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# Effect of Substrate on the Growth of *Grewia coriacea* (Malvaceae) Seedlings

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MJ performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Author BAM realized the protocol and managed the analyses of the study. Author NJD managed the literature searches and corrected the protocol and author NL wrote the protocol of the study. Author Attibayeba designed the study. All authors read and approved the final manuscript.*

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## ABSTRACT

Domestication of *Grewia coriacea* Mast. (Malvaceae) threatened to disappear in Lefini Basin requires looking for vegetable growth conditions of their seeds. The study is initiated to evaluate the effect of the substrate on vegetative growth of the seedlings from germination of the pretreated stones. Whole fruits harvested on 5 trees choosed in natural forest and entire stones resulting from decortication are material used for germination tests. Obtained stones were immersed in boiling water and nitric acid at 65% as well as scarification of their teguments made by using an abrasive paper. The stones grew in pots containing sand and mould. The following parameters are evaluated for the resulting seedlings: stems height, collet diameters, plastochrone, number of leaves, internodes, ramifications and roots length. Height of seedling growing on the mould is higher than

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that of those developing on the sand whatever the treatments. Height of stems resulting from the whole fruits is inferior to 6.3 cm compared to untreated stones, stones immersed in nitric acid at 65% during 15 min and that the scarified stones on mould and sand. Collet of seedlings developing on sand has a diameter of 0.47 cm compared to that of the seedlings developing in the mould which is 0.43 cm. On the two substrates, seedlings resulting from scarified stones, entire stones and those resulting from stones treated in the nitric acid and developing on sand and mould which have the most leaves, internodes, ramification and roots length compared with that recorded on entire fruits. Stones immersed in 65% nitric acid during 15 min and from the scarified stones present better height of the stems, a more significant collet diameter, a high leaves number, internodes and the stems ramification. On mould, the seedlings resulting from stones treated present a better vegetative growth compared to those of the sand.

*Keywords: Germination; seedling; pretreated stones; sand; mould; Grewia coriacea; Congo.*

## 1. INTRODUCTION

*Grewia coriacea* Mast. (Malvaceae) is one of the approximately 280 species of the genus *Grewia* distributed in tropical areas including Africa, Asia, Oceania and Madagascar [1]. More recent studies reveal that the genus includes approximately 600 species and subspecies of trees of small size and shrubs with null and void leaves. In the Republic of Congo, [2] reported nine species in the catalogue of the vascular plants: *Grewia barombiensis* K Schum., *G. coriacea* Mast., *G. hookeiana* Exell & Mendonça (Syn: *Grewia africana* (Hook. F.) Mast., *G. mildbraedii* Burret. *G. oligoneura* Sprague, *G. pinnatifida* Mast., *G. rugosifolia* De Wild., *G. seretii* De Wild and *G. setulosa* Mast. Of these species, *Grewia coriacea* is the only species which produces edible fruits for humans [3]. Fruits are whether sold in heap, bags, buckets or as drinks and generate additional incomes for harvesters and retailers otherwise a commercial value. Apart from the small consumers or seasoned fruits seller, transformation of fruits into bottled juice and other types of drinks is carried out by associations such as Gamboma Production and Primitaf [4]. However, these fruits rarefying because *Grewia* is characterized by a regressive situation resulting from an overexploitation, a considerable pressure exerted by rural populations and from a mode of fruits picking which is done by stripping or pure and simple cutting of the tree. This strong pressure prevents their natural regeneration. In Léfini basin where *Grewia coriacea* grows, it is noted a rarefaction or the absence of the seedlings in underwood of this fruit tree of the spontaneous Congolese flora [5,6,7,8,9]. In addition to the difficulties of seeds germination of *G. coriacea* in the underwood, the rare seedlings resulting from this regeneration have a poor vegetative growth. This does not make it possible to obtain viable trees, which leads to the difficulty of the reconstitution of the natural population. For its domestication, knowledge relating to the vegetable growth of *G. coriacea* appears necessary to avoid its extinction in Léfini basin of Congo. The present study is initiated to evaluate the effect of the substrate on the vegetative growth of the *G. coriacea* seedlings from germination of the pretreated stones for domestication.

## 2. MATERIALS AND METHODS

### 2.1 Material and Experimental Dispositive

*Grewia coriacea* seedlings come from germination of whole mature fruits. The mature fruits are dark red and were collected in Léfini basin of Congo, on five trees selected beforehand at the beginning of flowering. After collection, they are immersed in a bucket containing tap water in order to eliminate the nonviable supernatants. The viable fruits were set out in two lots:

Lot 1: whole fruits, i.e. with epicarp, mesocarp and endocarp;
Lot 2: fruits removed their epicarp and their mesocarp using a scalpel in order to obtain the stones.

The stones are in their turn divided into three sublots:

Sublot 1: 150 stones (control);
Sublot 2: 150 stones immersed in the 65% nitric acid during 15 min then rinsed abundantly with tap water during 10 minutes to eliminate the traces from nitric acid;
Sublot 3: 150 stones whose each endocarp is manually rubbed several times on the level

the zone of dehiscence of the stones in order to thin the teguments.

The 150 whole fruits and 150 pretreated stones are sown individually in pots filled with the ¾ with sand or mould to appreciate the aptitude for germination. These sowings are carried out in three completely randomized blocks including 25 constituent pots the experimental unit. In total, 150 whole fruits and 150 pretreated stones are respectively dumped in pots containing sand and the mould. Thus, 50 vigorous seedlings resulting from these germinations are used for the evaluation of the growth parameters.

This study was conducted in the site of scientific city forest (ex ORSTOM) in Brazzaville (4°16'42.4''S; 15°14'23.6''E). The site covers an area of 22.48 ha [10,11] and the altitude is 321 m. The ORSTOM forest ilot is mesophile and belongs, according to the phytogeographical point of view, to the natural area of Léfini. The climate is "equatorial of transition" and belongs to the type bas-congolais [12] and is characterized by two seasons: dry season from June to September and a rainy season from October to May, alternating with a small dry season from December to February marking a light deceleration of the rains. Monthly average temperatures lie between 26 and 27°C approximately. The annual average calculated from 1951 to 1970 gives 24.9°C. The thermal variations annual are weak and do not exceed 5°C. April and March seems the hottest months, July and August are regarded as freshest. One notes two types of seasons: one season dries. Annual rainfall average is about 1200 to 1500 mm.

### 2.2 Effect of Pretreated Stones on Seedlings Growth

The influence of the pretreated stones is observed on the growth in nursery of 50 seedlings chosen on a population of 150 stones sown in the mould and sand by treatment. The seedlings growth is followed over a three-month period and the following parameters are evaluated: stems height, collet diameters, plastochrone, number of leaves, internodes number, ramifications numbers and the roots length.

The stem height is measured once a week using a mm-graduated ruler from the collet to the insertion point of the final bud. The collet diameter is measured once a week using a slide caliper starting from the 119$^{th}$ day after sowing. The root length is measured at the 151$^{th}$ days after sowing using a mm-graduated ruler. From the 88$^{th}$ day after sowing, the leaves number, the number of internodes and the ramifications number are counted by seedling once a week. In addition to this counting, measurement of stem height, the collet diameter is carried out after the blooming of the leaves, the apparent plastochrone which is the interval of time which separates the appearance of two successive leaves out of the final bud at a given seedling [13] is observed. A leaf appeared when its apex is visible [14,15].

### 2.3 Statistical Analyses

Six variables are measured on each seedling resulting from untreated stone germination, scarified stones and stones immersed in 65% nitric acid during 15 min. The variables are: stems height, collet diameter, leaves number, internodes number, ramifications number and roots length. The software XLSTAT version 7.5.3 is used for all statistical analyses. In order to standardize the distributions and to equalize the variances concerning the influence of the pretreated stones on the growth of seedlings resulting from germination. All these variables underwent a transformation square. Comparison of the averages using ANOVA was carried on the variables according to the test of Student Newman-Keuls to the threshold of risk 5%.

## 3. RESULTS

### 3.1 Effects of the Pretreated Stones on the Stems Height

Stems height measured on the seedlings resulting from germination of the whole fruits, the untreated stones, scarified stones and treated stones by immersion in 65% nitric acid and growing on sand and mould is shown on Fig. 1. Analysis of these results reveals that the height growth is not uniform over all the experimentation period (14 weeks). This growth, slow during the first 5 weeks, regularly increases from the 5$^{th}$ to the 14$^{th}$ week. It is noticed however that in general, the height of seedlings growing on the mould substrate is relatively higher than that of those developing on the sand substrate whatever the treatments. Except for the stems height of stones treated with nitric acid where during the 3$^{rd}$ and the 4$^{th}$ week, the height of stem developing on sand, with 1.6 cm and 1.9 cm respectively, is appreciably higher than those

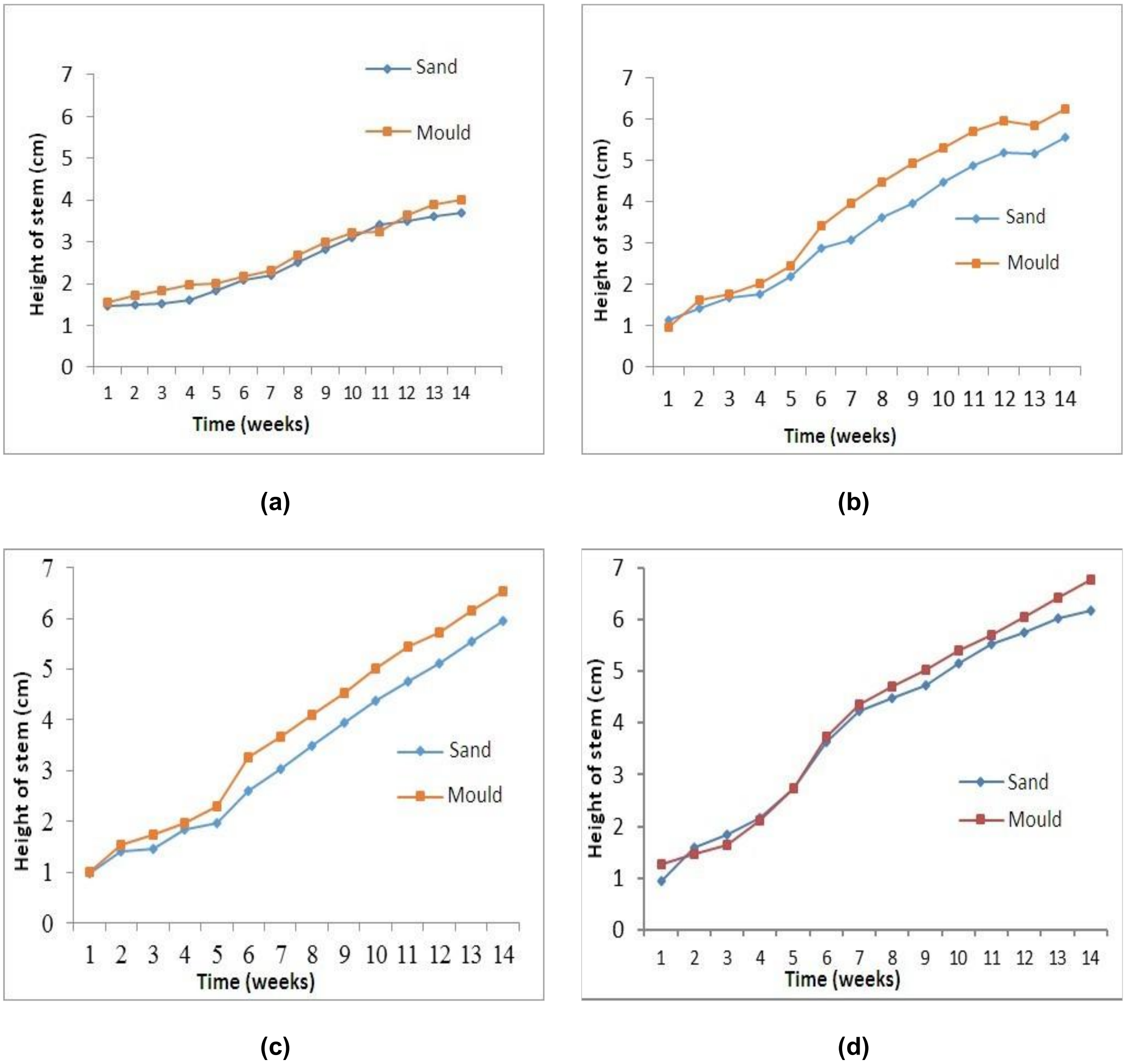



**Fig. 1. Variation of *Grewia coriacea* seedlings stems height resulting from seeds germination: whole fruits (a), untreated stones (b), scarified stones (c), stones immersed in 65% nitric acid (d) and developing on the substrates of sand and mould**

developing on the mould which have 1.5 cm and 1.6 cm respectively (Fig. 1b). At the 14$^{th}$ week for germination of whole fruits, seedlings developing on mould performed better than those on sand 4 cm of height against 3.7 cm respectively (Fig. 1a). This is also the case for seedlings resulting from germination of untreated whole stones developing on mould and sand with 6.2 cm high and 5.6 cm respectively (Fig. 1b); for seedlings heights resulting from germination of stones scarified with 6.5 cm (mould) against 6 cm (sand) (Fig. 1c); for seedling height resulting from germination of stones immersed in nitric acid with 6.8 cm (mould) against 6.2 cm (sand) (Fig. 1d). Seedlings resulting from germination of whole fruits develop approximately twice less than all the other cases.

Comparison of these averages shows that the height of stems resulting from the whole untreated stones, stones immersed in 65 % nitric acid during 15 min and that the scarified stones are not statistically different on mould (Table 1). No direct relation is established between the height of stems resulting from various treatments and the time after seeds germination on mould. This result reveals that the stones immersed during 15 min in 65% nitric acid significantly improve the seedlings growth on sand. That is confirmed by the analysis of the variance consigned in Table 1. There is a significant difference on seedlings growth obtained from the third week. The seedlings growth is more discriminatory at 5, 6, 7, 8, 13 and 14th week after seeds germination on sand.

### 3.2 Effects of the Pretreated Stones on the Collet Diameter of Seedlings

Fig. 2 illustrates the evolution of collet diameter of stems resulting from the whole stones, stones immersed in nitric acid and scarified stones. This result shows in general that the development of the collet is better for seedlings developing on sand compared to those developing on mould. Indeed, at the end of 7 weeks of development, the collet diameter of seedlings resulting from germination of whole fruits and developing on sand is 0.42 cm whereas that of the seedlings developing on mould is 0.41 cm (Fig. 2a). It is the same for that for the seedlings resulting from whole stones developing on sand with 0.43 cm against that from the seedlings developing on mould with 0.42 cm (Fig. 2b) and seedlings resulting from scarified stones developing in sand with 0.43 cm against that of the seedlings developing on mould with 0.43 cm (Fig. 2c).These differences are more perceptible on seedlings resulting from stones treated with nitric acid where one notices than, at the 7th week of development, the collet of seedlings developing on sand have a diameter of 0.47 cm compared to that of the seedlings developing in the mould which is 0.43 cm (Fig. 2c). But, in all the cases, they are the seedlings resulting from whole fruits which have the lowest collet diameters.

Table 2 presents the comparison of the average values of collet diameters of stems according to the treatments. The results of the statistical analyses show that the average diameter varies significantly according to the treatment and time after seeds germination on sand. This result shows that from the 6th to the 7th week after the germination of untreated whole stones and stones immersed during 15 in nitric acid significantly improve the collet diameter of seedlings on sand (Table 2). However, no significant difference between the treatments on the average diameter of collet seedlings on mould. Except the stones immersed in nitric acid, no direct relation is established between the collet diameter of stems resulting from various treatments and the time after seeds germination on mould (Table 2).

### 3.3 Effects of the Pretreated Stones on the Number of Leaves

Fig. 3 shows that the seedlings resulting from whole fruits give only few leaves (approximately 4 to 5 leaves) either for the seedling developing on sand or those developing on the mould (Fig. 3a) at the end of 10 weeks while in other cases, there is a good foliar production. Seedlings resulting from scarified stones and those

**Table 1. Comparison of average values the seedlings stem height resulting from germination of *Grewia coriacea* seed and developing on substrates**

| Time (Week) | Sand | | | Mould | | |
|---|---|---|---|---|---|---|
| | Untreated stones | Scarified stones | stones immersed in nitric acid | Untreated stones | Scarified stones | Stones immersed in nitric acid |
| 1 | 1.150a | 0.983a | 0.943a | 0.975a | 1.000a | 1.257a |
| 2 | 1.420a | 1.409a | 1.586a | 1.618a | 1.550a | 1.475a |
| 3 | 1.670ab | 1.474a | 1.839b | 1.757a | 1.746a | 1.636a |
| 4 | 1.778a | 1.848a | 2.168a | 2.023a | 1.971a | 2.103a |
| 5 | 2.205ab | 1.961a | 2.737b | 2.447a | 2.309a | 2.748a |
| 6 | 2.865a | 2.611a | 3.628b | 3.431a | 3.259a | 3.737a |
| 7 | 3.036a | 3.043a | 4.219b | 3.962a | 3.676a | 4.367a |
| 8 | 3.616a | 3.480a | 4.484b | 4.473a | 4.092a | 4.705a |
| 9 | 3.952a | 3.947a | 4.738a | 4.930a | 4.535a | 5.029a |
| 10 | 4.468a | 4.391a | 5.159a | 5.311a | 5.006a | 5.405a |
| 11 | 4.880a | 4.754a | 5.526a | 5.700a | 5.443a | 5.698a |
| 12 | 5.196a | 5.124a | 5,751a | 5.966a | 5.712a | 6.059a |
| 13 | 5.148a | 5.554ab | 6.033b | 5.858a | 6.159a | 6.424a |
| 14 | 5.556a | 5.961a | 6.18b | 6.235a | 6.524a | 6.778a |

*The values on the same line affected of the different letters are statistically different with the threshold from 5% according to the test of Newman and Keuls (p 0,05).*

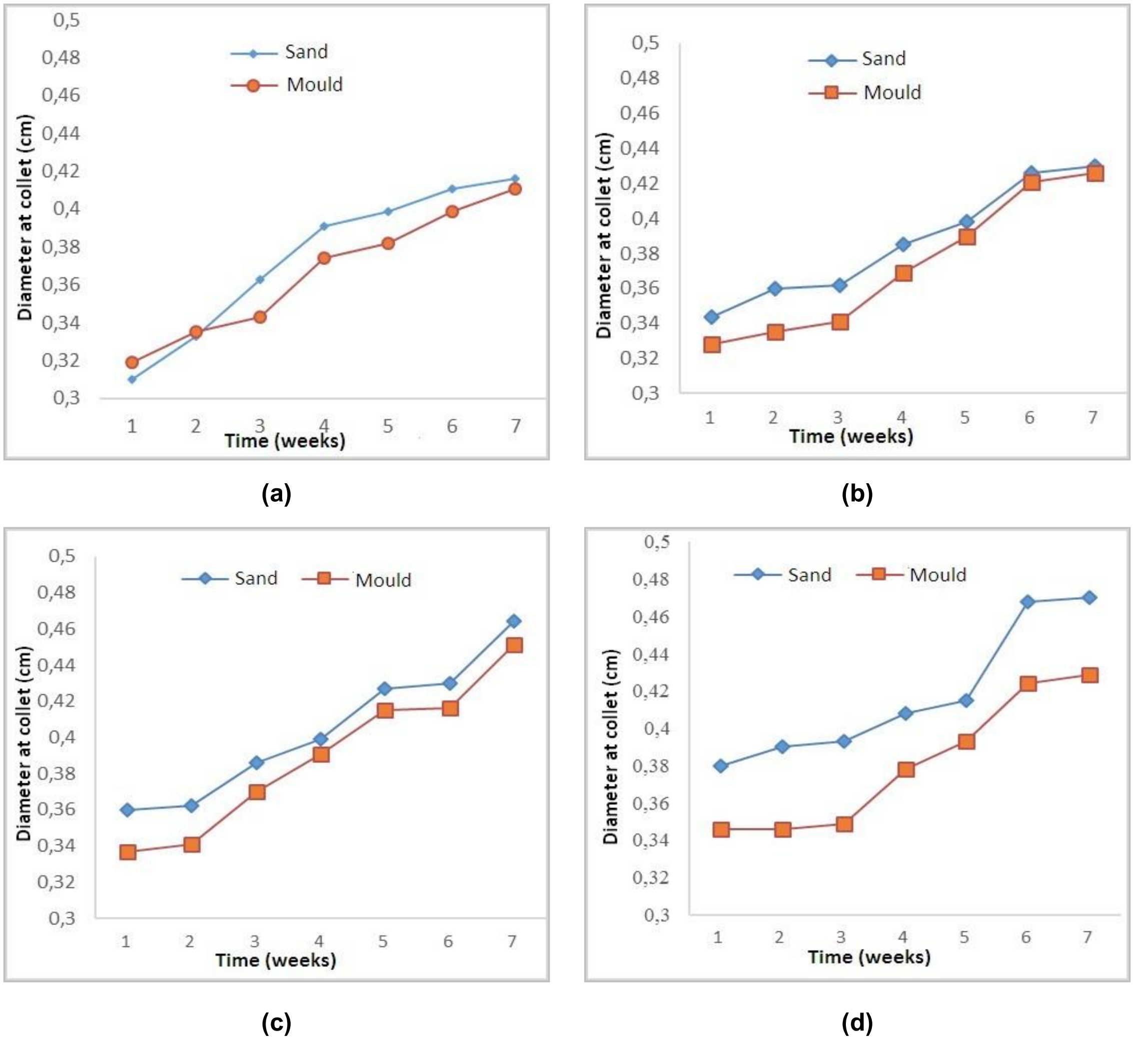



**Fig. 2. Variation of *Grewia coriacea* seedlings collet diameter of stems resulting from seeds germinations: whole fruits (a), untreated stones (b), scarified stones (c), stones immersed in 65% nitric acid (d) and developing on sand and mould**

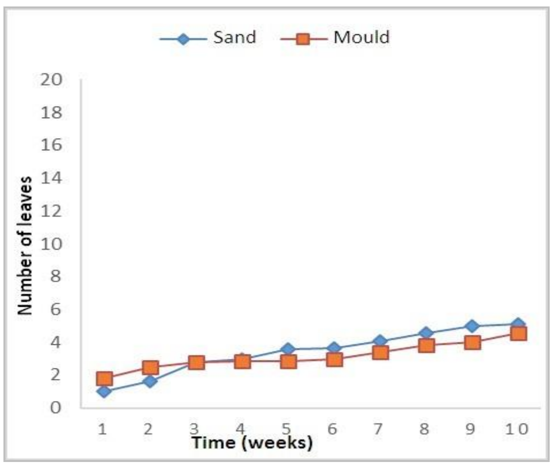
resulting from stones treated in the nitric acid and developing on sand which have the most leaves (approximately 15 to 16 leaves) at the end of 10 weeks (Fig. 3c-d). Contrarily, it is in the category of untreated whole stones that seedlings developed on mould gave more leaves (approximately 12) compared to those which developed on sand (approximately 10 leaves) as shown in Fig. 3b.

Analysis of the seedlings in the mould revealed no significant statistical difference (with the threshold of 5%) of the average leaves number produced according to the stones treated and the various times after the germination of seeds except for 1$^{st}$, 2$^{nd}$, 4$^{th}$ and 5$^{th}$ week. No close connection between the average leaves number released at the seedlings resulting from stones treated and time after germination except for 1$^{st}$, 2$^{nd}$, 4$^{th}$ and 5$^{th}$ week (Table 3). The mould does not make it possible to discriminate between the average emitted leaves number and stones treated. On sand, however, the results of statistical analyses show that the average leaves number counted by seedling varies significantly (with the threshold of 5%) according to untreated stones, stones scarified and stones immersed in the 65% nitric acid and various times after seeds germination. This result also reveals that the stones immersed in 65% nitric acid as well as stones scarified significantly improve the released leaves number on various times after stones germination (Table 3).

**Table 2. Comparison of the average values the collet diameter (cm) of seedlings stems resulting from seeds germination of *Grewia coriacea* Mast. and developing on sand and mould**

| Time (Week) | Sand | | | Mould | | |
|---|---|---|---|---|---|---|
| | Untreated stones | Scarified stones | Stones immersed in nitric acid | Untreated stones | Scarified stones | Stones immersed in nitric acid |
| 1 | 0.344a | 0.344a | 0.380a | 0.328a | 0.328a | 0.346a |
| 2 | 0.360a | 0.360a | 0.390a | 0337a | 0.335a | 0.346a |
| 3 | 0.362a | 0.362a | 0.393a | 0.341a | 0.341a | 0.349a |
| 4 | 0.386a | 0.385a | 0.408a | 0.370a | 0.369a | 0.378a |
| 5 | 0.399a | 0.398a | 0.415a | 0.391a | 0.390a | 0.393a |
| 6 | 0.427a | 0.426a | 0.468b | 0.415a | 0.421a | 0.424a |
| 7 | 0.430a | 0.430a | 0.470b | 0.416a | 0.426a | 0.429a |

*The values on the same line affected of the different letters are statistically different with the threshold from 5% according to the test of Newman and Keuls (p0,05)*

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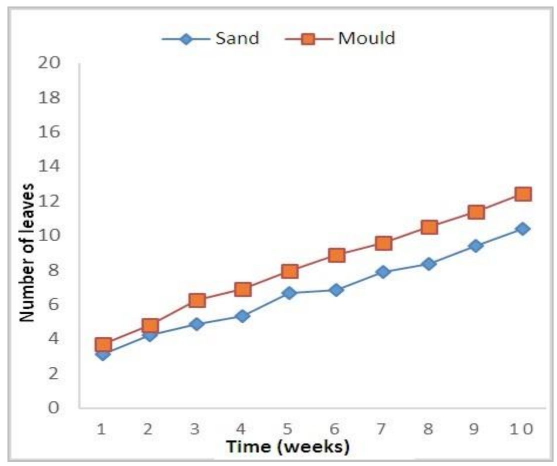


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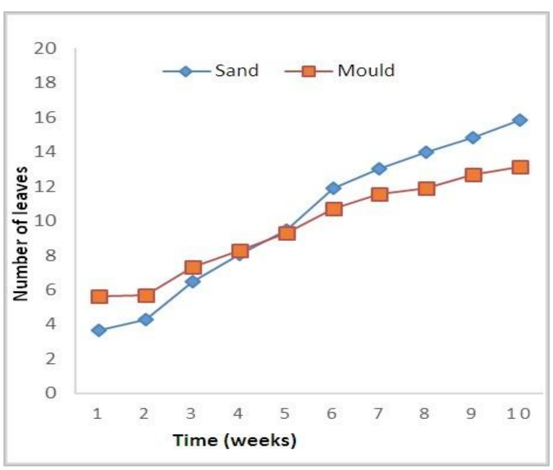


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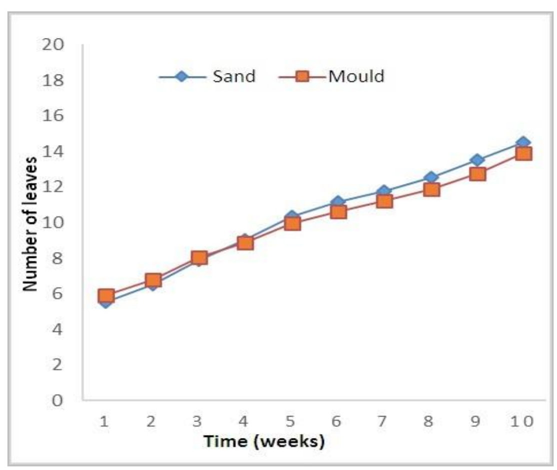



**(d)**

**Fig. 3. Variation of *Grewia coriacea* seedlings leaves number resulting from seeds germination: Whole fruits (a), untreated stones (b), scarified stones (c), stones immersed in 65% nitric acid (d) and developing on sand and mould**

**Table 3. Comparison the average values of leaves number for seedlings resulting from seeds germination of *Grewia coriacea* Mast. and developing on sand and mould**

| Time (week) | Sand | | | Mould | | |
|---|---|---|---|---|---|---|
| | Untreated stones | Scarified stones | Stones immersed in nitric acid | Untreated stones | Scarified stones | Cores immersed in nitric acid |
| 1 | 3.100a | 3.667ab | 5.522b | 3.696a | 5.615b | 5.933b |
| 2 | 4.200a | 4.267a | 6.529b | 4.806a | 5.715ab | 6.774b |
| 3 | 4.867a | 6.467ab | 7.912b | 6.244a | 7.346a | 8.032a |
| 4 | 5.316a | 8.067b | 9.029b | 6.922a | 8.308ab | 8.871b |
| 5 | 6.651a | 9.467b | 10.324b | 7.944a | 9.269ab | 9.968b |
| 6 | 6.863a | 11.900b | 11.147b | 8.893a | 10.714a | 10.645a |
| 7 | 7.906a | 13.000b | 11.765b | 9.569a | 11.536a | 11.226a |
| 8 | 8.333a | 13.950b | 12.529b | 10.517a | 11.893a | 11.903a |
| 9 | 9.379a | 14.850b | 13.529b | 11.373a | 12.679a | 12.742a |
| 10 | 10.379a | 15.850b | 14.529b | 12.424a | 13.107a | 13.903a |

*The values on the same line affected of the different letters are statistically different with the threshold from 5% according to the test of Newman and Keuls (p0,05)*

### 3.4 Effects of Pretreated Stones on Duration of Apparent Plastochrone

Table 4 illustrates the apparent plastochrone duration of young seedlings resulting from untreated whole stones, stones immersed in nitric acid and the stones scarified. The study of the foliar production for *Grewia coriacea* Mast. shows that the first two leaves emergent simultaneously 11 days after the germination of the stones pretreated followed by the 3rd leaf 10 days later. It is only after appearance of the 3rd leaf that the apparent plastochrone duration becomes more or less regular, and it is approximately 10 days.

**Table 4. Average apparent plastochrone duration of *Grewia coriacea* Mast**

| Leaves number | Date | Day number |
|---|---|---|
| 1st et 2nd – 3th | 20 nov. – 11 dec. | 21 |
| 3th – 4th | 11 dec. – 21 dec. | 10 |
| 4th – 5th | 21 dec. – 30 dec. | 9 |
| 5th – 6th | 30 dec. – 10 jan. | 11 |
| 6th – 7th | 10 jan. – 22 jan. | 12 |

### 3.5 Effects of Stones Pretreated on Number of Internodes

Fig. 4 shows for seedlings resulting from germination that the number of internodes increases during the development of these seedlings, and that whatever the substrate. It is noticed however that at the end of 10 weeks, the seedlings resulting from whole fruits have only approximately 3 internodes (Fig. 4a), i.e. three times less than the other seedlings. The high number of internodes (10) is recorded in seedlings resulting from stones scarified and developing on mould followed by those resulting from stones treated in nitric acid which have approximately 9 internodes (Fig. 4c) and those resulting from untreated whole stones which have approximately 8 internodes (Fig. 4b).

Table 5 presents the comparison of the average values of internodes number according to stones treated or not and the substrate. The results of the statistical analyses reveal that the average internodes number counted by seedling varies significantly (with the threshold of 5%) according to stone untreated, stones scarified and stones immersed in 65% nitric acid and various times after seeds germination on sand. 7th to the 10th week after seeds germination on sand, the result shows that stones immersed in 65% nitric acid as well as scarified ones significantly increase the internodes number (Table 5). On the contrary, the analysis of seedlings in the mould revealed, in the statistical plan, no significant difference (with the threshold of 5%) of the average internodes number counted according to the treatment and time after seeds germination on substrate (Table 5).

### 3.6 Effects of Stones Pretreated on Number of Ramifications

Fig. 5 shows seedlings resulting from stones treated in nitric acid with approximately three branches (Fig. 5d) ramify more and whatever the substrate on which they develop. The seedlings resulting from stones whole and those resulting from stones scarified and developing on sand or mould carry two branches (Fig. 5b-c). Seedlings resulting from fruits whole, however, hardly ramify and that whatever the substrate on which these seedlings developed (Fig. 5a).

Table 6 presents the comparison of the average values of ramification number per seedling according to stones treated or not and the substrate. Except the 1st and 2nd week for the stones scarified and immersed in 65% nitric acid, the results of the statistical analyses show that the average ramification number counted by seedling does not vary significantly (with the threshold of 5%), according to stones untreated, stones scarified and stones immersed in 65% nitric acid and various times after stones pretreated germination on the two substrates, sand and mould (Table 6).

### 3.5 Effects of Cores Pretreated on Roots Length

Fig. 6 shows well that the seedlings resulting from stones were deep-rooted and that whatever treatment and substrate on which these seedlings developed (Fig. 6). One notices, however, that the roots of seedlings resulting from whole stone measure 16.6 cm long, those of the seedlings resulting from stones scarified and stones treated in nitric acid have 15.4 cm and 15.1 cm in length respectively. Root elongation observed on fruits whole is relatively weak (4.3 cm) on the two substrates compared with that recorded on cores.

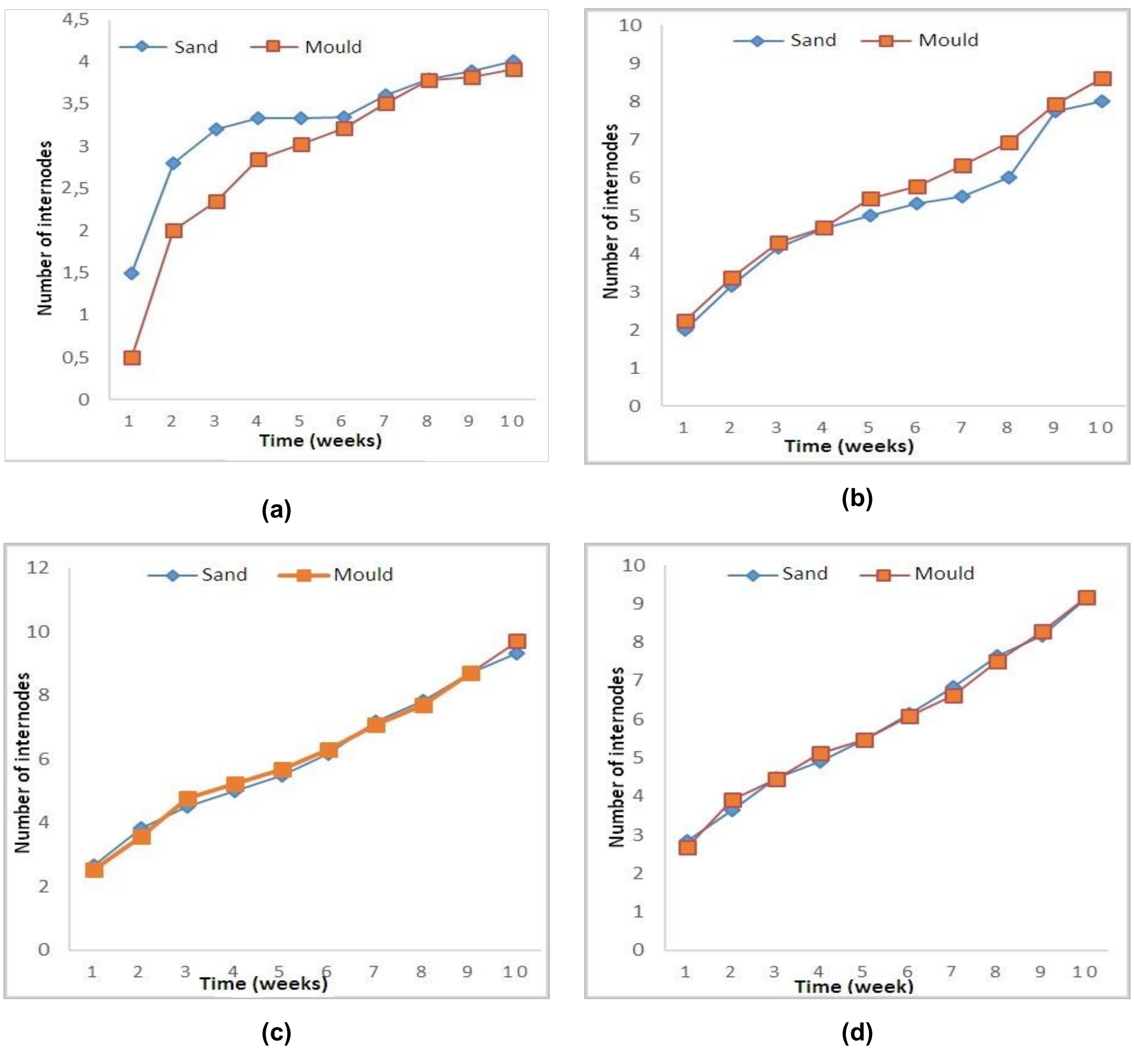
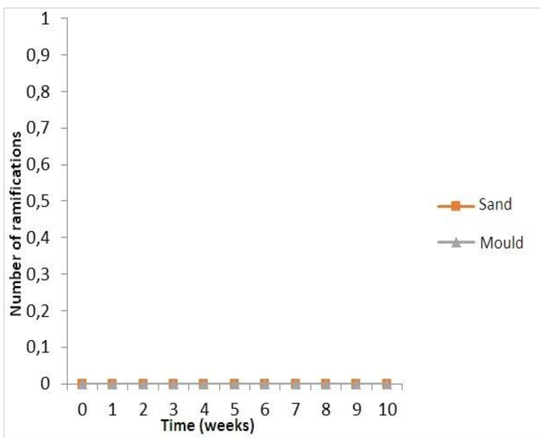



**Fig. 4. Variation of *Grewia coriacea* seedling internodes number resulting from seeds germinations: Whole fruits (a), untreated stones (b), scarified stones (c), stones immersed in 65% nitric acid (d) and developing on sand and mould**

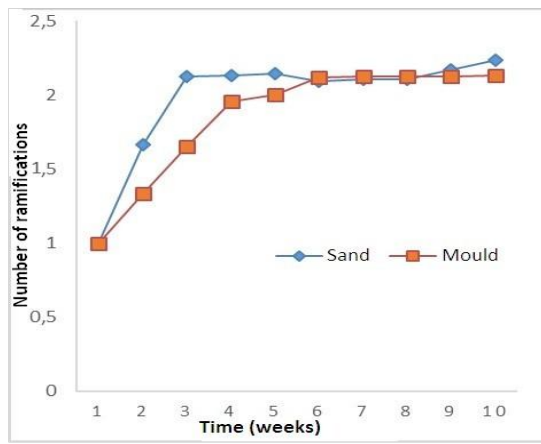
**Table 5. Comparison of the average values of internodes number seedlings resulting from seeds germinations of *Grewia coriacea* Mast. and developing on sand and mould**

| Time (week) | Sand | | | Mould | | |
|---|---|---|---|---|---|---|
| | Untreated stones | Scarified stones | Stones immersed in nitric acid | Untreated stones | Scarified stones | Stones immersed in nitric acid |
| 1 | 2.000a | 2.667a | 2.826a | 2.231a | 2.538a | 2.667a |
| 2 | 3.167a | 3.833a | 3.652a | 3.385a | 3.583a | 3.923a |
| 3 | 4.167a | 4.500a | 4.478a | 4.308a | 4.769a | 4.458a |
| 4 | 4.667a | 5.000a | 4.913a | 4.692a | 5.231a | 5.125a |
| 5 | 5.000a | 5.500a | 5.478a | 5.462a | 5.692a | 5.458a |
| 6 | 5.333a | 6.167a | 6.130a | 5.769a | 6.308a | 6.083a |
| 7 | 5.500a | 7.167b | 6.826ab | 6.308a | 7.077a | 6.625a |
| 8 | 6.000a | 7.833b | 7.652b | 6.923a | 7.692a | 7.500a |
| 9 | 7.333a | 8.692b | 8.174ab | 7.923a | 8.692a | 8.292a |
| 10 | 8.000a | 9.333b | 9.130b | 8.615a | 9.692a | 9.167a |

*The affected values of the different letters are statistically different with the threshold from 5% according to the test of Newman and Keuls (p.0,05).*




**(a)**

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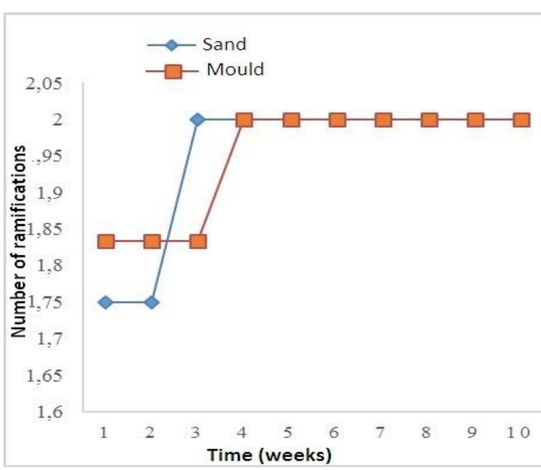
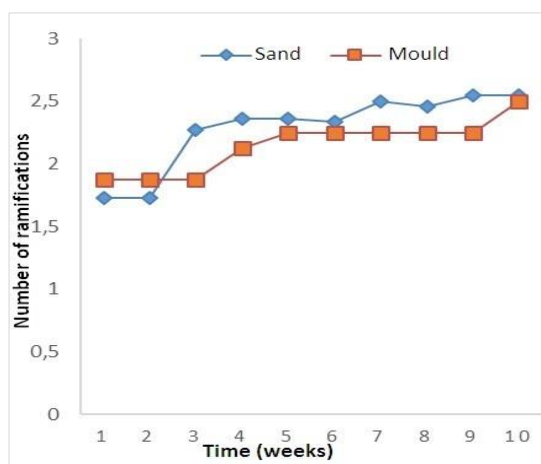



**(c)**




**(d)**

**Fig. 5. Variation of *Grewia coriacea* seedlings branches number resulting from seeds germinations: whole fruits (a), cores untreated (b), cores scarified (c), cores immersed in 65% nitric acid (d) and developing on sand and mould**

**Table 6. Comparison of ramifications number average per seedling according to seeds treated of *Grewia coriacea* Mast. and substrate of germination**

| Time (week) | Sand | | | Mould | | |
|---|---|---|---|---|---|---|
| | **Whole fruits** | **Scarified stones** | **Stones immersed in the acid** | **Whole fruits** | **Scarified stones** | **Stones immersed in the acid** |
| 1 | 1.000a | 1.750a | 1.727a | 1.000a | 1.833b | 1.875b |
| 2 | 1.667a | 1.750a | 1.727a | 1.333a | 1.833b | 1.875b |
| 3 | 2.125a | 2.000a | 2.273a | 1.650a | 1.833a | 1.875a |
| 4 | 2.000a | 2.000a | 2.364a | 1.960a | 2.000a | 2.125a |
| 5 | 2.146a | 2.000a | 2.364a | 2.000a | 2.000a | 2.250a |
| 6 | 2.091a | 2.000a | 2.333a | 2.121a | 2.000a | 2.250a |
| 7 | 2.107a | 2.000a | 2.500a | 2.105a | 2.000a | 2.250a |
| 8 | 2.059a | 2.000a | 2.455a | 2.125a | 2.000a | 2.250a |
| 9 | 2.171a | 2.000a | 2.455a | 1.974a | 2.000a | 2.250a |
| 10 | 2.237a | 2.000a | 2.545a | 2.053a | 2.000a | 2.500a |

*The affected values of the different letters are statistically different with the threshold from 5% according to the test of Newman and Keuls (p 0.05).*

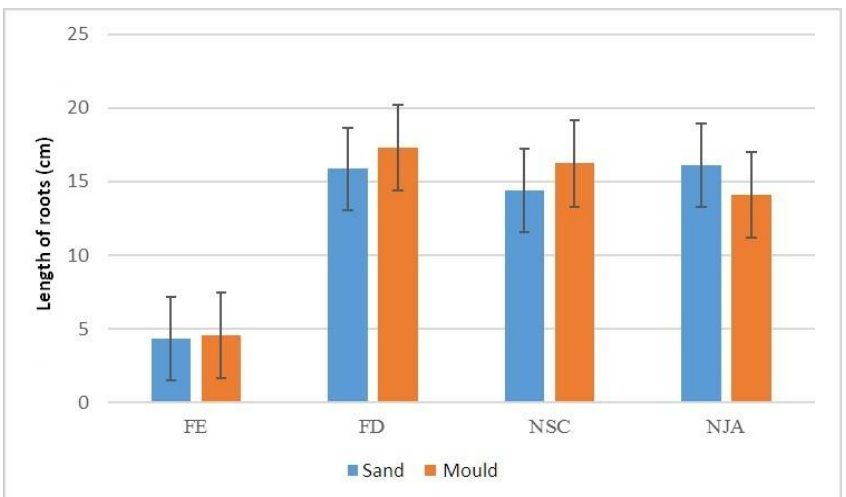



**Fig. 6. Variation of *Grewia coriacea* seedlings roots length resulting from germinations cores untreated, cores scarified and cores immersed in 65% nitric acid (d) and developing on sand and mould**

(*FE: whole fruits; FD: peeled fruits; NSC: scarified stones; NIA: stones immersed in the acid)*

Table 7 presents the statistical analysis comparison of the averages roots length according to treatment reveals that there is no significant difference between the treatments. Indeed, with the threshold of 5%, one notes that there is no statistically significant difference between the treatments the analysis of the variance also shows that the substrates do not affect the roots growth.

## 4. DISCUSSION

Behavioral study of the young seedlings resulting from fruits peeled and fruits or stones germinated enabled us to highlight the effect of the treatment and the nature of the substrate on their development. Indeed, the substrate made up of the mould appears favorable to the seedlings growth of *Grewia coriacea*. This substrate ensured a good development of seedlings. The similar results were obtained by [6] who reported the beneficial effect of mould on aspect qualitative of woody seedlings produced in nursery. The growth parameters were followed on seedlings resulting from stones pretreated as well as whole or peeled fruits in sand and mould. After germination, the first two leaves of the seedlings emergent simultaneously at the end of

11 days on the two substrates. On these seedlings, the foliar rhythm of release is approximately 10 days. It reported on this subject that, whatever the length of the cutting, at the young seedlings of *Grewia coriacea* Mast., the first leaves appear between 8 to 14 days, and apparent plastochrone varies between 3 and 4 days. This difference could represent the mode of multiplication used. It is possible that as the plant develops, the apparent plastochrone duration reaches values close to those obtained by this author. The plastochrone by seedlings moves from a duration of 21 days between the first two leaves and the third leaf to 10 days between third and the fourth leaf.

**Table 7. Comparison of the average roots number per seedling according to seeds treated of *Grewia coriacea* Mast. and substrate germination**

| Treatment | Substrate | |
|---|---|---|
| | Sand | Mould |
| Untreated stones | 15.858a | 17.241a |
| Scarified stones | 14.400a | 16.228a |
| Stones immersed in nitric acid | 16.121a | 14.076a |

In our study, it was shown that the best performances in term of growth in stem height, collet diameter, leaves numbers and roots length are observed on the seedlings resulting from stones immersed in nitric acid. The immersion in acid nitric accelerated germination as the results on the speed and the time of germination indicates it. The similar results are observed by [16]. The latter proved that the pretreatment in nitric acid would influence the development of the seedlings by decreasing the latency time of this one while eliminating from the possible physical obstacles or mechanics with their development. This seedling growth can be also explained by the fact that the acid might have broken the teguments. This crack would enable the future seedling to use less reserves at the time of the tegumentary opening, from where fast growth and a larger strength of the seedling. This observation joined that of several authors in particular [17], [18] who think that the largest or heavy seeds generally generate more vigorous seedlings which grow and resist the risks of the medium better. Thus, these results can allow us to say that the immersion in acid nitric would facilitate water to reach the stones, accelerates germination and supports the activation of the respiratory and mitotic processes necessary to the formation and the growth of the bodies of the seedling. However, the seedlings resulting from whole fruits germination have poor performances in terms of growth in stem height, collet diameter, leaves numbers and roots length. This weak performance would result from an exhaustion of the nutritive elements by the seedling before the opening of all seed teguments. This exhaustion would reduce the strength of the seedling impacting on its later growth on various substrates. These results corroborate those of [19]. In addition to nitric acid and the whole fruits, the performance in terms of growth of the seedlings resulting from the peeled fruits and fruits scarified are almost identical on sand and mould. These seedlings resulting from the stones treated as well as whole fruits, have a continuous growth, with little marked periods of rest. This period is characterized by the absence of the formation of axillary branches during the first 3 months. Comparison of the seedlings growth on two substrates reveal that the mould (black cotton soil and broken up dead sheets) appears definitely more favorable to the development of the seedlings of *Grewia coriacea* Mast than sand. The mould ensured a good seedlings growth. A good seedlings growth on mould can be explained by the fact that this substrate seems to be more or less natural. It is of this fact laid out with the good root anchoring and the development of the seedlings by the presence of the various nutritive elements. Our results are similar with those obtained by [20] working on *Ricinodendon heudelotui* (72% of germination) and [21] on *Coula edulis* (75% of germination) like by [22] on *Xylopia aethiopica* (60% of germination). In the same way, the beneficial effect of this black cotton soil mixture and broken up dead sheets were observed by [11] and [23]. Our results showed also that the growth of the roots at *Grewia coriacea* Mast. would depend on the composition on the substrate. The best growth root rates of the seedlings were recorded in mould. On this substrate, the presence of the nutritive elements and oxygen would support the root elongation. Oxygen can have a significant role in the root development. These results are concordant with those obtained by [24] showing that the absence or the lack of ventilation involves the mortality of the roots and would cause attacks by fungi. Sand proved to be the substrate which accelerates germination but does not support the root development stones during the experimentation. Indeed, sand would not contain enough nutritive elements compared to the mould for the development of the seedlings.

## 5. CONCLUSION

Seedlings resulting from stones treated present a better vegetative growth compared to those of the whole fruits. On sand, the more vigorous seedlings are those resulting from the stones immersed in 65% nitric acid during 15 min and from the scarified stones. These seedlings present better height of the stems, a more significant collet diameter, a high leaves number, internodes and the stems ramification. For the recolonisation of the natural surfaces where *Grewia* rarefies, the seedlings with strong vegetable growth potential exits of the stones immersed in nitric acid and stones scarified are recommended. It requires obtaining the vigorous seedlings in nursery, by the pretreatment of seeds for the domestication of *Grewia*. On mould, the seedlings resulting from stones treated present a better vegetative growth compared to those of the sand.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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