$) in the univariate analysis were entered into a multivariate linear regression model, so multivariate analysis were secondly performed to analyze significant determinant associated with roots system development as dependent factor. ß Coefficient of each independent variable associated with roots system development was defined. A P value of less than 0.05 was considered statistically significant.

5. Results

Different endomycorrhizal structures have been observed: arbuscules (figure 1) which seemed to ramify along the cortex of roots and vesicles (figure 2) often with an oval shape that were present between cells of the cortex.

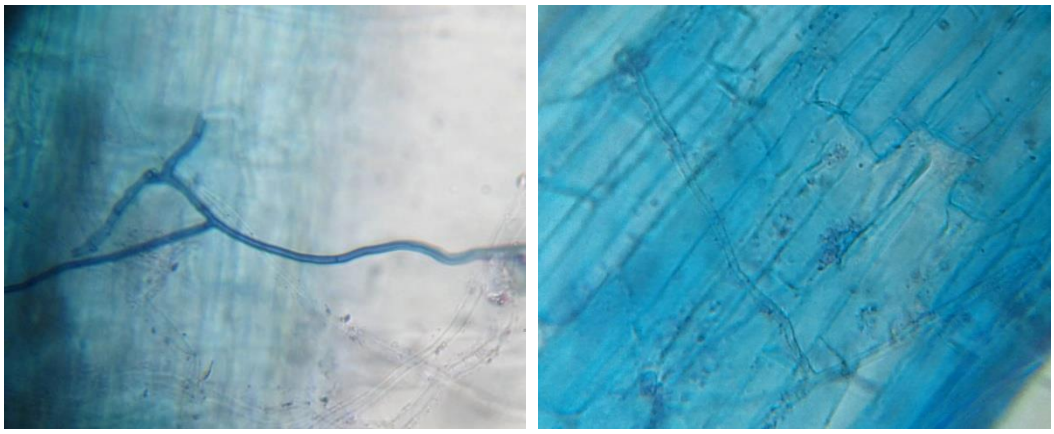

Figure 1. *Arbuscules.*

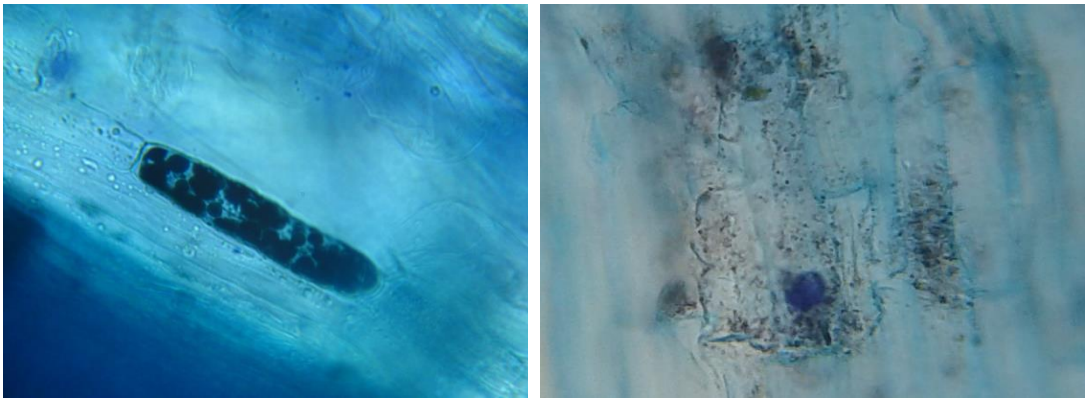

Figure 2. *Vesicles and others structures.*

The characteristics of parcel 1 as non fumigated soil and parcel 2 as fumigated soil are summarized in Table 2.

Table 2. *Samples characteristics.*

Characteristics	Soils N=30	Fumigated soil	Non fumigated soil	P
Spores number	114.6 ±51.3	69 ±12.3	137.5 ±47.8	<0.0001
Roots system development (VAS: 0-100 mm)	50 ±26.2	23.8 ±9.6	63.1 ±21.6	<0.0001

Spores number and roots system development in fumigated soil compared to non fumigated soil were respectively ((69 ± 12.3 vs. 137.5 ± 47.8; p<0.0001) and (23.8 ± 9.6 vs. 63.1 ± 21.6; p<0.0001)).

After identification of observed spores, according to the INVAM key, six genera, nine species were identified and three species were non identified. There were genera and species presents in 1st year parcel and absent in the 2nd year parcel and vice versa. The genera Glomus were the dominant one for the two parcels. The figures 3 and 4 demonstrate appearance frequency of species and genera in the 1st and 2nd year of strawberry culture.

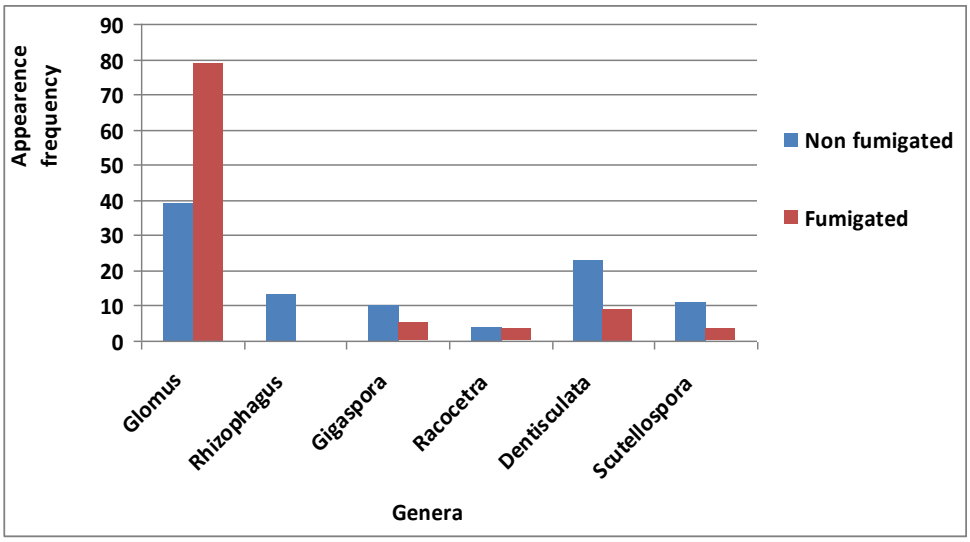

Figure 3. *Genera appearance frequency in fumigated and non fumigated soil of strawberry culture expressed in percentage.*

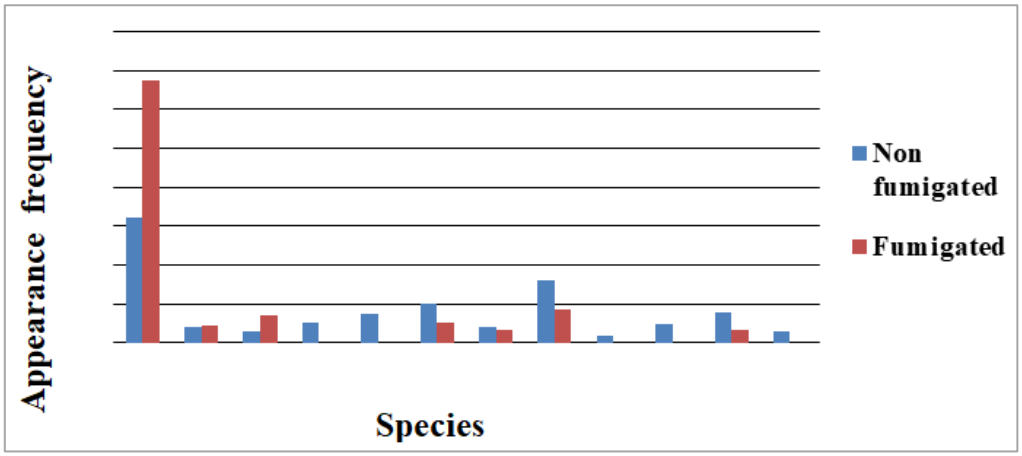

Figure 4. *Species appearance frequency in fumigated and non fumigated soil of strawberry culture expressed in percentage.*

The data on the table 3 demonstrate that the roots system development was correlated to the mycorrhizal frequency (r=0.392; p=0.03), mycorrhizal intensity (r=0.464; p=0.01), the number of spores (r=0.926; p<0.0001), the vesicular content (r=0.637; p<0.0001) and the arbuscular content (r=0.695; p<0.0001).

Table 3. *Correlation analysis.*

	Roots system development	
	r	**P**
Mycorrhizal frequency (%)	0.392	0.03
Mycorrhizal intensity (%)	0.464	0.01

	Roots system development	
	r	P
Vesicular content (%)	0.637	<0.0001
Arbuscular content (%)	0.695	<0.0001
Spores number	0.926	<0.0001

Table 4. *Linear regression analysis with roots system development as dependent variables and arbuscular content, vesicular content, number off spores, soil, mycorrhizal frequency and mycorrhizal intensity characteristics as independent variables.*

	Roots system development	
	β	p
Mycorrhizal intensity (%)	0.385	0.03
Vesicular content (%)	0.525	0.003
Arbuscular content (%)	0.595	0.001
Spores number	0.947	<0.0001
Fumigate (yes/no)	-0.719	<0.0001

The linear regression for the dependant variable as demonstrated in table 4, the first variable was dependant to arbuscular content (β=0.595; p=0.001), vesicular content (β=0.525; p=0.003), number of spores (β=0.947; p<0.0001), mycorrhizal intensity (β=0.385; p=0.03) and fumigate (yes/no) (β=-0.719; p<0.0001).

6. Discussion

Mycorrhization status and roots system development were significantly important in the non fumigated soil than fumigated one. Plants grown in fumigated soil were less mycorrhizal than others grown in non fumigated soil. That joins what was demonstrated by A.C Mc Graw in 1986 [13].

Fumigation is used to eliminate the pathogenic agents of the ground before the installation of a culture. In soilless substrates lacking the indigenous mycorrhiza or under the conditions where field soils are fumigated and most of the indigenous mycorrhizal fungi are eliminated as demonstrated by Vosatka [14].

Our study demonstrate that the AMF diversity was less important in fumigated soil than non fumigated one and that joins the work of Brazanti et al., in 2002 where they explain that fumigation reduced AMF root colonization [15].

All soil treatments studied reduced mycorrhizal colonization of strawberry plants. Strawberry growth and production were higher with biofumigant treatments. Leaf mass of non-inoculated and inoculated plants was higher for almost all non-chemical soil fumigation treatments compared to the control. The number of strawberry fruits for non-inoculated biofumigant treatments was the highest [16]. Despite reduced AMF colonization, the biofumigant resulted in higher fruit numbers and leaf mass.

Reeve et al. [17] proposed that repeated long-term use of fumigants may have more significant and long-lasting effects on soil microbial populations than those measured in a single study of the effects and incidences of fumigation.

In recent study, the soil microbial biomass in the non-fumigated 33-year-old site was higher than in the 33 years after fumigation site [18]. By taking in account the positive actions of mycorrhizae on plants hosts, there is evidence that the damages from pathogenic fungi are limited when plants are colonised by mycorrhizae as demonstrated by Caron et al., 1985 [19], while plant tolerance against metal toxicity and unfavorable environmental conditions is increased as well, as figured in the studies of Al-Karaki et al., 2001, Harrier and Watson, 2003 [20, 21]. So, Thanks to the beneficial effects associated with symbiotic soil microorganisms, such as AMF rhizosphere bacteria [22].

In strawberry breeding program, the increased growth obtained by inoculating transplants with mycorrhizae could be useful since low vegetative vigour has been observed during plantation establishment in non-fumigated soil [23].

The strawberry culture is a great consumer of the phytosanitary products to control plant diseases. These products are fatal for the health of the Man and the environment causing so a lot of damage.

Excessive use of pesticides can damage the flora of agricultural land by harming species of beneficial soil microorganisms and worms that naturally limit pest populations and maintain soil health [24]. There are a multitude of approaches to prevent, mitigate or control plant diseases. These agricultural inputs have contributed significantly to the dramatic improvement in crop productivity and quality over the past 100 years. However, environmental pollution caused by excessive and misuse of conventional and agrochemical products as well as awareness campaigns against pesticides have led to considerable changes in the attitudes of farmers and consumers towards the use of pesticides in agriculture [6]. And while their presence can present various challenges to an infectious pathogen, the absence of a measurable decrease in pathogen infection or disease severity is indicative of commensal interactions. in some cases, fumigation reduced the P concentration in plant leaf tissues. With other crops, the effects of fumigation on yield could be explained by the destruction of AMF [25].

Under controlled conditions and with substrates receiving fumigation, the measured beneficial effects of mycorrhizal inoculation on plant growth can vary from zero to 2,600% for citrus and to 1,000% for cassava. Under field conditions and on non-fumigated soils, these responses are of lesser magnitude but can reach 300%. The magnitude of the response is unpredictable because it depends on factors inherent to the host plant,

the environment and the fungus itself [26].

Therefore, it represents a non chemical soil fumigation method that should be applied in sustainable strawberry production [16].

7. Conclusion

Fumigated strawberry fields had lower AMF populations than their non-fumigated sites, which presents a risk to fumigated fields because AMF play a major role in soil health and fertility. Crop rotations and cover crops are probably alternatives to maintain or improve bacterial proportions; However, this study suggests that the fumigation has a significant effect on the mycorrhization colonization of roots, diversity of AMF and roots system development of strawberry. This study highlights the need for further researches into the long-term impact of alternative treatments to chemical soil treatments in order to select soil disinfestation methods that have low negative environmental impacts, maintain the health of the soil and improve sustainable agricultural production.

Abbreviations

INVAM	International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi
AMF	Arbuscular Mycorrhizal Fungi
P	Phosphate
KOH	Potassium Hydroxide
H_2O_2	Hydrogen Peroxide
ml	Milliliter
g	Grams
cm	Centimeter
M.F.	Mycorrhizal Frequency
M.I.	Mycorrhizal Intensity
A.C.	Arbuscular Content
V.C.	Vesicular Content
L	Liter
RPM	Rotation Per Minute
A.F.S	Appearance Frequency of Spores
A.F.G	Appearance Frequency of Genera
VAM	Vesicular Arbuscular Mycorrhizal Fungi
VAS	Visual Analogical Scale
SPSS	Statistical Package for Social Sciences Software

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Author Contributions

Madiha Bahouq: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Hanane Bahouq: Conceptualization, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Abdelmajid Soulaymani: Conceptualization, Formal Analysis, Supervision, Validation, Visualization, Writing – review & editing

Conflicts of Interest

The authors declare no conflict of interest.

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