Journal of Water Sciences & Environment Technologies

Cit this: *JOWSET,* ***2018*** *(02), N°02, 359-366*

Physico-chemical optimization of growth substrates for better production aboveground of plant in Tunisia

Youssef M'SADAK*, 1, Mohamed Aymen ELOUAER1

[1] *Sousse University, High Institute of Agronomy, Chott-Mariem, 4042, Tunisia*

**Corresponding Author: Tel.: +21673327534; fax: +21673327591; e-mail: msadak.youssef@yahoo.fr*

In order to physically and chemically optimize *Acacia cyanophylla* compost, screened Acacia compost was incorporated to rabbit manures compost, according to different ratios. The various growth substrates obtained were subjected to physical analysis relating to water and air retentions (Total porosity, aeration porosity and retention porosity) and chemical analysis concerning to pH, organic matter, nitrogen and C/N ratio. Simple screening technique, using 6 and 8 mm meshes, are physically and chemically better than double and triple screening. The best ratio mixture between *Acacia cyanophylla* compost and rabbit manures compost was respectively 75% and 25%. For a better use of those substrates, further studies are needed to optimize their biological characteristics.

Received: 27 June 2017
Accepted: 20 April 2018
Available online: 16 September 2018

Keywords:
Composts
Screening
Mixture
Growth substrates
Physical behavior
Chemical behavior

Introduction

Depletion of a non-renewable resource such as peat and its high price in the market has favoured the utilization of alternative materials as growth substrates [1]. Developing inexpensive and nutrient-rich organic media alternatives cannot only eliminate environmental impacts, but it also means to reduce fertilization rates, irrigation rates and nursery costs [2]. Nowadays, numerous studies have demonstrated that these organic residues, after proper composting, can be used with very good results as growth media instead of peat [3] [4] [5] [6] [7]. Compost from green residues is among organic residues that are used successfully as plant growing media. [8] have demonstrated that compost improves seedling development by improving the physical proprieties of growth substrates, such as electrical conductivity, water retention, porosity and pore spaces; the increases in oxygen content directly affect the root zone, which influences seedling growth. This fact was demonstrated by several research studies, which have shown that the nature of the products used for the manufacture of growth substrate has a major influence on its physicochemical properties [2] [9].

In fact, a successful seedlings production requires in prior a growth substrate with adequate physical characteristics.

The physical properties of a substrate affect its aeration characteristics and water holding capacity [10] [11] [12]. For this reason, it is essential to know physical, chemical and biological characteristics of the material for its better use as a growth medium.

Substrates containing composted bio-solids and yard debris had superior performance, but not all their physical properties are within the suggested optimum range [13]. That's why; they require some optimization actions for their better use as growth substrate. This study aims the physico-chemical improvement of *Acacia cyanophylla* compost for its optimum use as growth substrate mostly for the production of forest seedling, through adjusting its particle size (Change in particle size compost through different screening techniques) or composition (variation in mixture ratio of *Acacia cyanophylla* compost with rabbit manures compost).

Materials and methods

Substrates Being Tested

The compost used in the present study was manufactured from fresh woody materials (branches, and leaves) of *Acacia cyanophylla*. Windrow piles, 1.5 m high by 10 m long, were constructed using shredded materials. Forced aeration was used for the first eight weeks (bio-oxidative phase), followed by a six-month maturation period during which the piles were turned periodically to maintain adequate O_2 levels. During the maturation phase, the pile was turned every 15 days in order to improve both the O_2 level inside the pile and the homogeneity of the material. Ammonium nitrate was added to windows to ensure Carbon-Nitrogen (C/N) nutritional balance. Pile moisture was controlled weekly by adding enough water to obtain moisture content of not less than 50%. For its optimum use as growth substrate in forest nursery, the end product was passed through different size screening meshes (Tab. 1). Taking into account Acacia cyanophylla compost characteristics, it was mixed with rabbit manures compost, at different ratio, in order to prepare suitable growth media for forest nursery. Tab. 2 shows the volumetric formulations of the different media used in this study.

Tab 1: Screening techniques and meshes used for the preparation of screened compost

Screening Technique	Meshes used (mm)	Screened compost
Simple Screening (SS)	6 mm	SS1
	8 mm	SS2
	12 mm	SS3
Double Screening Technique (DS)	(6) And (8) mm	DS1
	(8) And (12) mm	DS2
	(6) And (12) mm	DS3
Triple Screening Technique (TS)	(12), (8) and (6) mm	TS1
	(6), (8) and (12) mm	TS2

Analytical Methods

Crude *Acacia cyanophylla* compost, crude rabbit manures compost, screened *Acacia cyanophylla* compost by diverse screening techniques and meshes and different substrates mixtures were analyzed for total porosity (sum of space available for water and air), aeration porosity (air capacity or space available for air) and retention porosity (water capacity or space available for water). To determine the different porosities of a substrate, we use the method described below. We take a container, which we blocked its drainage hole, and we fill it with water (WV is the volume of water in the container). We remove water and fill the container with the substrate. We add water very slowly into the entire surface of the container until the water appears on the surface. The volume of water added (VWA) represents the volume of air and water in the substrate. We obstruct drainage holes and we collect water that drains (VCW) for 10 minutes (VCW or volume of water collected is the volume of air in the substrate). Porosities of substrate (total porosity, aeration porosity and retention porosity) were calculated according to formulas below.

Total porosity (TP) = (VWA / WV) x 100 (%)
Aeration Porosity (AP) = (VCW / WV) x 100 (%)
Retention Porosity (RP) = TP - AP (%)

Chemical Characterization of Substrates

Raw Acacia cyanophylla compost, rabbit manures compost, simple and double screened *Acacia cyanophylla* compost by diverse screening meshes and different substrates mixtures were chemically analyzed according to the following parameters: pH, Organic Matter (OM), Nitrogen (N) and Carbone-Nitrogen ratio (C/N).

pH

pH measurement was carried out according to international standard [14]. pH was analyzed in a 1:5 (v/v) water extract. Dried substrate sample (20 g) was diluted in 5 times its volume (1⁄5) of water (100 ml distilled water). Suspension was put to stirring for 5 minutes then left to settling for at least two hours. The pH reading was done through a pH meter.

Organic Matter

The determination of OM rate at each substrate involves the following two steps:
20 g of each substrate sample was put in the oven for 24 hours at 70°C. 3 g of previously dried substrate sample was put for 2 hours in 900°C oven for at least 6 hours in a muffle. Dry residue was determined after calcinations. The OM content was determined according to the following equation:
OM (%) = [(M1-M2)/M1] x 100

M1: Weight before sample calcinations (mg);
M2: Weight after sample calcinations (mg).

Nitrogen and C/N Ratio

Nitrogen (N) was determined according to Kjeldahl method [15]. 200 mg of the substrate and 5 ml of sulfuric acid ($H2SO_4$) was put in a flask (mineralization phase). After 30 min, 200 mg of selenium catalyst was added to the suspension and passed in the digester heating for 1 hour until an appearance of yellow color (digestion phase). After cooling, 30 ml of distilled water was added to the flask; 30 ml of sodium hydroxide was added to alkalinize the medium (distillation phase). Nitrogen content on each substrate was displayed directly on a sheet computer.

Experimental Design

The physical suitability of all substrates (Tab. 1- 2) was carried out via porosity test. A randomized block design was used, with four replications for each porosity treatment. All results

reported in the text are the means of determinations made on four replicates. RACC was considered as control substrate and the different media (Tab. 1- 2) as treatments.

Tab 2: Growing media used in the study

Media	Formulation
RACC	RACC (100%)
CRMC	RRMC (100%)
M1	RACC (75%) + RMC (25%)
M2	RACC (50%) + RMC (50%)
M3	RACC (25%) + RMC (75%)
M4	SS3 (75%) + RMC (25%)
M5	SS3 (50%) + RMC (50%)
M6	SS3 (25%) + RMC (75%)

RACC: Raw *Acacia cyanophylla* compost; **RMC**: Rabbit manures compost; **SS3**: 12 mm screening mesh *Acacia cyanophylla* compost; % Volume in brackets.

Statistical Analyses

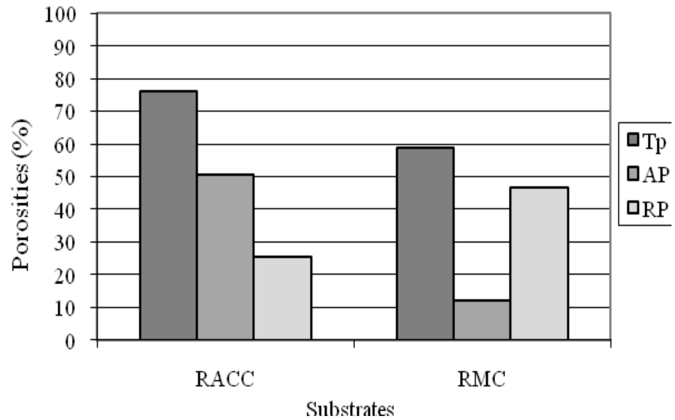
Porosities parameters of substrates were evaluated with analysis of variance (ANOVA) and Duncan multiple ranges test ($p < 0.05$) using the SPSS (13.0) System. Differences were considered significant at the 5% level (means followed by different letters).

There should be sufficient information to allow other researchers to repeat the experiment by clearly defining the experimental design. A clear description or a specific reference to all biological, analytical, and statistical procedures is required. All procedure modifications must be explained. Field experiments that are sensitive to interactions and where the crop environment cannot be rigorously controlled, such as crop production and yield component assays should usually be repeated for time and/or space, in order to ensure representative results.

Results and discussion

Fig. 1 shows the main porosities (total porosity, aeration porosity and retention porosity) of crude *Acacia cyanophylla* compost and rabbit manures compost.

[16] defined the requirements of an 'ideal substrate', they are inspired from Canadian norms [17]; the optimal substrate should exhibit 50% total porosity, 20% aeration porosity and 30% retention porosity. Crude *Acacia cyanopylla* compost used in the study approaches the most from the definition of 'ideal substrate', but its retention porosity is below the ideal range, so, it need inclusion of more water retentive substrate to correct its water holding capacity. For the rabbit manures compost (RMC), just the AP (%) value falls outside the ideal range, therefore, it requires the incorporation of other substrate, having more aeration porosity, to improve its air capacity. Total porosity and retention porosity were adequate in two cases of growing media. Accordingly, it seems interesting to make a mix of *Acacia cyanophylla* compost and rabbit manures compost to correct their physical unbalance. The ultimate goal is to find the best mixing ratio for better optimization of porosities.

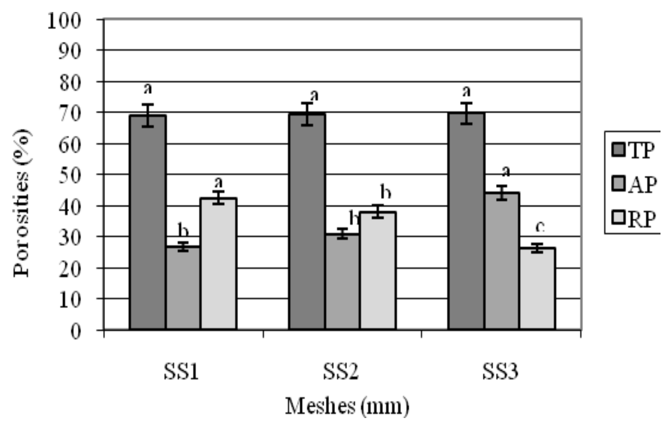


(*) RACC : Raw *Acacia cyanophylla* Compost; RMC : Rabbit manures compost

Fig. 1: Porosities of purs compost

Simple Screened *Acacia cyanophylla* Compost

Fig. 2 shows the effect of simple screening techniques on the values of porosities (total porosity, aeration porosity and retention porosity).



(*) Means followed by the same letter are not significantly different at 5% level according to Duncan test.

(**) SS1: Simple screened compost using 6 mm mesh;
SS2: Simple screened compost using 8 mm mesh;
SS3: Simple screened compost using 12 mm mesh

Fig. 2: Porosities of substrates from simple screening methods

Total porosity is within the ideal range (TP ≥ 50%) for all types of meshes (6, 8 and 12 mm) used in simple screening technique.

According to aeration and retention porosities values, results revealed that when particle size increases (size of mesh used increases), the substrate air capacity increases and its water holding ability decreases and inversely. This observation is in

accordance with results of [18] who stated that the particle size distribution of a substrate is important because it determines pore space, bulk density, air and water holding capacities. An excess of fines clogs pores, increases non-plant-available water holding capacity and decreases air filled porosity.

The screened composts obtained from 6 mm and 8 mm meshes are within the norm for all porosities, however, in case of 12 mm mesh, retention porosity is insufficient and under the average (RP < 30%) and need to be optimized by adding more water retentive substrate to correct its water holding capacity.

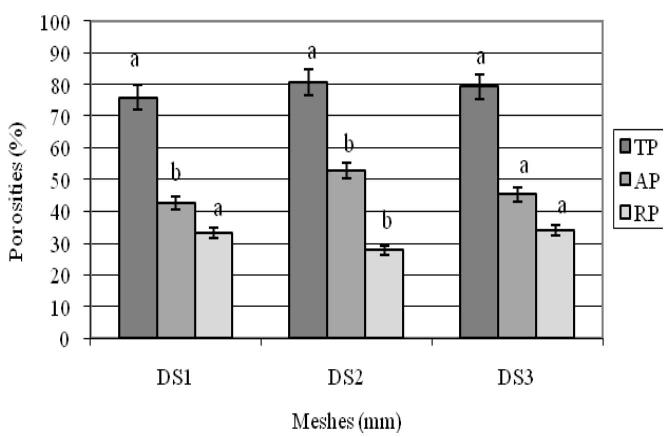
10 mm screening mesh should be tested in future. It seems to be the best; it could give screened compost with optimum aeration and retention porosities. Simple screening needs to be adopted to improve particle size distribution of forestry compost products (saving time and money compared to double or triple screening techniques).

For the substrates with coarse particles, the water penetration is relatively short compared to the fine-textured substrates [19].

It will be interesting to opt for the simple vibrating screen-grid (10 x 10) mm, because of the highest quality of underflow that can be produced and time and money saved [20].

Double Screened *Acacia cyanophylla* Compost

Fig. 3 shows the effect of double screening techniques on the values of porosities (total porosity, aeration porosity and retention porosity).



(*) Means followed by the same letter are not significantly different at 5% level according to Duncan test.

(**) DS1: Double screened compost using consecutively (6) and (8) mm meshes; DS2: Double screened compost using consecutively (8) and (12) mm meshes; DS3: Double screened compost using consecutively (6) and (12) mm meshes

Fig. 3: Porosities of substrates resulting from double screening

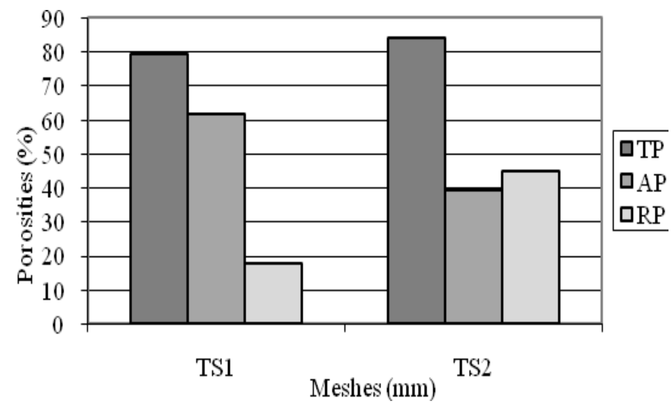
Total porosity (TP) and Aeration porosity (AP) are within the ideal range (TP ≥ 50% and AP ≥ 20%) for all types of meshes used: (6 and 8 mm), (8 and 12 mm) and (6 and 12 mm). Retention porosity is under the optimum range (RP < 30%) for double-screened composts using consecutively 8 and 12 mm meshes.

Double screening technique is less good than simple screening technique in terms of substrate water holding capacity and air capacity, so, it is not recommended to use it (losses in time of screening and cost).

The screening method used (single pass, second pass to screen the material initially sifted out or second pass to screen the sifted material twice) had a significant impact on the physical characteristics of the substrate obtained (total porosity, aeration and retention capacity) [21].

Triple Screened *Acacia cyanophylla* Compost

Fig. 4 shows the effect of triple screening techniques on the values of porosities (total porosity, aeration porosity and retention porosity). Total porosity (TP) and Aeration porosity (AP) are within the ideal range (TP ≥ 50% and AP ≥ 20%), however, retention porosity is under the norm (RP < 30%) when using consecutively (12), (8) and (6) mm meshes (TS1). So, it is not suggested to use triple screening technique because of the additional costs and time of work incurred in comparison to the simple screening technique (SST).



(*) TS1: Triple screened compost using successively (12) and (8) and (6) mm meshes. TS2: Triple screened compost using successively (6) and (8) and (12) mm meshes

Fig. 4: Porosities of substrates resulting from triple screening methods

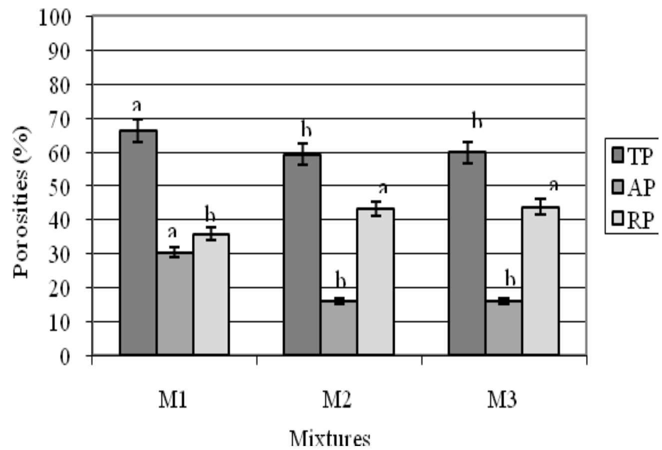
***Acacia cyanophylla* Compost and Rabbit Manures Compost Mixtures**

Fig. 5 shows the results of porosities (total porosity, aeration porosity and retention porosity) and Fig. 6 the change in total porosity and aeration porosity of the different mixtures of *Acacia cyanophylla* compost and rabbit manures compost.

All mixtures ratio (M1, M2 and M3) of *Acacia cyanophylla* compost and rabbit manures compost are in agreement with the nom of total porosity (TP ≥ 50%) and retention of porosity (RP ≥ 30%), however, the aeration porosity is below the range (AP < 20%) for mixtures M2 (1⁄2 RACC + 1⁄2 RMC) and M3 (25%

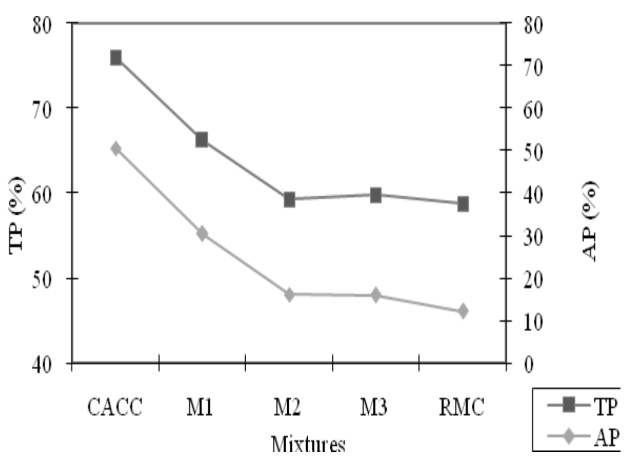
RACC + 75% RMC). We can say that the best mixture ratio between *Acacia cyanophylla* compost and rabbit manures compost is M1 (75% – 25%).

Fig. 6 shows that when the ratio of rabbit manures compost increases (from 25% to 75%) in substrate mixtures, retention porosity increases and aeration porosity decreases. From these findings, we can say that the two considered composts could be used in combination with supremacy of *Acacia cyanophylla* compost to provide a balance between substrate air capacities and water holding capacity.



(*) Means followed by the same letter are not significantly different at 5% level according to Duncan test.
(*) M1: 75% RACC + 25% RMC; M2: 50% RACC + 50% RMC; M3: 25% RACC + 25% RMC

Fig. 5: Porosities of substrates resulting from mixtures of rabbit manures compost and *Acacia cyanophylla*-based compost

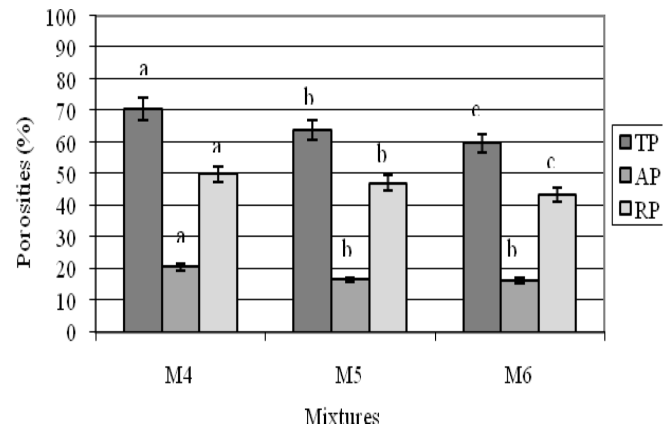


(*) M1: 75% CACC + 25% RMC; M2: 50% CACC + 50% RMC; M3: 25% CACC + 25% RMC; CACC : Crude *Acacia cyanophylla* compost

Fig. 6: Variation in total porosity and aeration porosity of substrates resulting from mixtures of rabbit manures compost and *Acacia cyanophylla*-based compost

Mixtures between SS3 and Rabbit Manures Compost

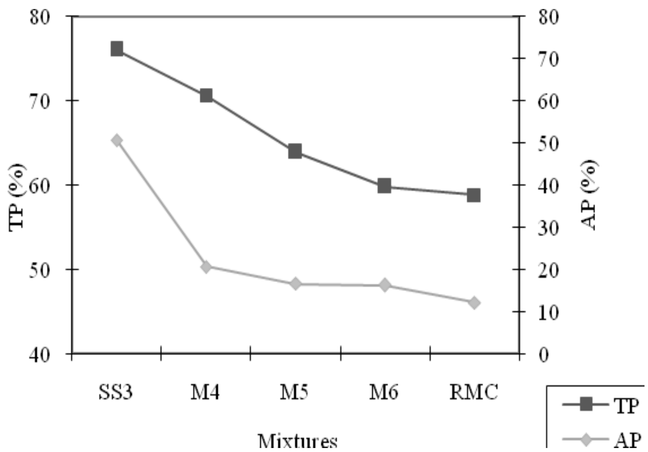
Fig. 7 shows the results of porosities (total porosity, aeration porosity and retention porosity) and Fig. 8 the variation in total porosity and aeration porosity of the different mixtures of 12 mm mesh screened *Acacia cyanophylla* compost (SS3) and rabbit manures compost (RMC). According to Fig. 8, total porosity and retention porosity are within the norm for all types of mixtures (M4, M5 and M6), however, aeration porosity is below the range (AP < 20%) for mixtures M5 and M6; which means that the increase in rabbit manures compost ratio mixture beyond 25% (or the decrease in SS3 ratio mixture away from 75%) decreases the air capacity of the final substrate (Fig. 8). We can say that the appropriate ratio mixture between SS3 and RMC is respectively 75% and 25%, which correspond to M4 mixture.



(*) Means followed by the same letter are not significantly different at 5% level according to Duncan test.
(**) M4: 75% SS3 + 25% RMC; M5: 50% SS3 + 50% RMC; M6: 25% SS3 + 75% RMC;
SS3: 12 mm mesh screened *Acacia cyanophylla* compost

Fig. 7: Porosities of substrates resulting from mixtures of rabbit manures compost and 12 mm mesh screened *Acacia cyanophylla* compost (SS3)

The gradual substitution of peat with increasing percentages (25, 50 and 75%) of Co-compost or compost significantly affects total porosity, aeration porosity and retention porosity of the resulting growth substrate. Results show that certain mixtures containing peat and Co-compost meets standards in term of porosities; however, those standards are not complied in term of the aeration porosity for the two following proportions: (50% peat + 50% Co-compost) and (25% peat + 75% Co-compost). If the incorporation of Co-compost with peat is not interesting beyond 25%, the partial substitution of peat with 50% screened forestry compost revealed encouraging results [22].



M4: 75% SS3 + 25% RMC; M5: 50% SS3 + 50% RMC; M6: 25% SS3 + 75% RMC; SS3: 12 mm mesh screened *Acacia cyanophylla* compost

Fig. 8: Variation in total porosity and aeration porosity of substrates resulting from mixtures of rabbit manures compost and 12 mm mesh screened *Acacia cyanophylla* compost

pH

Table 3 reports pH results of different growth substrates tested. pH of different substrate samples are within the optimum range (pH is close to neutral) which is appropriate to the assimilation of nutrients. It should be noted that there is a significant difference in pH between the tested substrates. The pH of the RACC was smaller than RMC. Substrate resulting from simple screening with 6 mm mesh has the lowest pH, while the substrate resulting from double screening using successively 12 mm and 8 mm meshes has the highest pH, in comparison with others screened composts, however, this pH remains lower than of Raw Acacia cyanophylla compost and Rabbit manures compost. Accordingly, screening significantly lowers pH of substrates; it's the same case for mixtures.

The pH values of the different substrates were in the optimum range of neutrality and this irrespective of the screening procedure or the nature of the mixture. However, [23], [24] showed that the relatively neutral pH of mature Acacia cyanophylla-based compost, combined with the poor quality of irrigation water could negatively affect the nutrient availability in the root plants. Indeed, irrigation water in nurseries are generally loaded with bicarbonate and carbonate, the gradual accumulation of those mineral in growth substrate cause an increase in pH, which has a direct effect on nutrient availability, even if they are present in the nutrient solution. This effect on nutrient availability can be explained by the appearance of marked symptoms of micronutrient deficiency particularly in woody and deciduous plant [24] [25]. At a pH of 7.5, the absorption of iron by the plant becomes very limited and for a pH above 8.5, growth medium becomes alkaline and the assimilation of Cu, Zn, Mn, Fe and N decreases gradually [26].

Tab 3: Growing media used in the study

Media	pH
RACC	6.7^{c}
CRMC	7.7^{a}
M1	6.0^{g}
M2	6.1^{fg}
M3	6.3^{de}
M4	6.2^{ef}
M5	6.4^{d}
M6	6.9^{b}

Organic Matter

Table 4 below reports the results observed in the rate of Organic Matter of different growth substrates. Results show that the rate of organic matter in different growth substrates is influenced by particle size obtained from simple or double screening. When particles size increases, organic matter content increases and inversely. The organic matter content is significantly lower in substrates resulting from a double screening using consecutively 12 mm and 8 mm meshes than from substrates resulting from double screening using repeatedly 8 mm and 12 mm meshes. Organic matter is significantly higher in substrates resulting from simple screening with 12 mm mesh and double screening using consecutively 8 mm and 12 mm meshes. From these findings, we suggest that screening operation leads to a loss in organic matters of substrates. The loss in OM was also noted in studied mixtures.

Nitrogen and C/N Ratio

Table 5 below reports results of Nitrogen content (N) and C/N ratio of different growth substrates tested. There were significant differences in terms of nitrogen contents and C/N ratio. The lowest nitrogen content was recorded in RACC while the highest rate was noted in rabbit manures compost. Concerning simple screened compost, the more the mesh used in screening is smaller, the more the rate of Nitrogen is lower. Screened compost resulting from double screening using consecutively 8 mm and 12 mm meshes has lower N content than screened compost resulting from double screening using repeatedly 8 mm and 12 mm meshes. M2 mixture (75% SS3 + 25% RMC) has better N content than M1 mixture (75% RACC + 25% RMC). Concerning C/N ratio, the highest value corresponds to the RACC, whereas the lowest was recorded in simple screened compost using 6 mm mesh.

Tab 4: Growing media used in the study

Media	Organic Matter (%)
RACC	64.3[c]
CRMC	67.2[a]
M1	60.6[h]
M2	61.4[g]
M3	63.0[f]
M4	63.8[e]
M5	53.6[i]
M6	65.0[b]

The non-availability of nitrogen for plants in growth substrate is one of the most important factors inducing crop loss [27]. According to [11], growth medium with low C/N ratio are not suitable, as they evolve over time through mineralization process, this lead to substrate subsidence, changes in porosity related to dry matter losses and clogging by fine particles. Competition for oxygen between microorganisms appears especially as the porosity decreases. According to [28], mature compost should have a C/N ratio between 8 and 15. These data was not in agreement with our results, which involve that the tested substrates are not yet ripe. It should be suggested that other factors were responsible for high values of C/N ratios. It is also evident to make mixture between rabbit manures compost, which is rich in minerals ions, and Acacia cyanophylla compost, which has low mineral elements contents, by respecting a good balance between the different elements.

Tab 5: Growing media used in the study

Media	Total Nitrogen (%)	C/N
RACC	1.23[g]	29.0[a]
CRMC	1.81[a]	20.6[g]
M1	1.76[b]	19.1[h]
M2	1.46[d]	23.3[e]
M3	1.45[d]	24.1[d]
M4	1.31[f]	27.0[b]
M5	1.37[e]	21.7[f]
M6	1.37[e]	26.3[c]

Conclusions

Physical and chemical proprieties are the mains qualities of a growth substrate, which are the subject of this study. From the results obtained, we can draw the following conclusions:

Raw *Acacia cyanophylla* compost is not a suitable substrate for containers forest seedling growth, especially because of its relatively poor water holding capacity, which justifies its screening or mixing with rabbit manures compost to improve its water capacity. Porosities (total porosity, aeration porosity and retention porosity) of substrates resulting from single, double or triple screening aren't always in the range of acceptance; however, simple screening have shown best results; therefore, double and triple screening aren't recommended. Rabbit manures compost should not be used alone because of its low aeration porosity; hence, it is interesting to mix it with *Acacia cyanophylla* compost, which has a high air capacity. According to our experiment, the best mixture ratio obtained is 75% *Acacia cyanophylla* compost (crude or screened) and 25% rabbit manures compost. Other ratios need to be tested in the future, such as 65% and 35% or 60% and 40%, respectively between *Acacia cyanophylla* compost and rabbit manures compost. According to chemical proprieties, *Acacia cyanophylla* compost is not a suitable substrate for forest seedling growth because of its poor carbone and nitrate content, which justifies its screening or mixing with rabbit manures compost to improve its nutritive proprieties. The best mixture ratio is 75% simple screened with 12 mm mesh Acacia compost and 25% rabbit manures compost.

Separate section, in accordance with research objectives, the conclusions should clearly state the main experimental results without using abbreviations, acronyms, or references. If the results have no implications, this fact should be mentioned.

Acknowledgements

The authors wish to thank the Rabbits Society of Sahel, Ksibet Sousse (Sousse, Tunisia) for providing composts. This study was supported by the High Institute of Agronomy, Chott Mariem (Sousse, Tunisia).

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