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Research Article

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Germinative performance of seeds of 14 species of medicinal plants

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ABSTRACT

In order to estimate the germinative performance of seeds of medicinal plants an experiment was performed with a pretreatment of seeds *in vitro* culture. The effect of pretreatments (Soaking in water and sulfuric acid "SA") on the GC and *in vitro* germinative performance of the seeds of fourteen medicinal plants namely: *Nigella sativa*, *Lupinus mutabilis*, *Ricinus communis*, *Glycine max*, *Peganum harmala*, *Lepidium sativum*, *Hyoscamus muticus*, *Petroselinum crispum*, *Anacyclus valentinus*, *Ajuga iva*, *Salvia hispanica*, *Sesamum indicum*, *Eruca sativa* and *Portulaca oleracea* were studied.The seeds of the plants studied are germinated in the soil firstly and in a Murashig and Skoug (MS) culture medium. Germination performance (germination capacity"GC" and germination rate"GR") were measured for each treatment. The results obtained showed that the pretreatments make it possible to demonstrate the germination, and to determine the optimal pretreatments for each species studied. *L. sativum*, *E. sativa*, *R. communis*, *N. sativa*, *H. muticus*, *P. harmala*, *P. crispum* are germinating species easy in the soil, while *L. sativum* and *P. harmala*, *N. sativa*, *L. mutabilis*, *R. communis* are species that require pretreatment with soaking in water. While soaking in SA is necessary for germination of *H. miticus* and *P. crispum*. In MS culture medium, the best GR were observed for all seeds except *A. valentinus*, *A. iva*, *P. harmala* and *S. indicum*. The species studied showed a varied behavior with respect to pretreatments at the time of their germination.

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INTRODUCTION

According to estimates, 80% of the world's population still depend on medicinal plants for different treatments. Plant production and the establishment of good agricultural crops are closely dependent on seed germination, which is a crucial step in the life cycle of higher plants [1]. However, germination can be heterogeneous because the seeds do not germinate in the same way or at the same time. In medicinal plants, the seed ensures reproduction, it is most often a resistance organ capable of waiting a very long time. The germination corresponds to the transition from a slower state of life to an active state of life, whereas reserves that until then had ensured the residual metabolism of the embryo will be actively metabolized to ensure the growth of the seedling [2]. The increase in the germinative performance of seeds of medicinal plants would allow defining their germinative capacity. As well as a pretreatment used to facilitate seed germination during *in vitro* culture. Pretreatments are for the removal of dormancy that is done naturally or artificially. During germination tests in the laboratory, it is conceivable that when one type of dormancy is lifted, another one appears. On the other hand, the seeds do not go from the dormant state to that of "ready to sprout" brutally. Rather, it is thought that, gradually, the seeds of a population become more receptive to the range of environmental conditions to which they are able to germinate and less sensitive to the range of conditions that impede their germination [3]. Our work consists in determining the effect of pretreatments (soaking in water and in SA acid) on the *in vitro* capacity and germinative performance of seeds and to have, *in vitro*, responses to preliminary seed treatments. Define appropriate pretreatments for the species studied and reduce latency, optimize the percentage and speed of seed germination.

MATERIALS AND METHODS

Plant Material (Seeds and Plants)

The seeds used in our tests are seeds come from the herbalists of M'sila in January 2018 (Figure 1). They were kept in paper bags with a tag with the name of the species.

Germination in the Soil (Pots)

To get an idea of the GC of the seeds of the studied species, a direct sowing of seeds in the substrate (Soil of best growth type serving as

Figure 1: Species studied (seeds)

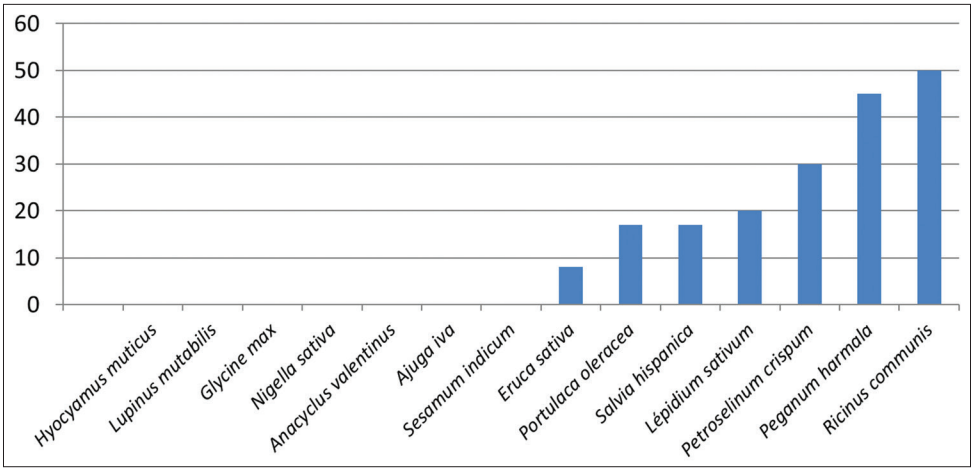

Figure 2: Number of seeds germinated in the soil of the studied species after 60 days

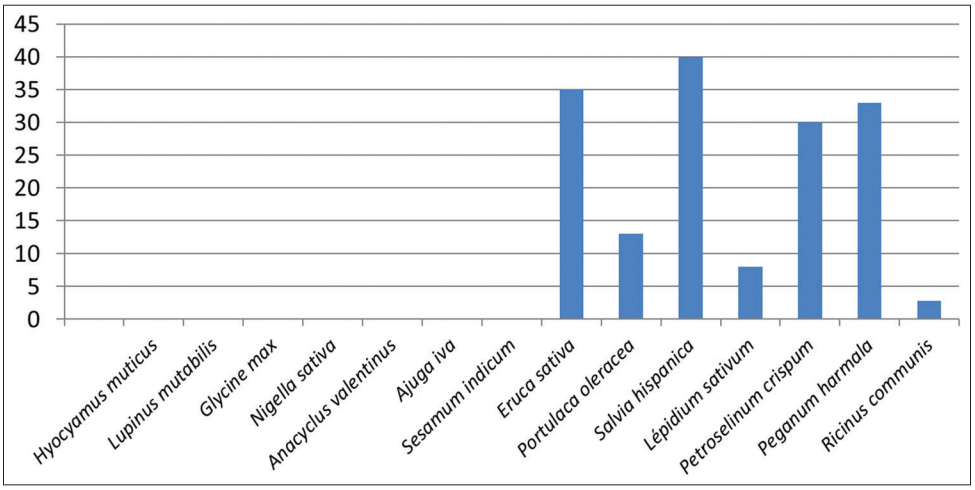

Figure 3: Germination time in the soil of the different species studied

germiners like Nitrogen (N) phosphate (P2O5) Potassium (K2O) with animal wastes and plants) was also studied. The seeds were sown at a depth equal to 1 to 2 times their diameter. 14 plastic pots are used for each species. Seeding was carried out in plastic boxes (7 cm wide, 15 cm long and 4 cm high) open, drilled at the bottom and filled with soil. Each bin is identified with a plastic tag bearing the origin of the seeds. We did a daily count of the seedlings that had emerged. Sprouted seeds are torn off to avoid double counting. The emergence corresponds to the appearance of a seedling with two cotyledonary leaves. Growth monitoring of young seedlings lasted 60 days. During the experiment, watering was done every day. The variables observed were the germination time (Days) and the number of germinated seeds (60 days).

Germination Tests

Germination in petri dishes

In order to understand the germinability of the seeds of the species studied, there is a certain degree of dormancy, and if pretreatment of the seeds can influence or improve germinability, we have done four pretreatments:

- A batch of seeds is germinated directly without dipping (without treatments).
- The second batch of seeds is imbibed in distilled water for 1h at room T.
- The third batch stays for 24 h in distilled water at room T.
- A fourth chemical treatment for the seeds was provided by soaking in a concentrated solution of SA acid H2SO4 for 5 min.

The seeds are thus thoroughly rinsed with sterile distilled water. Following this operation, the seeds are germinated in petri dishes lined with paper soaked in distilled water at a rate of 15 to 80 seeds per box depending on the size of the seeds. Firstly, arrange one or two layers of blotting paper in the bottom of the petri dishes, then moisten with a spray bottle or pipette and be careful not to soak it, when returned, the water should not flow, at risk of seed rot. (From the moment when there is a medium saturated with moisture, there is a risk of rot). After place the seeds to be tested in petri dishes, lined with a paper soaked in 20 ml of distilled water. They must not touch each other (space of three times the size of the seed at least). The dishes are placed in an incubator set at (25 ° C ± 2 ° C) T [4]. Then, moisten the paper towels every two days or so (when the paper starts to desiccate) taking care not to soak it. It is considered that a seed germs when the radicle pierces the integument [5,6].

Germination in culture medium

Since the young root only uses cotyledonary reserves, to provide nutrients to the culture medium, we used the nutrient medium of Murashige and Skoog [7].

All metal instruments (forceps, tips, scalpels...) or glassware (beakers, culture tubes, petri dishes...) are coated with aluminum foil, and are put in an oven at a temperature of 170 ° at 200 ° C for 2 h of time. During the manipulations the metal instruments are immersed in 70% alcohol and then burned in the Benzene burner to burn the alcohol. The manipulations are carried out under horizontal laminar flow hood (cleaned with 70 ° alcohol), sterilized with a UV lamp. The seeds are sterilized by soaking in 70% ethanol for 70 seconds and then transferred into a solution of sodium hypochlorite (NaOCl) 6% for 15 min and rinse with sterile distilled water three successive times for 10 min. Min each time. The seeds are thus placed in a petri dish in a solution of sterile distilled water for seeding.

Seeding of the Seeds

Referring to the results obtained during the first experiment (germination test in a petri dish), only the pretreatments of seeds suitable for each species subjected to culture in MS medium, were chosen. Seeding of pretreated seeds is done in MS medium previously poured into sterile flasks (25 ml of culture medium in each box) at the rate of 3 seeds per flask (Table 1).

The flasks were placed in an oven at 25 ± 1 ° C in the dark, after resumption of development (the radicle pierced the cuticle), the flasks will be transferred to the chamber of culture (23 ± 2 ° C), 16/8 photoperiod).

RESULTS AND DISCUSSION

Germination in the Soil (Pots)

The results obtained for the germination time and the number of germinated seeds after 60 days appear on the (Figures 5 and 6). Seeds that responded positively germinated at least after 8 days (E. sativa) and at maximum after 50 days (R. communis) and between the two times for *P. oleracea*, *S. hispanica*, *L. sativum*, *P. crispum* and P.

It is noted that the best germination times in soil are for *E. sativa*. After 60 days of germination we recorded the data in (Figure 4). It is noted that the best GRs are recorded for the species *S. hispanica*, and the low rate was for *R. communis*. While the seeds of *Hyoscyamus muticus*, *A. iva*, *S. indicum*, *L.m utabilis*, *A. valentinus*, *N. sativa*, and *G. max* that they submit to soil do not record any germination during the test (60 days).

Table 1: Pretreatment of seeds before culture in MS medium

Species	Pretreatment	Methods
A. valentinus	Without pre-treatments	
A. iva		
S. hispanica		
S. indicum		
E. sativa		
P. oleracea		
N. sativa	Soaking in distilled water	During (24h)
L.m utabilis		
R.com munis		
G. max		
P. harmala		During (1h)
L. sativum		
Hyosyamus muticus	Tremper 5 min dans l'acide sulfurique (H2SO4)	
P. crispum		

Figure 4: Germination results in the soil of the different species studied

In accordance with our observations and those made by Werker [8] for seeds sown in the soil (pots containing the potting soil) A.*valentinus*, A.*iva*, S.*indicum*, N. *sativa*, L. *mutabilis*, G. *max*, H. *muticus*. We have noticed a lack of germination, which can be explained by the hardness of the integuments' seed, which has not undergone any pretreatments. This performance confirmed the results in the other tests (germination in the molded dishes and in the MS medium)., the seeds of S. *hispanica*, E. *sativa*, P. *oleracea*, R. *communis*, L. *sativum*, P. *crispum*. P. *harmala* are well germinated with a long germination time that can reach 50 days compared with *in vitro* germination.

Germination of Seeds in Petri Dishs

The objective of this step is to define the effect of treatment and genotype on the seed response of the species studied to germination. The results of the comparison of seed GRs for the four treatments are presented in the Figures 5 and 6.

The comparison between the 4 treatments for the 14 species revealed a difference for germination. *P. boleracea*, *S. hispanica and E. sativa*, show the highest seed GR and this without preliminary seed treatment (control), with a rate of more than 60% and 3.5% for A. valentinus compared to other species that do not. did not germinate (Figure 5). For preliminary treatment with soaking in water for 24 h, except *R. communis*, *G. max*, *L. mutabilis*, *N. sativa*, *P. crispum* which germinated (0.45 and 4.75%) (Figure 5). Seeds of the species *N. sativa*, *P. harmala*, *L. sativum*, *R. communis* germinated but with seed soaking treatment in water for 1 h, with high GRs for *L. sativum*, *P. harmala* (10.5 and 62.4 %), and low for *R. communis* and *N. sativa* (0.15 and 0.75%) (Figure 5). The species *Hyocyamus muticus*, *N.sativa*, *P. crispum* and *G. max* revealed a GR after treatment with soaking in *SA acid*. Note that *Hyocyamus muticus* shows the highest rate (56%) while *N. sativa*, *P. crispum* and *G. max* show low rate (between 0.45 and 1.2%) (Figure 5). From the results obtained, comparison of the effect of preliminary seed treatments on germination showed that soaking in water

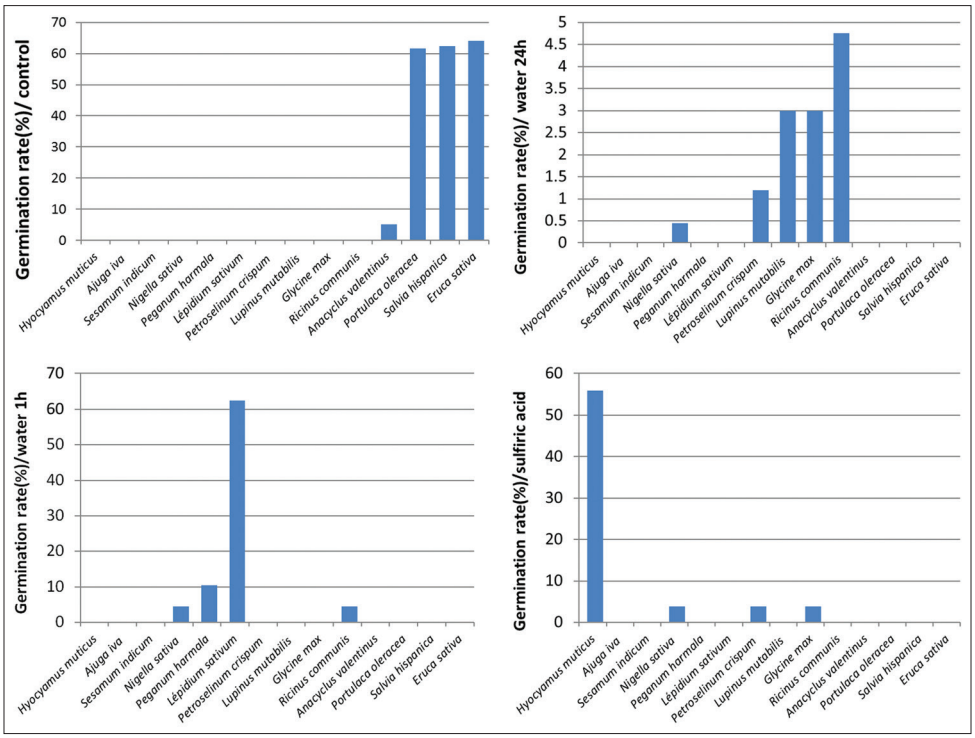

Figure 5: Comparison of seed germination rate in Petri dishes for the 14 species studied

for 24 h was effective for the species studied. The comparison between the 4 treatments for the 14 species revealed a difference for germination. *P. boleracea*, *S. hispanica* and *E. sativa*, show the highest seed GR and this without preliminary seed treatment (control) (more than 60% and 3.5%) for A. *valentinus* compared to other species that did not germinate (Figure 5). For preliminary treatment with soaking in water for 24 h, except *R. communis*, *G. max*, *L. mutabilis*, *N. sativa*, *P. crispum* which germinated (between 0.45 and 4.75%) (Figure 5). Seeds of the species *N. sativa*, *P. harmala*, *L. sativum*, *R. communis* germinated but with seed soaking treatment in water for 1 h, with high GRs for *L. sativum*, *P. harmala* (10.5 and 62.4). %), and low for *R. communis* and *N. sativa* (0.15 and 0.75%) (Figure 5). The species *Hyocyamus muticus*, *N.sativa*, *P. crispum* and *G. max revealed* a germination after treatment with soaking in SA acid. Note that *Hyocyamus muticus* shows the highest rate (56%) while *N. sativa*, *P. crispum* and *G. max* show low rate (between 0.45 and 1.2%) (Figure 5). From the results obtained, comparison of the effect of preliminary seed treatments on germination showed that soaking in water for 24 h was effective for the species studied.

The calculated GR for the four treatments (control, soaking in water (24 h/1 h), and with SA acid) allowed us to plot the curves of the GR as a function of time (Figure 7). According to the Figure 7, the germination of seeds of *E. sativa* without treatments (control) is manifested during the second day of sowing with a speed of 60 germinated/days and increased gradually until reaching 80 germinated/days in the seventh day, and the same kinetics almost for *S. hispanica* and *P. oleracea*. While for *A. valentinus*, seed germination occurs on the third day and increases to a maximum of 8 sprouts/days on the seventh day. On the other hand no seeds germinated for the other species. The results obtained for the seed GR of the studied species as a function of time for pretreatment batches with soaking in water for 24 h appear in the Figure 8. The seeds of L. mutabilis appear at the first day with a speed of 1 germinated/days and quickly increased to 8 sprouts/day on the second day, consequently they arrive at 11 sprouts/day to the fifth day. The same kinetics was recorded for the species G. max. while the seeds of *N. sativa* recorded a speed of 2 germinated/day in the third day which remained constant until the fifth day then increases gradually until reaching 3 sprouted/days on the seventh day. For the germination of *R. communis* seeds, are started to germinate on the second day of sowing and increased sharply at a rate of 10 germinated/days until 18 sprouts/days arrive at the end of the test. For *P. crispum* there was delayed germination on the third day with a very weak rate on the fifth day (2 germinated/days), this percentage of germination remains variable as a function of time and reaches up to 8 germinated/days in the seventh day. The other seeds soaked in water for 24 h showed no germination. The results concerning the germination speed of the species studied for pretreated batches with soaking in water for 1 h appear in Figure 9). The treatment of *Lepiduim sativum* seeds by soaking in water for 1 h gave a high number of sprouted seeds (60) on the second day until the fourth day with 78 sprouts/seeds, these rates remained stable until the end of the test. But for *P. harmala*, no seed germinated during the first five days of germination until the seventh day, when we notice 21 sprouted seeds. Also *P. crispum* seeds have a low GR for the last four days (4 sprouted/day). And the same results were almost recorded for *N. sativa*. No seeds sprouted every day for seeds of other species. Figure 10. shows the seed GR of the species studied for soaking in SA acid for 5 min. It is noted that seed germination of *H. miticus* starts on the first day with low speed and then increases gradually to about 11 germs/day at the

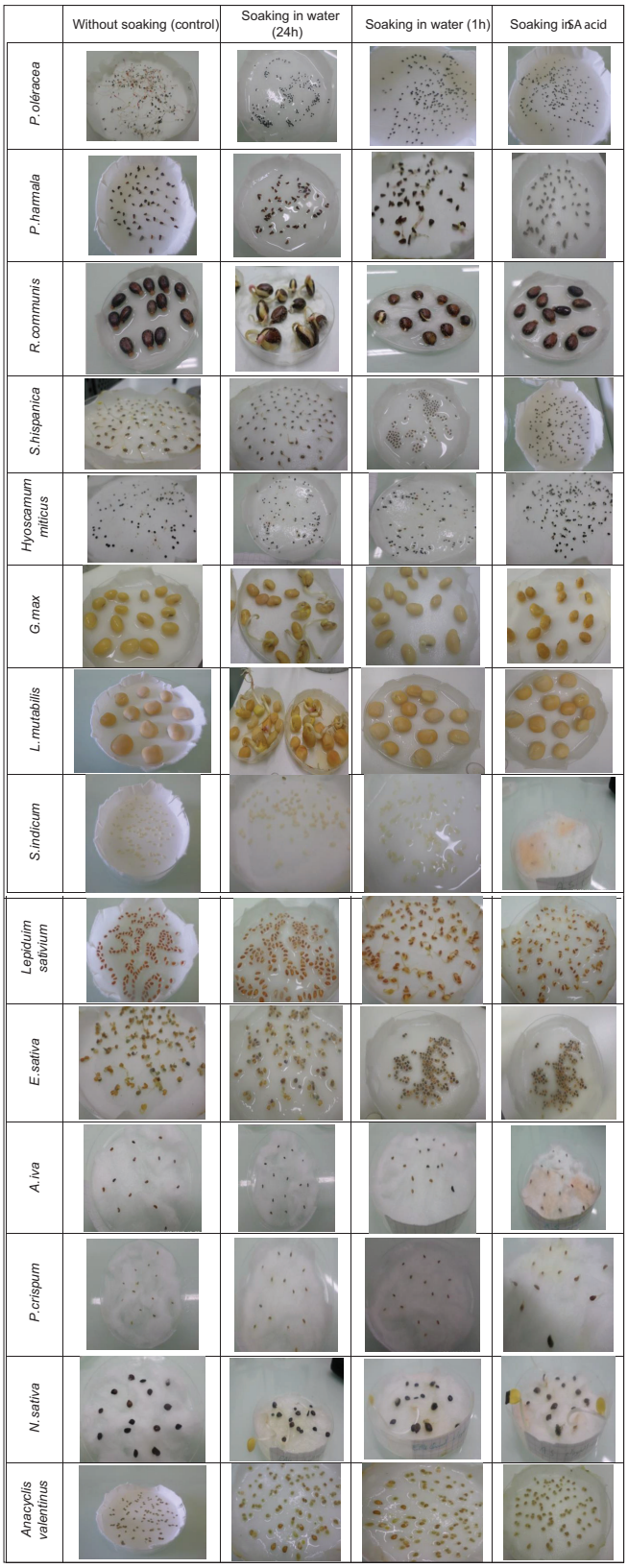

Figure 6: Results of seed germination in the Petri dishes for the four pretreatments of the 14 studied species

end of the test. For seeds of *Pertoselinum crispum*, germination starts after the fourth day and increases to 8 seeds germinated on the seventh day. While for *N. sativa* seeds, the GR is low, less than 1 germinated/day at day 4 and remains stable until the end of the test (1 seed germinated). While for G. max, germination begins after day 5 and 2 sprout/day is seen from day 7. Seeds of other species showed no germination throughout the trial period.

Precocity of Germination

From the results of the GR, early germination was recorded in *E. sativa*, *S. hispanica* and *P. oleracea* for controls (no treatment). Also for soaking in water for 24 h, we see precocity of germination that has been recorded in *L. mutabilis*, G. max, *R. communis* and *N. sativa*. While for soaking in water for 1 h, except the species Lepiduim sativum that has precocity of germination. Same thing for soaking in SA acid for 5min, except *H. miticus* which has precocity of germination. The germination tests carried out on the seeds of the species studied under different pretreatments show an absence or presence of germination, this germination performance is linked to the expression of optimal conditions of germination [9]. For the *A. iva* and *S. indicum* shows a zero germination performance, this raises several hypotheses such as: a low viability of the tested lots and this may be due to the harvest problem or this lack of germination can This is explained by the hardness of the seed coat, thus preventing the development of the embryo and the emergence of the radicle. The degree of dormancy differs from one species to another [10]. For all the species studied for which morphological dormancy was assumed, this is the case of *A. valentinus*, *S. hispanica*, *E. sativa*, *P. oleracea* these are annuals we have shown that to have a fast and homogeneous germination of the seeds of A. valentinus, *S. hispanica*, *E. sativa*, *P. oleracea* in the case of control (without pretreatments). Those seeds that have been pretreated before sowing have shorter waiting times than those that have not been treated. With regard to germination time, the study also showed that short germination times are obtained with treated seeds. We suspected a physiological dormancy on which *N. sativa*, *L. mutabilis*, *R. communis*, *G. max*, *P. harmala*, *L. sativum*, the soaking time of seeds in water depends on the thickness and hardness of the seed coat. In fact, the use of water in the context of this study not only reduced the delay between sowing and first germination, the germination time of the seeds but also increased the GR. For the seeds *P. harmala*, *L. sativum* soaked in water for 1 h have a GC significantly higher than untreated seeds. Thus the soaking of the seeds in the water favors the speed of germination and has made it possible to extract the inhibitors which act on the development of the radicle. At the level of the seed, the albumen and the integument may be the potential cause of this type of dormancy because they can store the inhibitors [11]. As indicated, the unscreened seeds of N. sativa, L. mutabilis and R. communis not treated even at a long incubation period of 7 days did not germinate, same result for treatment with SA acid, indicating the inhibitory effect of the integument making them impermeable to water, a phenomenon typical of legume species. This corroborates the results of Venier *et al.*[12] (2012) who reported that non-scarified seeds of leguminous trees did not show imbibition or germination because of the hardness of the integument. The most effective treatment is immersion in water for 24 h. According to Dracup *et al.* [13], shows that water

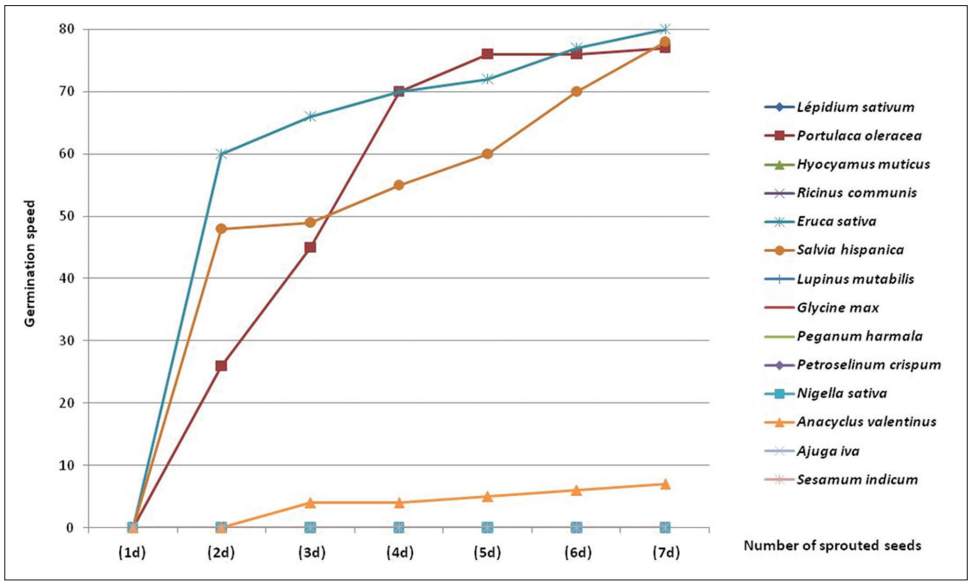

Figure 7: Germination rate of seeds of the species studied for the controls (without treatments)

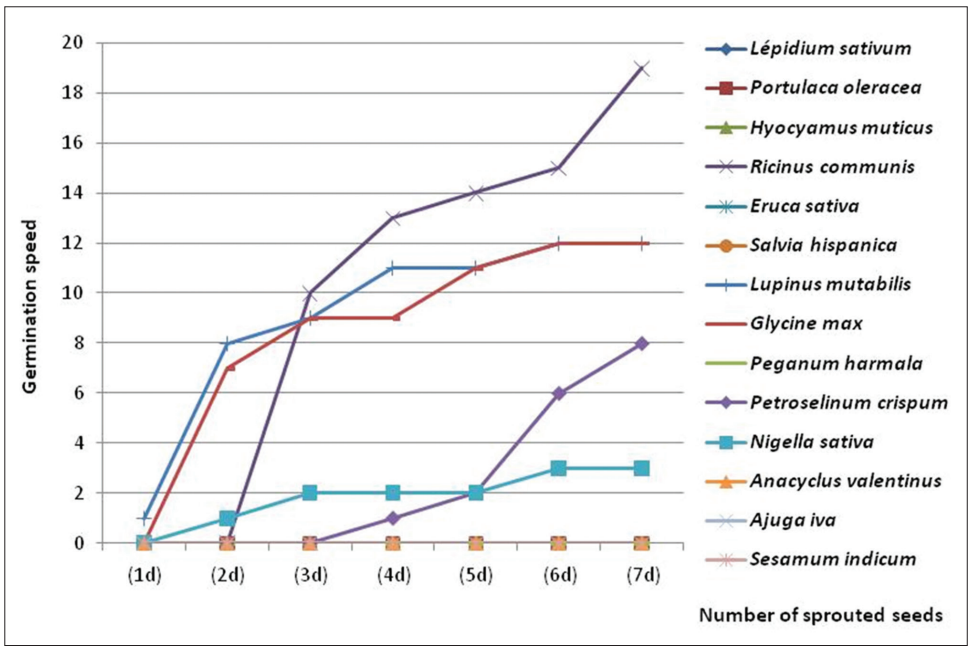

Figure 8: Speed of seed germination of the species studied for soaking in water for 24 h

is a more important factor for the germination of Fabaceae seeds (G. max, L. mutabilis). Water is first absorbed by the natural openings of the seed and then diffused through its tissues [14]. The cells of the seed then become turgid. The GC, time and GR are higher when the seeds of Hyosyamus muticus and P. crispum are chemically scarified with SA acid during (5 min). We found a same GR for the seeds P. crispum53% when these are treated with concentrated SA acid for 5 min and soaked in distilled water for a period of 24 h but the early germination is different and faster for SA acid. The germination behavior of these species in response to these treatments therefore indicates that they have a physical dormancy. This pretreatment is known for the elimination of integumentary chemical inhibitions and embryonic dormancy [15,16]. The work of Neffati *et al.* [17] related to the study of seed viability of some Tunisian species show that the effect of this pretreatment can be positive or negative depending on the species.

Germination in the Culture Medium (ms)

Germination rate

The higher GR (100%) is observed after 30 days was recorded in the seeds of *G. max*, *P. crispum*, *L. mutabilis*, *S. hispanica* and *R. communis*. While in *E. sativa*, seed GR is moin significant after 30 days also same for L. sativum, common purslane and

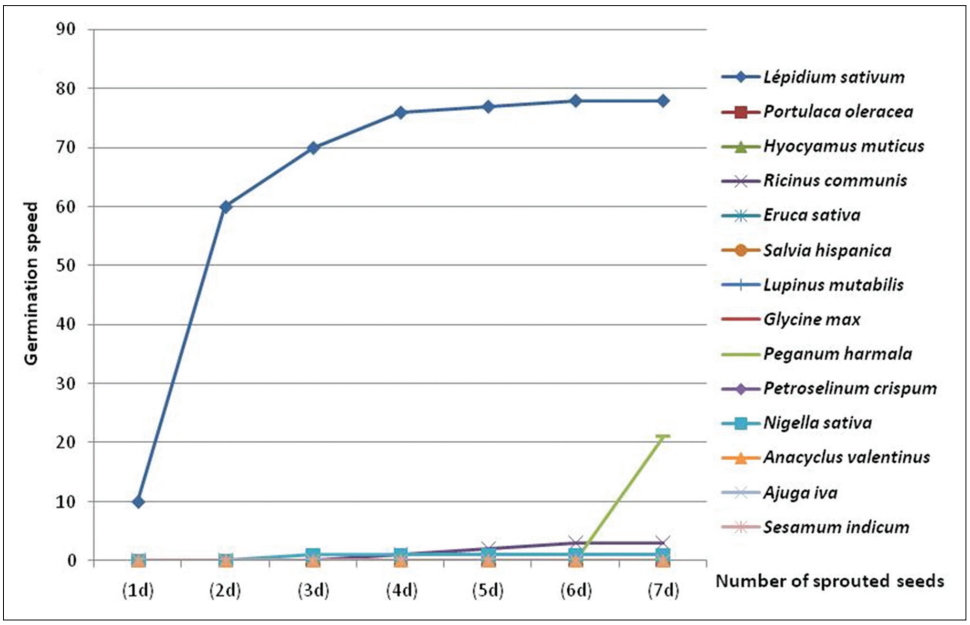

Figure 9: Germination rate of seeds of the species studied for soaking in water for 1 h

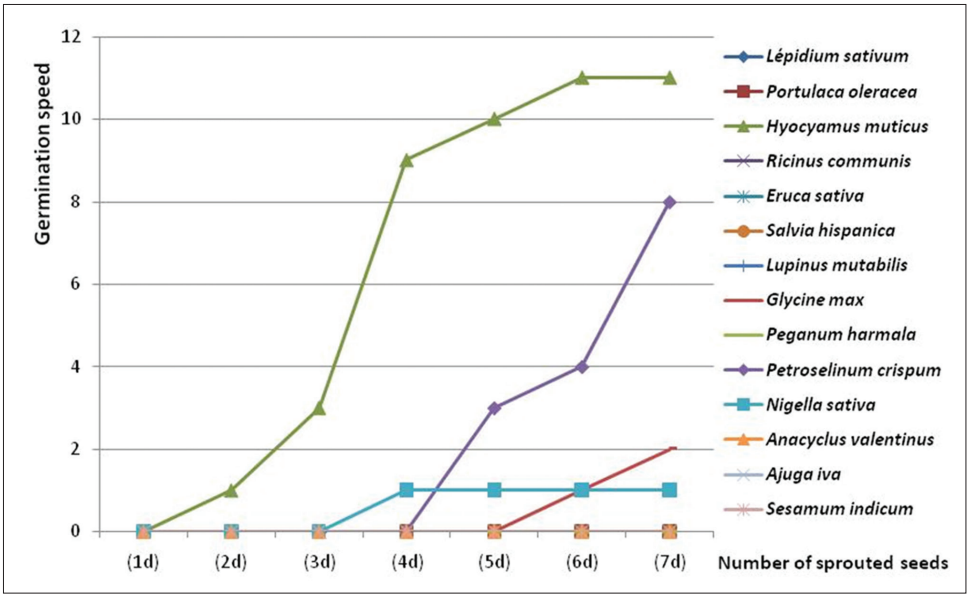

Figure 10: Germination rate of seeds of the species studied for soaking in SA acid for 5 min

Hyocyamus muticus and N. sativa with lower rate germ with only 20%. The other four species did not germinate completely (Figures 11 and 12).

Precocity and Germination Time in ms Culture Medium

Except the species of *E. sativa* which showed a precocity of germination of the seeds submitted in the culture medium MS. While the other species are sprouted genal after a period of five and eight days and fifteen days for *N. sativa*. No sprouting was recorded for *A. iva*, *S. indicum*, *P. harmala* and *A. valentinus* species.

The tests carried out led to the determination of the regeneration capacity of whole plants from seed in the MS culture medium without the addition of growth regulators, and a methodology for better improvement of the species. Regarding the micro-propagation from sowing seeds, the results obtained showed that most of the seeds studied are germinated at reduced time compared to traditional trials (sowing in soil). We recorded zero growth throughout the 30-day period for A. iva S. indicum, P. harmala, A. valentinus. For the last two species we have noticed their germinative power in the molded boxes. This result is the best by our previous tests in soil and in molded boxes. We also noticed that the percentage of seed germinated was high after 7 days in the MS culture medium which promotes the germinative capacity that ensures the good development of vitro plants, which explains the increase in the speed of growth of hypocotyls and roots of both H. muticus and

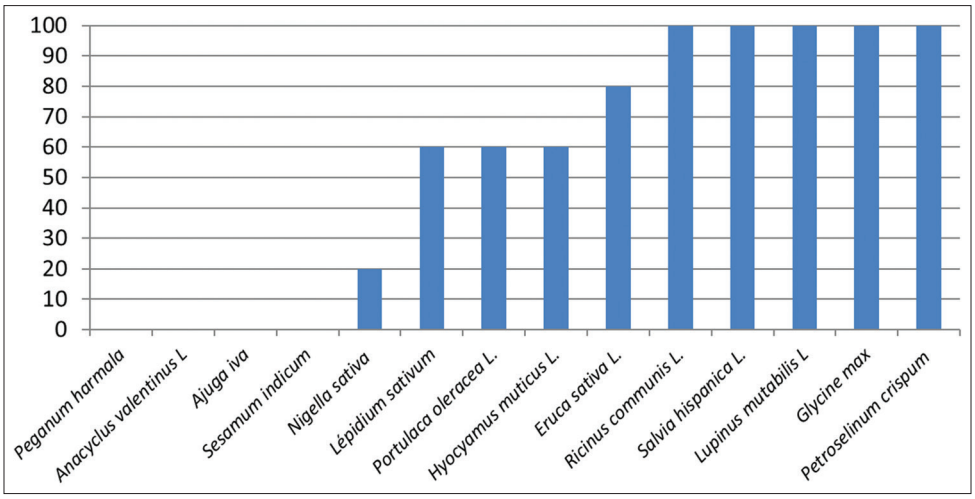

Figure 11: Seed germination rate results in the MS culture medium for the 14 species studied

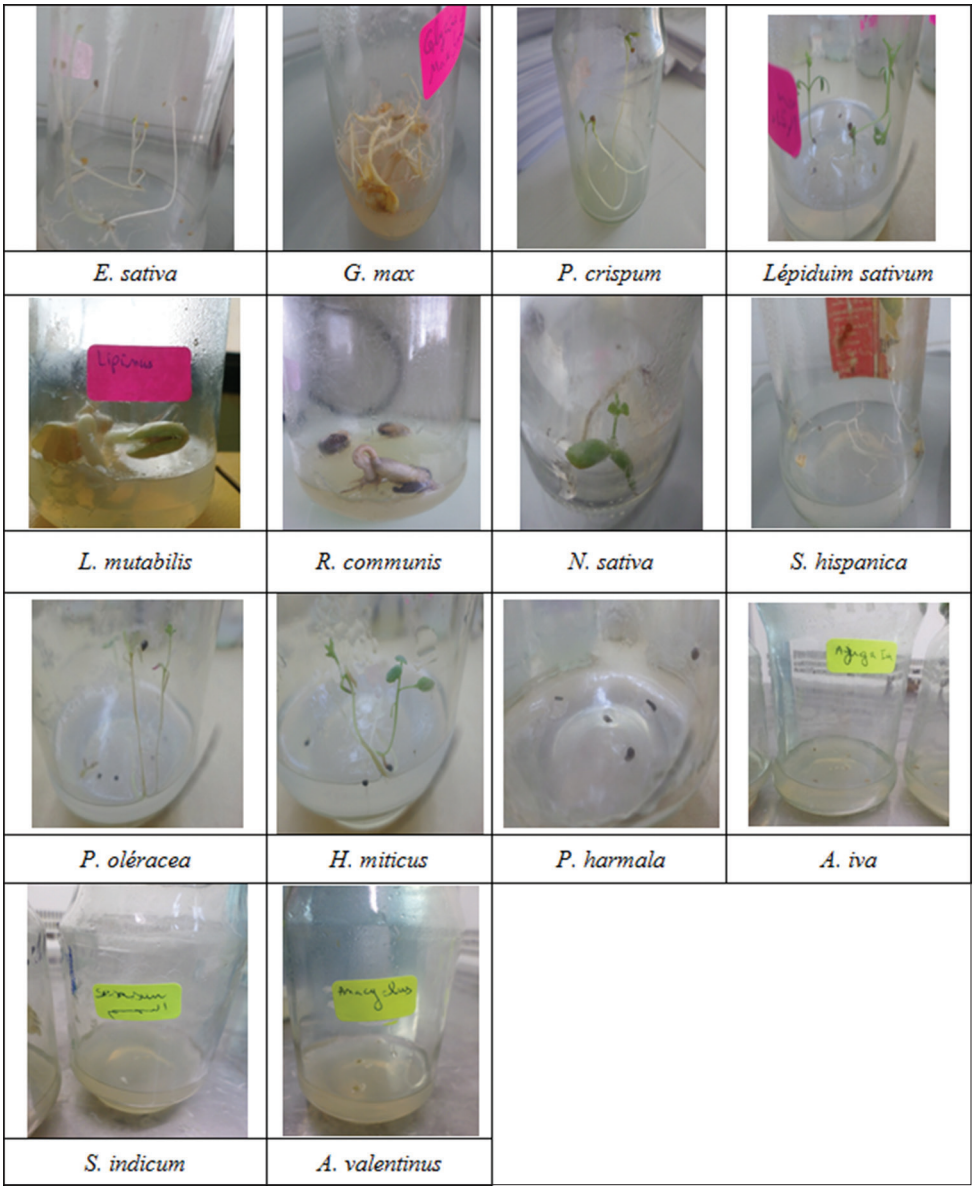

Figure 12: Results of seed germination in the MS culture medium for the species studied

P. oleracea species in the 30-day period. Germination in the MS medium is very successful, the majority of the seeds marked a significant recovery, especially between 1 to 15 days, with good growth for both N. sativa and E. sativa species. The best values of the germ speed of E. sativa are obtained after 1 day against the seeds of N. sativa which characterize by slow germination the latter join the results of Hera *et al.* [18] which showed that the complete germination with the leaves hypocotyls and roots during 11- 15 days.

The results showed that seed germination of S.hispanica, P.crispum, G.max, L. mutabilis and R. communis depended on the composition of the nutrient medium. The most effective was found to be a basal MS medium without the addition of growth regulators, and ensuring 100% seed germination between 5-8 days after sowing. For the other tests, the percentage of germinated seeds was 3-64% after 7 days of culture. He might suggest that the favorable influence of the MS culture medium components resulted in better seed germination compared to the other trials. This combination was effective in increasing GR and seedling development [19].

CONCLUSION

We undertook this experimental work on the germinative performance of the seeds of medicinal plants in order to determine the appropriate pretreatment. The study carried out in the laboratory shows an effect of pretreatments on the germination of seeds of the studied species. Comparisons classify the studied species as follows:

- Species with better germination times in soil such as E. sativa.
- Species with high germinative capacity: P. oleracea, S. hispanica, E. sativa and A. valentinus which are shown an aptitude for germination without preliminary treatment of the seeds.
- Species require preliminary treatment with soaking in water to germinate: G. max and P. crispum, N. sativa, P. harmala, L. sativum and R. communis.
- Species with early germination E. sativa, S. hispanica and P. oleracea for untreated seeds (controls). Also for soaking in water for 24 h, early germination was recorded in L. mutabilis, G. max, R. communis and N. sativa. While for soaking in water for 1 h, except the species Lepiduim sativum that has a precocity of germination. And for soaking in SA acid, except H. miticus, which has a precocity of germination. Germination in the MS culture medium gave the best growth performance for all seeds of the species studied except for the four species that have germination difficulty: A. valentinus, A. iva, P. harmala and S. indicum. It can be said that the studied species exhibit very different behaviors with respect to pretreatments at the time of their germination. Our work is just an introduction to research on seed germination factors of medicinal plants. But to reach this goal it is essential to make a more complete study. it will therefore be more interesting to spread it on all the parameters influencing germination, namely intrinsic and extrinsic, as well as the influence of seed conservation conditions, while also reducing the number of species and families to allow a more synthetic and complete, and highlight the importance of increasing the germinative performance of the seeds of medicinal plants, to better exploit and preserve them at the same time.

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