ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Impact Of Osmotic Stress On Seed Germination Of Two Varieties Of Pine Characteristic Of The Region Of Taza.

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ABSTRACT

The pine is the conifer the most wide-spread and the most known in the Mediterranean Basin. Its great plasticity and robustness have made him a pioneer species for large-scale production and protection reforestation. The study of the germination of two pine varieties of the Taza region (Pinus halpensis and Pinus pinaster) under osmotic stress conditions was carried out in order to control better the first problem encountered during the reforestation trials of this forest species. The effect of osmotic stress on pine seed germination using polyethylene glycol (PEG 6000) at different concentrations (from 0 to 8 g / 100 ml) has shown that the Pinus halpensis variety is more drought resistant than the Pinus pinaster variety and therefore may be recommended in arid and semi-arid areas.

Keywords: Pinus halpensis; Pinus pinaster; germination; osmotic stress

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ISSN: 0975-8585

INTRODUCTION

Currently, many natural Mediterranean forests and reforestation are imbalanced.

The Pin, by its plasticity and its low requirements, presents the specie most used in the reforestation and in the reconstitution of the arid and semi-arid degraded zones. It adapts to all soils, when the climatic conditions are favorable to it.

This specie is present throughout the Mediterranean region which makes it an interesting model for studying the impact of climate change that may affect this zone [1].

The region of Taza which is characterized by the presence of two endemic pine varieties namely, the Aleppo pine (*Pinus halpensis*) and the maritime pine *(Pinus pinaster)*.

However, few studies have until now focused on the germinative behavior of seeds of this species under controlled conditions.

It is in this context that we chose to study the first factor who is the drought and which prevents the germination of the pine in the Taza region and that by using different concentrations of PEG 6000.

MATERIALS AND METHODS

1- Plant material and culture conditions

The seeds of *Pinus halpensis* and *Pinus pinaster* used come from the region of Taza (Tazekka National Park). Indeed, Taza is a city located in the north-east of Morocco (figure 1), characterized by a Mediterranean-type climate: cold and wet in winter and semi-arid in summer.

According to the ombrothermal diagram of Bagnouls and Gaussen (figure 1), the city of Taza is characterized by a dry season that extends over almost 6 months starting in May and ending in October, with a maximum temperature (30,09°C) in the month of August, and avery low level of rainfall (6.5 mm). Therefore, during this period, the tree suffers from water stress, which can adversely affect pine growth.

Seed collection was carried out on natural aged trees. Indeed, the full and viable seeds are sterilized with bleach and distilled water for 10 minutes. They are then rinsed abundantly with running water and then 3 times with distilled water [2].

The seeds are then immersed in tap water for a time (48H), then washed with distilled water and germinated, in batches of 80 seeds for the control and for each pretreatment.

2- Effect of osmotic stress on germination

The germination tests were carried out in sterile plastic Petri dishes (diameter = 90 mm) at a rate of 20 seeds / dishes, ie 4 replicates by treatment, on a filter paper wet with 5 ml of distilled water for the controls and 5 ml of a solution of PEG 6000 (polyethylene glycol) for treated; 1,5 g/100ml (-10 joules/mole); 2,5 g/100ml (-17 joules/mole); 3 g/100ml (-20 joules/mole), 4,5 g/100ml (-30 joules/mole) and 8 g/100ml (-53 joules/mole).

The PEG 6000 (Polyethylene glycol: HOCCH2 (OCH2CH2) is a relatively stable, inert osmotic agent that is very soluble in water and non-toxic.

The dishes are placed in an incubator at a temperature of 25 °- 27 ° C. The duration of the experiment is 21 days, during which we counted daily the number of seeds sprouted. The appearance of a radicle of about 1 mm was used as a germination criterion [2].

The germination rate was calculated using the following relationship:

ISSN: 0975-8585

The Germination rate (%) = (Nx/N0)x100

Nx: number of germinated seeds
N0: total number of seeds

The results are submitted to a two way analysis of variance (ANOVA) and a PCA with SPSS 20 software.
The PCA (Principal Component Analysis) which is a multicriterion classification method, represented on a plane with 2 factorial axes or on a multidimensional space (3 or more) which is used to classify individuals and / or variables into clouds relatively homogeneous.

RESULTS AND DISCUSSION

The study of the effect of different concentrations of PEG 6000 on the evolution of the germination rate of Aleppo pine and maritime pine seeds (figures 3, 4 and 5), shows that the percentage of germination of stressed seeds is reduced compared to the control regardless of the variety studied.

However, the study shows a variable effect of osmotic stress on the germination percentage of the two pine varieties studied.

For radicle breakthrough, two-way variance analysis revealed very significant differences in PEG 6000 concentrations for both pine varieties. Also, average numbers comparison of sprouted seeds shown at the end of the four 5-day slices using the two-way analysis of variance shows that the differences are highly significant.

In other words, their germination rates during the 20 days of follow-up are significantly different (figure 4, 5 and table 1).

The main results obtained in the present work on the germination of the seeds of Aleppo pine and maritime pine under osmotic stress is that the latter has the highest germination rate (figure 3). Indeed, for the controls, the germination rate of Aleppo pine and maritime pine is respectively 68,75% and 81,25%.

By increasing the PEG 6000 concentration, a 62.5% decrease in the optimal germination rate is observed in Aleppo pine when the concentration is 8g / 100ml. While for the maritime pine, it appeared that the osmotic stress of 8g / 100ml PEG 6000 is the limit value for the germination of this variety since no seeds could germinate at this high concentration.

Similar results have been obtained from various studies in *Pinus ponderosa* and *Pinus eldarica* [3], *Pinus brutia* and *Pinus eldarica* [4], *Pinus halepensis* [5, 6] and *Pinus taeda* [7].

Principal Component Analysis (PCA)

The results obtained gave rise to a multivariate analysis and the first two factorial axes summarize more than 99.7% of the information for both Aleppo pine and maritime pine (Tables 2 and 3). We conclude that the graphs obtained (graphs of the variables and graphs of the individuals) will be significant to make the following interpretations (see diagram 1 and 2).

Indeed, the proximity of a variety relative to a given concentration means that the impact of that concentration is preponderant and vice versa, as the variety is remote from a concentration level indicates that the concentration effect is less significant (Diagram 1 and 2). A variety is poorly represented if its cos^2 is close to 0.

For both varieties of pine (Aleppo pine and maritime pine):

C3 (3 g/100ml (-20 joules/mole)) and C4 (4,5 g/100ml (-30 joules/mole)) are far from the ordinate at the origin, so the proximity of these 2 variables means that they are very strongly correlated.

- A proximity between period 3 and period 4 (Diagram 1' and 2') means that these 2 individuals have the same behavior with respect to the variables studied.
- The proximity between a variable point and an individual point means that this variable has a high value for that individual. So, C3 and C4 are close to period 3 and period 4 means that the longer the drought period, the more the drought effect is visible.

Amamou [8] was able to find, by studying the effect of water stress on the germination of the seeds of twelve maritime pine origins of its Mediterranean-Atlantic geographical area, that there are different responses of the seeds with respect to stress but that the geographical origin also significantly affects the germination rate of the provenances. Similar results have been reported in other Pinus species such as *Pinus brutia* [9] and *Pinus halepensis* [10, 11], but also in other species [12, 13].

Our results are consistent with those obtained by the dendroclimatological study conducted on three forest species in Tunisia (*Pinus halepensis Mill., Pinus pinea L.* and *Pinus pinaster Sol*.) and which showed that the Aleppo pine shows a strong resistance to drought when it is in climatic conditions or the most difficult, while the maritime pine shows the highest sensitivity to drought [14]. Indeed, Aleppo pine is characterized by exceptional plasticity [15]. It is considered one of the most tolerant pines for pronounced temperature and drought [16].

El Khorchani and al [14] indicate that the Aleppo pine shows a high resistance to drought when it is in the most difficult climatic or edaphic conditions while the maritime pine shows the highest sensitivity to drought.

The specie that best resists stress would be the specie that would be able to modulate its growth based on water availability. Such behavior is characteristic of Aleppo pine. Indeed, studies have shown that this specie has both an ability to prevent drying out, thanks to a reactivity of the stomata, and an ability to withstand drying while maintaining a minimum process of photosynthesis at very low water potentials [17]. In both cases, these two skills are what allow the Aleppo Pine to survive under strong stress conditions.

Furthermore, in the national reforestation policy, taking into account the requirements of this species, while maintaining optimal conditions for its germination in the nursery, is more than necessary for the rehabilitation of evergreen Aleppo pine stands.

The exploitation of this tree on a large scale requires a good control of the conditions of germination and the growth of the plants.

CONCLUSION

Drought is a major abiotic stress that is, on the one hand, an important factor influencing the vitality of plant species and, on the other hand, a limiting factor for the extension of the forest.

The objective of this study is to analyze and compare the responses of two endemic pine varieties to the Taza region under water stress using the PEG 6000 osmotic agent.

The results obtained for the 2 varieties studied of pine, revealed a more or less variable variability for the resistance to the early water deficit which is the germination.

Indeed, this study has shown that the variety of Aleppo pine can be recommended to planters and nurseries because it is the most drought resistant variety.

Although the maritime pine has the highest germination power under good conditions, it seems to be the most sensitive when conditions become unfavorable.

In susceptible trees, the potential risk of drought could lead to a physiological imbalance that may expose them to insect attack and thus accelerate their dieback. The risk of drought is greater on sandy soils because on this type of soil the availability of water decreases sharply [18]. This can cause abrupt drying and

ISSN: 0975-8585

sometimes death of trees. The decline of maritime pine observed in recent years in coastal dunes may have been caused by this type of accidental desiccation [14].

Thus, some species currently widely used for reforestation, such as the maritime pine on the coastal dunes, could in the future limit its use to the benefit of other more drought-resistant species such as the Aleppo pine [14].

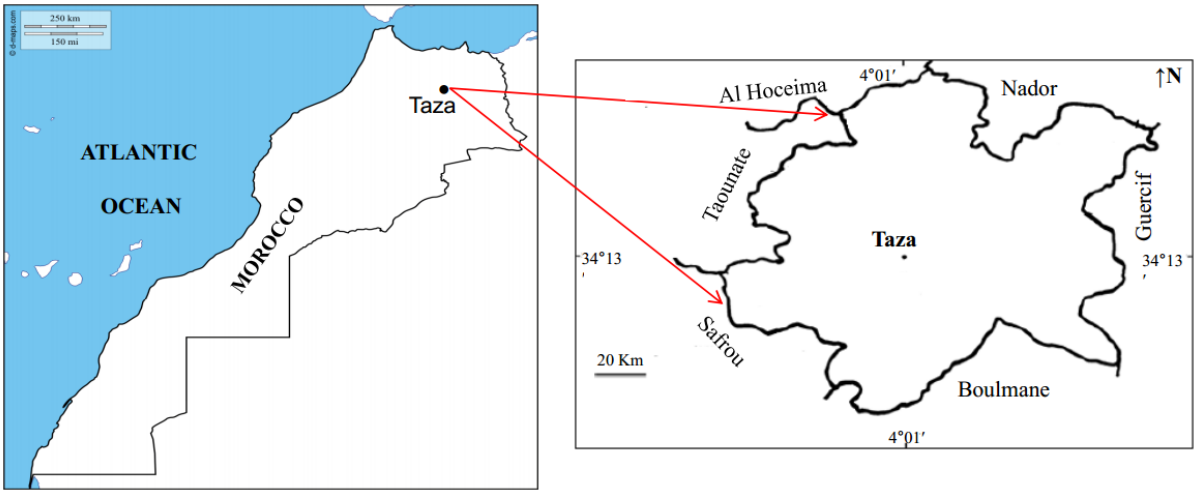

Figure 1: Geographical location of the city of Taza [19].

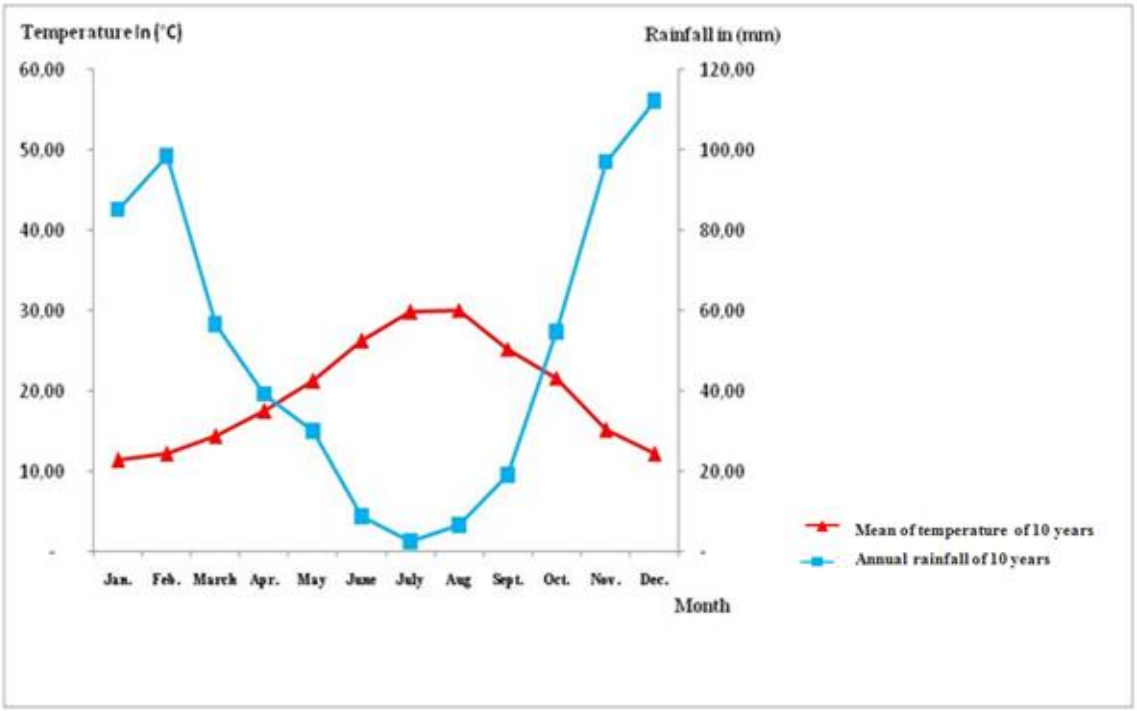

Figure 2: Ombrothermic diagram of BAGNOULS ET GAUSSEN of Taza station (Period: 2008-2017)

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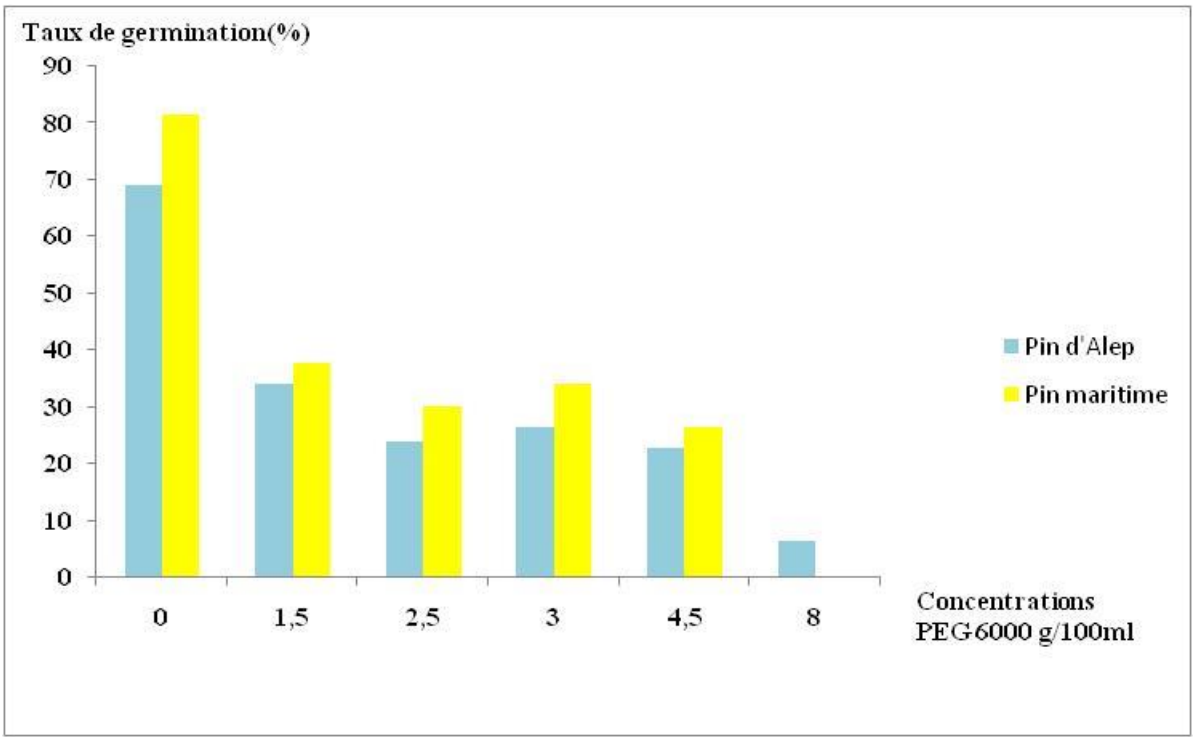

Figure 3: Effect of PEG 6000 on the breakthrough of the radicle after 20 days of germination in Aleppo pine and maritime pine

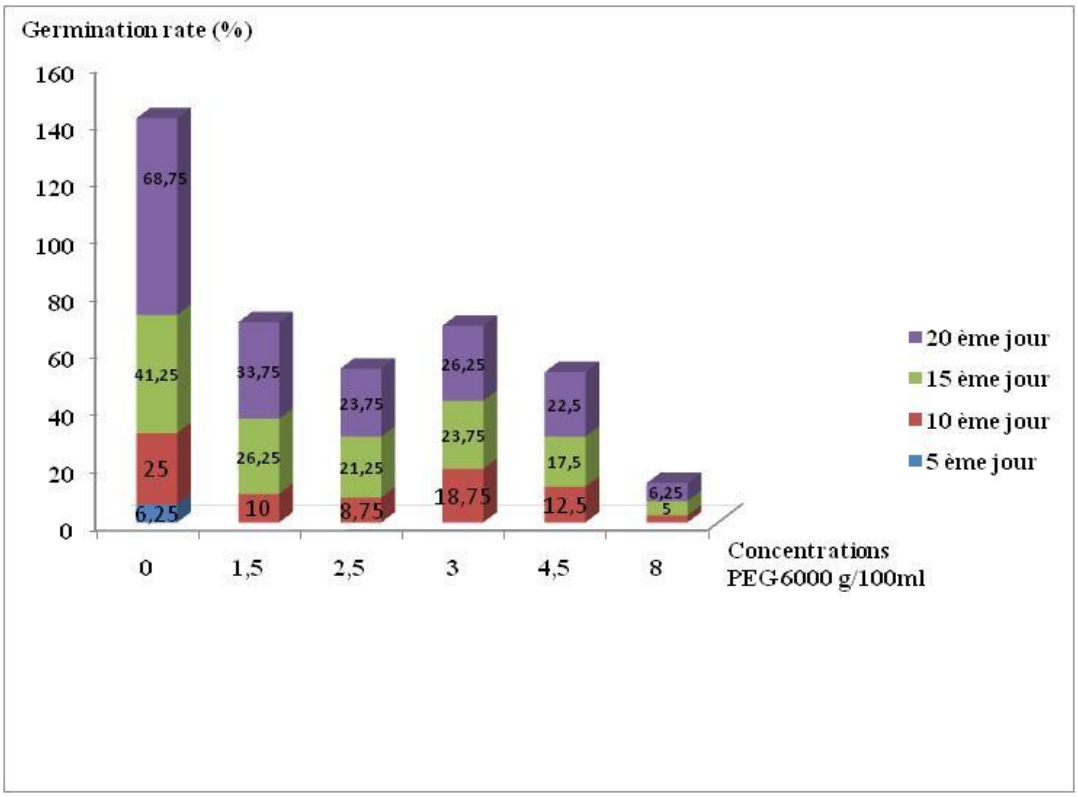

Figure 4: Effect of PEG 6000 on breakthrough of radicle in 5-day increments in Aleppo pine

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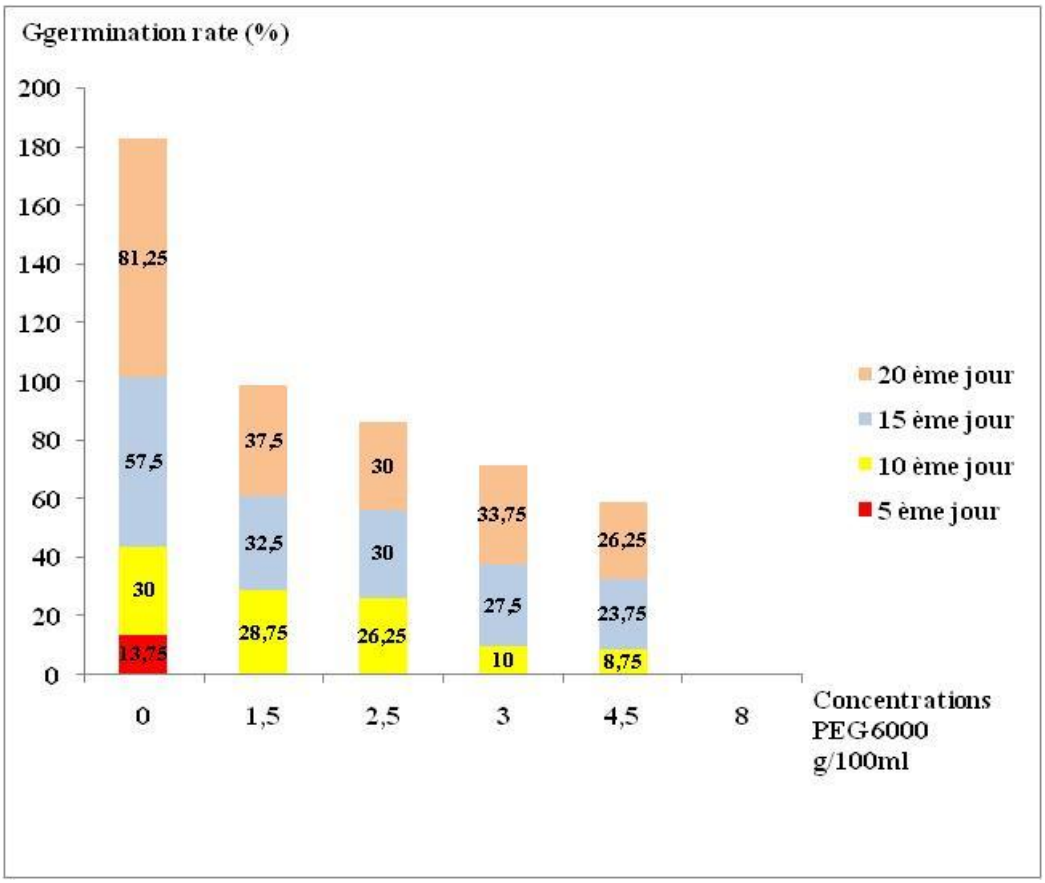

Figure 5: Effect of PEG 6000 on radicle breakthrough in 5-day increments in maritime pine

Table 1: Analysis of variance on the effect of PEG 6000 on the germination of two **varieties of pine characteristic of the region of Taza**

ANOVA II , P	**Pin d'Alep:**	Maritime pine**:**
Between concentrations	P<0,01	P<0,01
Between time	P<0,001	P<0,001

ANOVA II , P	**Pin d'Alep + Pin maritime :**
Between concentrations	P> 0,05
Between varieties	P<0,01

P< 0,05 : Significant differences at the threshold 5%, P< 0,01 : Very significant differences at the threshold 1%.

P< 0,001: Highly significant differences at the threshold 1‰, P> 0,05:

Differences not significant at threshold 5%, P : Probability.

ANOVA II : Two-way analysis of variance (concentrations and time); (concentrations and varieties)

Table 2: Total variance explained for the Aleppo pine

Component	Extraction sums of squares of the factors retained		
	Total	% of the variance	% cumulated
1	4,861	97,226	97,226
2	,125	2,506	99,732

Extraction method: Principal component analysis.

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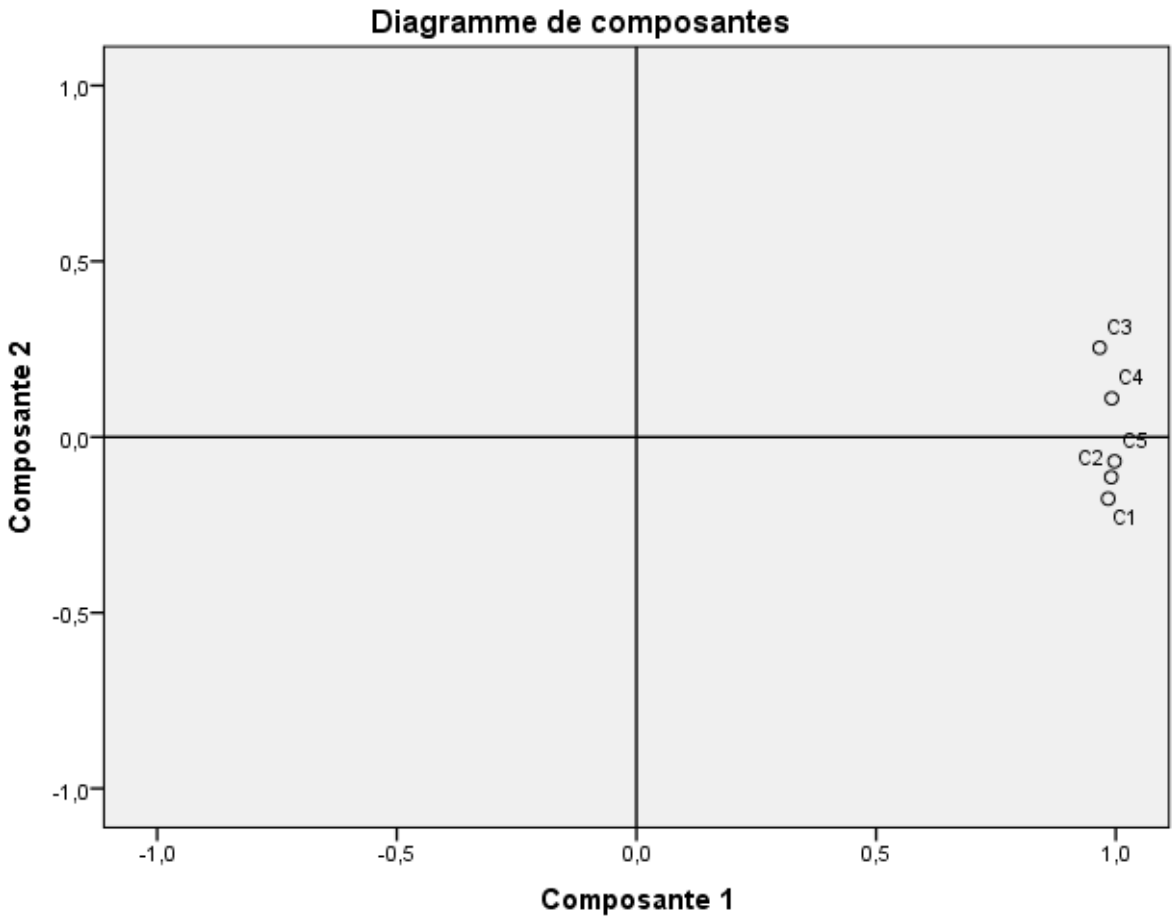

Diagram 1: Principal Components for the Aleppo pine

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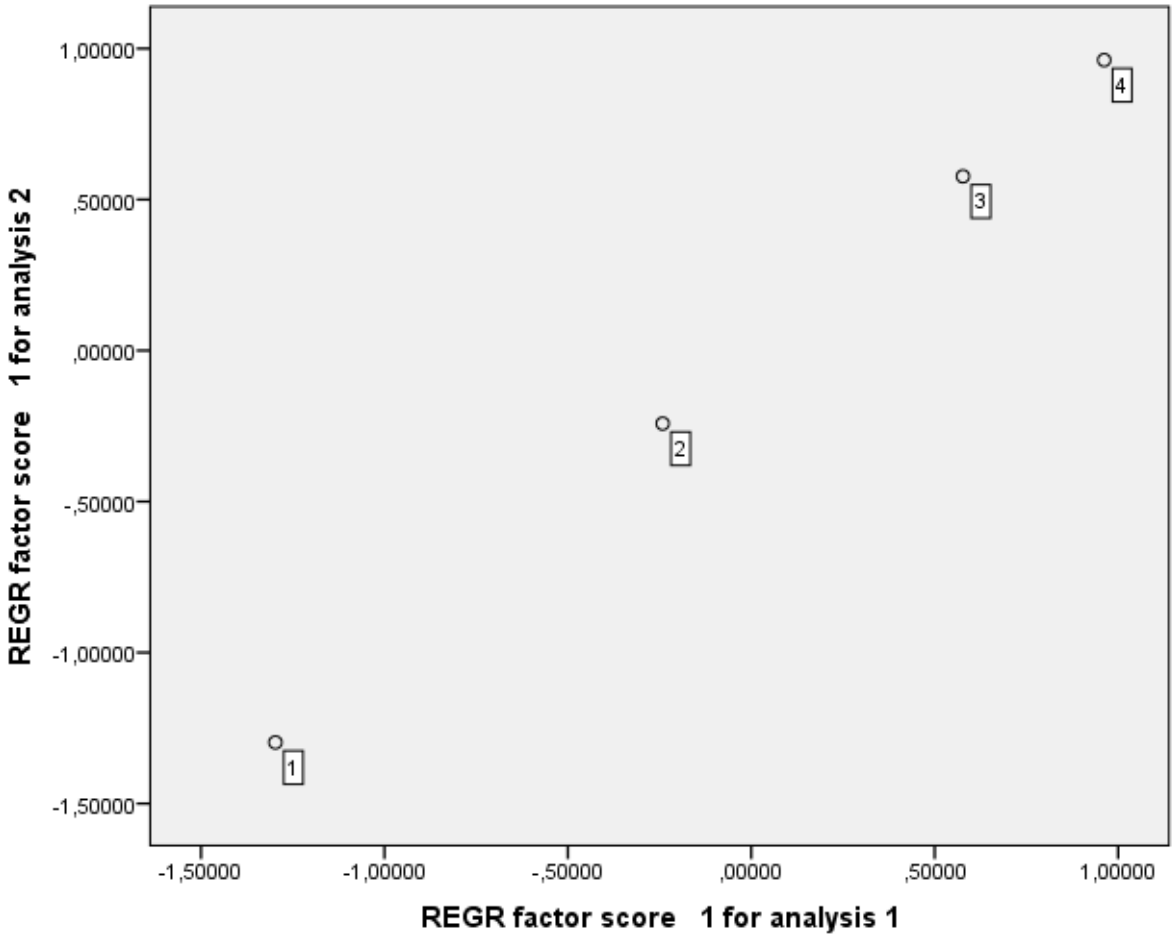

Diagram 1'. Dispersion diagram for the Aleppo pine

1: Période 1 = 5 days; 2: Période 2 = 10 days; 3: Période 3 = 15 days; 4: Période 4 = 20 days

Table 3: Total variance explained for the maritime pine

Component	Extraction sums of squares of the factors retained		
	Total	% of the variance	% cumulated
1	3,718	92,938	92,938
2	,273	6,825	99,763

Extraction method: Principal component analysis.

ISSN: 0975-8585

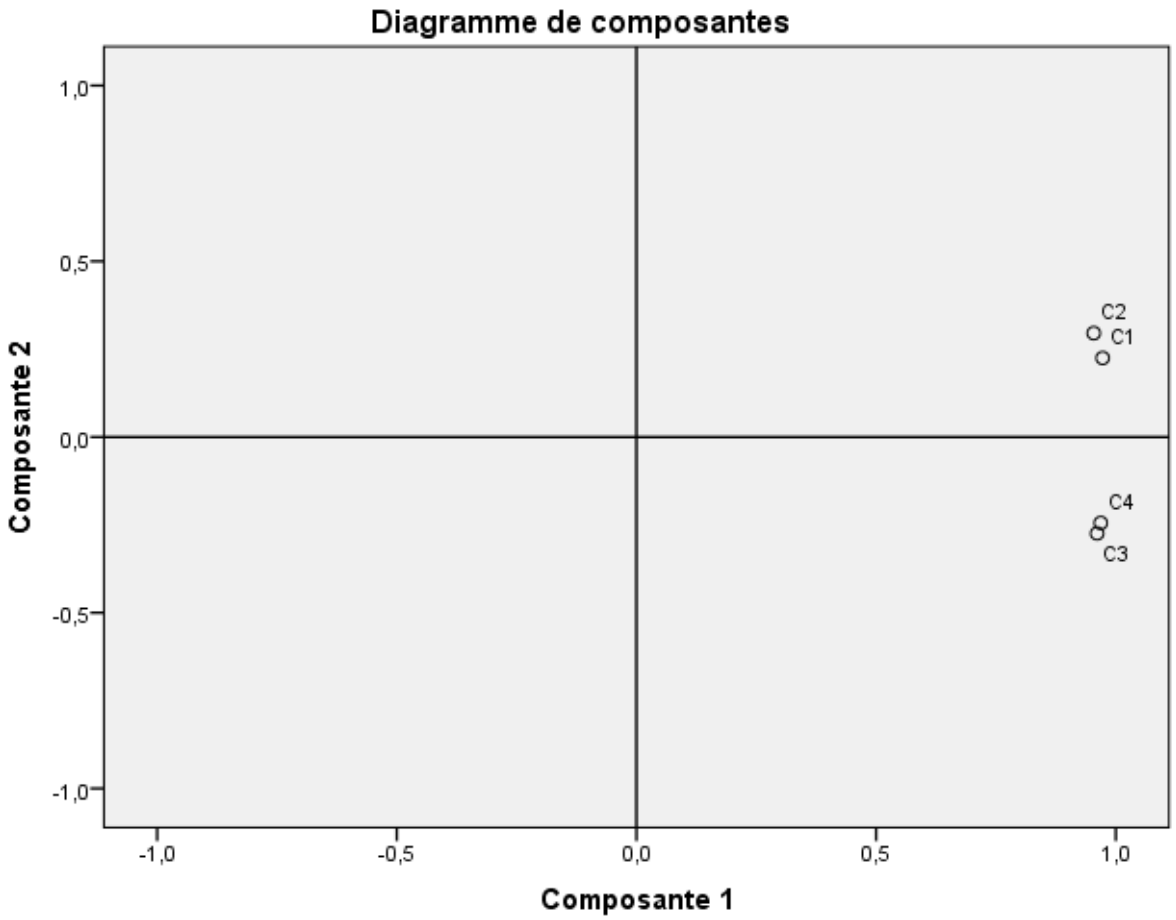

Diagram 2: Principal Components for the maritime pine

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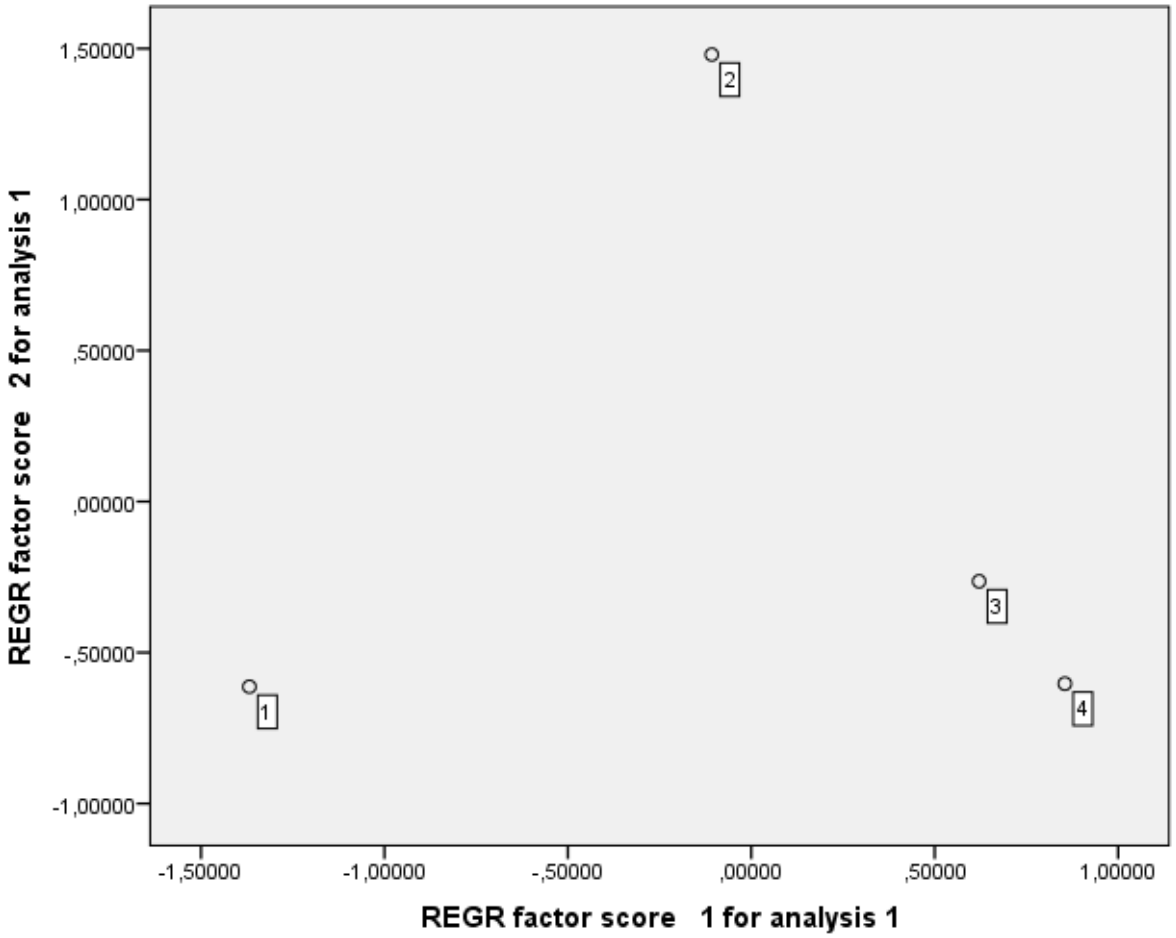

Diagram 2'. Dispersion diagram for the maritime pine

1: Période 1 = 5 days; 2: Période 2 = 10 days; 3: Période 3 = 15 days; 4: Période 4 = 20 days

ACKNOWLEDGEMENTS

We would like to thank **Mr El Haouari Mohammed** Professor of Higher Education Assistant Regional Center of the Trades of Education and Training of Taza (CRMEF-TAZA), Morocco, for his help.

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