
Effect of some litter based substrates on germination and growth of allepo pine plants *Pinus halepensis* (Mill.) in the nursery

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Abstract Chemical analysis of the litter showed that the mixed litter contained a high concentration of essential fertilizing elements (N, P, K, Ca and Mg) and has a pH close to the neutrality, which is conducive to the plant growth. The mixed litter gave the best germination rate with an average of 90%. In terms of growth parameters such as height, diameter at the base, and biomass of both aboveground and belowground part, the mixed litter outperformed the other types of litter as well as the control group. The findings of this study hold significant importance for reforestation practices, emphasizing the benefits of avoiding monoculture.

Keywords: Litter, Desertification, Green dam, Monoculture, Aleppo pine

Introduction

The Aleppo pine (*Pinus halepensis* Mill.) is considered a major and essential component of the Mediterranean forest and represents a valuable forestry asset in most countries around the Mediterranean, particularly in Algeria (Nahal, 1962; Boudy, 1950). In addition to their rapid growth and resistance to xeric conditions, Aleppo pines are also able to replenish degraded areas and occupy bare land (Bentouati, 2006; Zavala and Zea, 2004).

In Algeria, Aleppo pine covers 35% of forest areas in the north i.e. around 850000 ha. It forms significant forests with variable ecological value (Bentouati, 2006; Guit, 2015). This species, which is found in all bioclimatic zones from the coastal to the Saharan Atlas, finds its optimal growth mostly in semi-arid zones (Kadik, 2005; Djerrad, 2016). Due to its great adaptability and weather resistance, it has become essential for reforestation in semi-arid areas in order to prevent desertification (Kadik, 2005; Guit, 2015; Djerrad, 2016). Despite their economic value (wood, essential oils, seeds, tannins, etc.), along with the ecological services (soil conservation, carbon sequestration, habitat for biodiversity), Aleppo pine forests face strong pressures from rural populations

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(overexploitation, overgrazing, tree cutting, etc.) (Le Houerou, 1969; Benmessaou). Khouja *et al.* (2020). These pressures are exacerbated by the lack of a sustainable and harmonious silvopastoral management approach that balances resource utilization with conservation efforts. Consequently, this management method has resulted in forest degradation (tree mortality, lack of regeneration) and its resources (Abdessemed, 1984). In addition to these threats, Aleppo's pine forests are also facing the effects of climate change, characterised by rising temperatures, more frequent summer droughts, persistent heat waves, and the intensity of forest fires (Menasri, 2014). These climate changes make the forests more vulnerable to diseases and opportunistic pests, as well as forest fires, which result in reduced surface areas and, consequently, the advance of the desert.

Faced with the degradation of the Aleppo pine forests and to halt the advance of the desert towards the northern regions of Algeria, an ambitious reforestation project was launched in 1970, introducing the inception of the green dam project. The aim of this was to reforest three million hectares by creating a green barrier connecting the western and eastern borders of Algeria along a 1,500 km stretch. However, the outcomes achieved thus far have not aligned with the initial expectations and aims of the project. The reforestation efforts have been plagued by high mortality issues primarily attributed to technical challenges, parasitic infestations, wildlife intrusions, climatic adversities such as frost, wind, and drought, forest fires, and to some extent, social factors that were overlooked during the planning phase.

The initial decade of reforestation activities conducted without thorough assessments has led to various issues stemming from the mono-specific cultivation of Aleppo pine. This approach, known as monoculture, poses significant risks to the environment due to the proliferation of detrimental insects like the processionary caterpillar (*Thaumetopoea pityocampa*) and other pests that can cause substantial damage to young tree stands. If left unchecked, the infestation of these insects can result in complete defoliation of entire plantations, ultimately leading to the demise of the trees. Moreover, the persistent use of pesticides to combat these pests can inadvertently give rise to resistant strains, fostering a reliance on chemical interventions. This overreliance on chemical agents not only impacts the quality of the final product but also depletes and degrades the soil over time. Monoculture exhausts the soil by depleting essential nutrients required for growth, consequently necessitating the application of fertilizers. Furthermore, it diminishes the biodiversity of the ecosystem by eradicating vital microorganisms crucial for soil health and resilience against external threats.

In light of these challenges, the overarching aim is to enhance the success rates of mono-specific Aleppo pine reforestation endeavors by transitioning towards mixed cultivation methods to mitigate the prevalent failure rates observed in practice, thereby promoting plant development and curbing desertification. The objectives delineated for this investigation encompass evaluating the impact of mono-specific and mixed forest litter on the growth and germination of Aleppo pine cultivated in ground nurseries, as well as comparing these outcomes with plants grown in a control substrate devoid of litter, incorporating both mono-specific and mixed forest litter components.

Materials and methods

Experiment site

This experiment was carried out in the experimental nursery of the Regional Forestry Research Station (RFRS) located approximately 12 km west of the capital of the city of Jijel (Algeria). The study area has a sub humid bio climate, with cold, wet winters and hot, dry summers. this work was carried out between March and September 2023.

Commodities used

For this study we require the plant material (the seeds of Aleppo pine), the preparations of forest litter, the soil and the growing pots:

The plant material used for this study was a lot of Aleppo pine seeds, which was provided to us by the services of the forested conservation of Mila (Algeria) (City adjacent to Jijel).

The litter used in this study was from two different species: the litter of a coniferous tree which is the Aleppo pine (*Pinus halepensis* Mill) and the other of a leafy one which is the holm oak (*Quercus ilex*). These litters were taken from the forests of the wilaya of Mila (Algeria). These are fresh Aleppo pine litter (needles harvested directly from Aleppo pine trees), old Aleppo pine litter from the previous year of brown color in the process of decomposition and old Holm oak litter.

The soil was taken from the surface (30 cm deep) of an Aleppo pine forest in the city of Mila (Algeria). The main physicochemical properties of the soil sampled were: a pH of 7.85 indicating alkaline soil, while the electrical conductivity was 1.05 mS.cm⁻¹. An average rate of organic matter (1%) and a clay-silty texture.

For the growing pots we used a plastic pots dimension of: Depth: 25.5cm; Top diameter 24cm; Bottom diameter; 18.5 cm. The bottom of these pots is perforated with 4 holes to allow the drainage of excess water.

Conduct of the test

Once the soil is removed, it is brought back to the place of experimentation we sieved it to 3 mm, in order to eliminate the residues not crushed and to have a homogeneous material. The pots were filled with a shovel through the ground. The provided Table 1 presents the names and composition of the different treatments tested.

Table 1. Name and composition of treatments tested

Treatment	Soil	Litter (%)
Treatment (T1)	75	25 Fresh Aleppo pine litter (LF)
Treatment (T2)	75	25 Old Aleppo pine litter (LO)
Treatment (T3)	50	25 Old Aleppo pine litter + 25 old Holm oak litter (LM)
Witness (T4)	100	/

The nursery study was conducted according to a device in random blocks complete with 4 repetitions (4 blocks), each block is composed of 4 treatments and each treatment is composed of 10 pots (10 plants) giving 40 plants per block, and 160 plants for the entire experimental set-up. The breeding of the plants was conducted in nursery on the ground and under a shade allowing about 50 to 60% of sunshine. The sowing operation took place on March 15, 2022, the seeds were deposited at a depth of 2 to 3mm to optimize germination. After seeding, routine weeding and watering was provided as needed throughout the 4-month rearing cycle.

Measurements and observations

Seedling emergence

The seeds were considered germinated when an Aleppo pine seedling appeared and grew. Each time a seedling appears it is counted, until the last emergence. The rate of plant development was measured twice a week for two months.

Seedling survival

We calculated the survival rate of the plants. It represents the total number of plants remaining alive compared to the total number of germinated seeds.

Morphological parameters

In order to avoid any errors during the measurements, we numbered the samples of each Aleppo pine plant, and the different parameters measured were carried out on the same plants. We opted for an optimal and feasible sampling, 70% of the population with a total of 112 plants measured at each date.

Throughout the experiment, the measurements concerned the Stem heights and crown diameters: The height (cm) of plant stems was measured by a graduated ruler from crown to apical bud and diameter (mm) the crown of the plants was measured at the same time using a digital caliper with an accuracy of 1/100 mm.

Fresh and dry biomass

At the end of the experiment, seven (07) plants were randomly selected. For each plant, the root system is carefully separated from the substrate to keep the maximum root mass. The aerial part is separated from the root system using a blade at the collar. The fresh weight of both parts (the photosynthetic apparatus and the roots) is determined using a precision balance (Adventurer OHAUS) and expressed in mg. Subsequently, the dry biomass is determined after passing through the oven (model MENNERT) set at 80°C for 48 hours.

Data analysis

The data collected during this experiment concerning the development of Aleppo pine plants in the different substrates tested were statistically interpreted by a single variation factor analysis of variance (treatment) using the STATITCF software. If the differences were significant, the Newman and Keuls test was used to classify the substrates into homogeneous groups at the 5% probability threshold.

Analysis at the forest litter laboratory

The different litters used in this work were analysed chemically or we measured total nitrogen by the Kjeldahl method, phosphorus by the Joret-Hubert method, 1955, total elements (calcium, potassium and magnesium) by the flame photometry method and finally the pH was determined using a Metrom pH meter ratio 12.5 according to the glass electrode method coupled to a reference electrode.

Results

Litter chemical analyses

According to mixed bedding showed the values in major fertilizing elements (N.P.K) very important compared to old litter and by far for fresh litter which gave a growth advantage for plants raised at the level of mixed litter (Table 2). The content of major elements for mixed bedding was three times higher compared to fresh bedding and especially for nitrogen. The same was found to be true for calcium and magnesium, mixed bedding always has high values compared to old and fresh bedding. It found that fresh and old litter had acidic pH values with respectively 5.71 and 5.88, while the pH of mixed litter was closed to neutrality 6.77.

Table 2. Litter chemical analyses

Litter type	LF	LO	LM
Total Nitrogen (%)	0.58	0.85	1.85
Phosphorus (%)	0.12	0.14	0.50
Potassium (%)	0.10	0.18	0.35
Calcium (%)	3.72	4.40	5.00
Magnesium (%)	0.13	0.17	0.45
pH	5.71	5.88	6.77

Seedling emergence

The results of the showed that the highest rate of germination of Aleppo pine seedlings was recorded at the T3 treatment level with an average rate of 90%, T4 treatments gave the lowest emergence rate at 55% (Figure 1). After two months of nursery rearing the highest survival rate of the plants is marked for T3 treatment (Figure 2), with an average rate of 80% while the lowest rates were recorded for treatment T1 and T4 with respectively 65,5 and 45%.

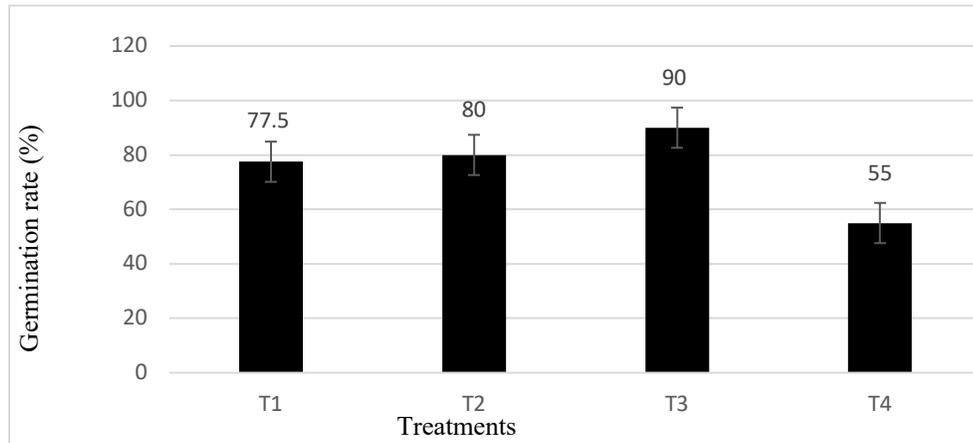


Figure 1. Germination rate by type of treatments

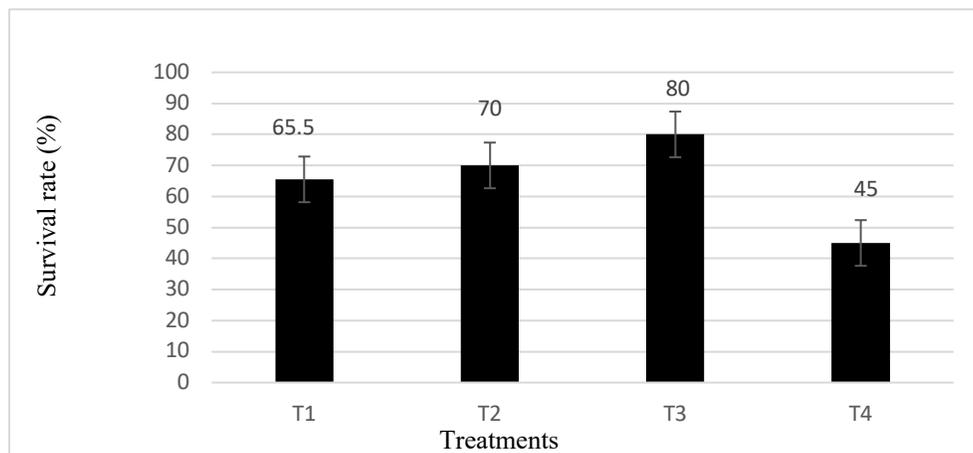


Figure 2. Survival plants rate by type of treatments

After emergence, the stem elongated, and the apical meristem formed new vegetative sequences. Aerial growth including stem growth, diameter change, root system and stem biomass were noted at the end of the experiment (after 4 months).

Stem height

The results of the analysis of the variance at the threshold of 5% of the stem height of Aleppo pine plants in showed that there was highly significant difference ($F_{OBS} > F_{THE}$) between the different treatments tested (Table 3).

Table 3. Analysis of variance (stem height)

Source of variation	SCE	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	295	63	4.96			
Var-fact1	90.15	3	30.05	43.51	2.18	S***
Var-fact2	152.9	3	50.98	73.89	2.81	S***
Var-interf1.f2	8.01	9	0.89	1.39		
Var-Block	19.23	3	6.41	9.28		
Var-Residual	31.05	45	0.69			

S***: very very highly significant, F OBS: Factor observed or calculated, F THE: Factor theoretical

The test of NEW MEN and KEULS at the threshold of 5%, highlights 4 homogeneous groups is shown in Table 4 and Figure 3. A dominant group A which gave the average height of the highest stem with an average height of 8 cm represented by the treatment T3, the lowest values of the stem height are recorded at treatment level T1 and T4, represented by groups C and D were 6.31 cm and 4.77 cm, respectively.

Table 4. NEW MEN and KEULS Test (Stem Height/Treatments)

Treatment	Medium(cm)	Groups
T3	8	A
T2	7.12	B
T1	6.31	C
T4	4.77	D

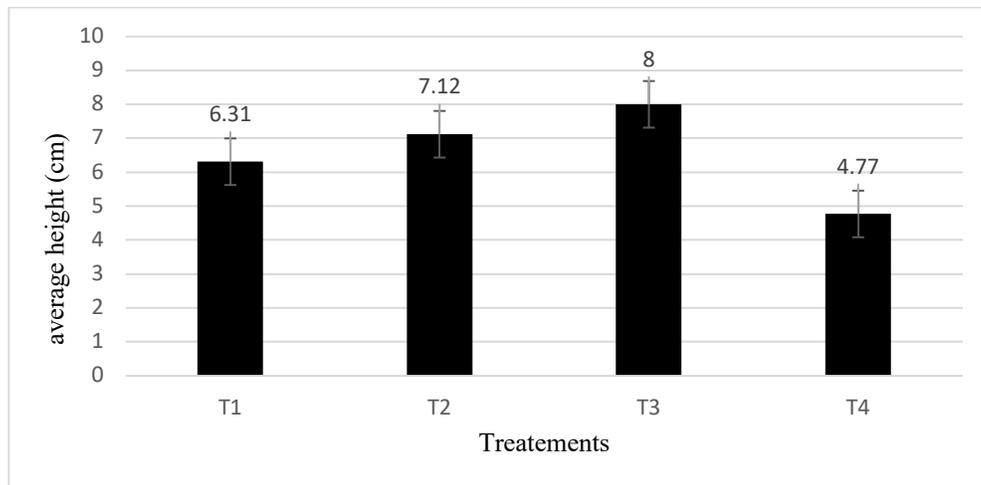


Figure 3. Average stem height by type of treatment

Diameter at the collar

The results of the analysis of variance at the 5% stem diameter at the collar threshold of Aleppo pine plants is shown in Table 5. It showed that there was highly significant difference ($F_{OBS} > F_{THE}$) between the different treatments.

Table 5. Analysis of Variance (Diameter at the collar)

Source of variation	SCE	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	10.38	63	0.16			
Var-fact1	3.78	3	1.26	41.21	2.18	S***
Var-fact2	4.56	3	1.52	49.68	2.81	S***
Var-interf1.f2	0.45	9	0.06	1.33		
Var-Block	0.03	3	6.01	0.46		
Var-Residual	1.35	45	0.03			

S***: very very highly significant, F OBS: Factor observed or calculated, F THE: Factor theoretical

The test of NEW MEN and KEULS at the threshold of 5%, brings out 4 homogeneous groups are shown in Table 6 and Figure 4. A dominant group A which gave the average diameter of the highest stem with an average of 1.84 cm which recorded for the treatment T3, the smallest values of the stem diameter are recorded at the treatment level T1 and T4, represented by the last groups C and D 1.42 cm and 1.17cm, respectively.

Table 6. NEW MEN and KEULS Test (Collar diameter/treatments)

Treatment	Medium(cm)	Groups
T3	1.84	A
T2	1.55	B
T1	1.42	C
T4	1.17	D

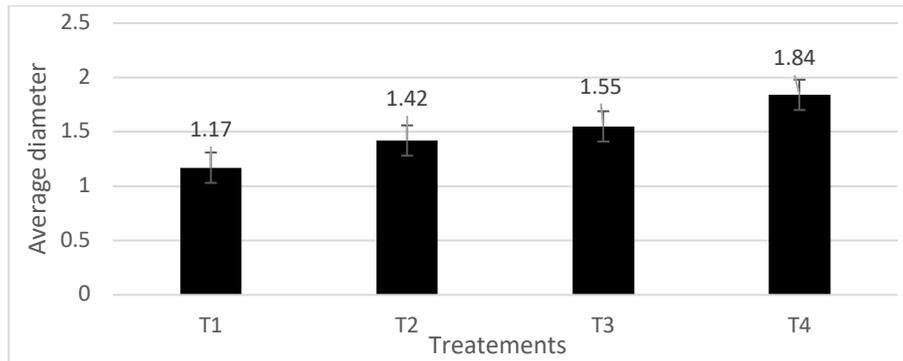


Figure 4. Average collar diameter by type of treatment

Biomass growth

Fresh biomass and dry stem biomass

The results of the variance analysis at the 5% threshold of fresh and dry biomass of the stems of Aleppo pine plants in table 7 showed that there was a significant difference ($F_{OBS} > F_{THE}$) between the different treatments tested.

The test of NEW MEN and KEULS at the threshold of 5%, highlights 3 homogeneous groups in table 8, were represented by the dominant group, which was obtained by the T3 treatment, which yielded fresh and dry biomass of the stems of the Aleppo pine with respective averages of 2,29g and 1.88g, a second group B represented by T2 and T1 treatments which gave values lower than those of the T3 treatment and a last group C which gave the lowest values in fresh and dry biomass with respectively 0.77g and 0.35g.

Table 7. Analysis of the fresh and dry biomass variance of the stem

Medium fresh biomass of stem					
Source of variation	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	15	0.90			
Var-fact1	3	1.65	3.99	3.86	S
Var-fact2	3	0.55	0.71		
Var-Residual	9	0.77			
Medium dry biomass of stem					
Source of variation	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	15	0.90			
Var-fact1	3	1.63	3.88	3.86	S
Var-fact2	3	0.52	0.69		
Var-Residual	9	0.78			

S***: very very highly significant, F OBS: Factor observed or calculated, F THE: Factor theoretical

Table 8. NEW MEN and KEULS (fresh biomass and dry biomass of stem/ treatment)

Treatment	Fresh biomass		Dry biomass	
	Medium (g)	Groups	Medium(g)	Groups
T1	1.29	B	0.84	B
T2	1.68	B	1.08	B
T3	2.29	A	1.88	A
T4	0.77	C	0.35	C

Fresh biomass and dry root biomass

The results of the the 5% threshold variance analysis of fresh and dry biomass from Aleppo pine roots in Table 9 showed that there was a significant difference (F OBS> F THE) between the different treatments tested.

Table 9. Analysis of the fresh and dry biomass variance of the roots

Medium fresh biomass of stem					
Source of variation	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	15	0.90			
Var-fact1	3	0.22	10.07	3.86	S
Var-fact2	3	0.04	1.74		
Var-Residual	9	0.02			
Medium dry biomass of stem					
Source of variation	DDL	Medium Square	F OBS	F THE	Significant
Var-tot	15	0.02			
Var-fact1	3	1.06	3.90	3.86	S
Var-fact2	3	0.01	1.41		
Var-Residual	9	0.01			

S***: very very highly significant, F OBS: Factor observed or calculated, F THE: Factor theoretical

The test of NEW MEN and KEULS at the threshold of 5%, highlights 2 homogeneous groups in Table 10, a dominant group A represented by the treatment T3 which gave the fresh and dry biomass of the roots of the pine plants of Aleppo with averages respectively 0,70 g and 0.39g, a second group B represented by treatments T2, T1 and T3 which gave low values compared to treatment T3 with respectively 0.34g, 0.28g, 0.16g for fresh biomass and 0.24g, 0.18g and 0.11g for dry biomass.

Table 10. NEW MEN and KEULS (fresh biomass and dry biomass of roots/treatment)

Treatment	Fresh biomass		Dry biomass	
	Medium (g)	Groups	Medium(g)	Groups
T1	0.28	B	0.18	B
T2	034	B	0.24	B
T3	0.70	A	0.39	A
T4	0.16	B	0.11	B

Discussion

Duchaufour (1983) indicated that resinous (Pine, spruce etc.) are species whose bedding is acidifying which cause a slowdown in bio-degradation and form humus of the type Moder or Mor characterized by a high lignin and lipid content with C/N greater than 50 are thus formed under the acidifying bedding, poor in nitrogen, Duchaufour, 1968, added that the type of litter that improves soil is the product of hardwoods in temperate climates (Aulnes, etc.). Their leaves are little lignified, rich in nitrogen and water-soluble compounds with C/N of 25. Their lipid and lignin content are remarkably low. They exert an influence on biological activity, resulting in good decomposition and rapid humification of organic matter. Mull humus is generated under the ameliorating litter. According to Leghari *et al.* (2016) nitrogen activates plant growth, promotes the growth and development of all aerial parts: twigs, stems and leaves which will give an advantage to the plants raised at the level of this substrate. We also found that fresh and old litter has acidic pH values which will acidify the soil more and more and causes problems of chemical assimilation and the activities of soil bacteria, while the pH of mixed litter is close to neutrality. Doucet (2006) states that pH is a significant element of soil chemistry and determines the availability of nutrients for plants and soil microorganisms.

The emergence is considered a first diagnosis of the success of a culture. However, a bad emergence can have several causes, namely the depth of the seedling (too deep or too superficial), drought or parasites. For all growth parameters, the T3 treatment gave the best results and the most efficient Aleppo pine plants, come in second position the T2 treatment followed by the T1 treatment compared to the control T4 without litter, these results have clearly shown the positive effect of litter on the development of Aleppo pine seedlings in nurseries, because litter constitutes the plant mass from leaves, branches and stems still less transformed that cover the soil. It contributes significantly to soil organic matter, which is an important source of plant nutrients (Young, 1989). It improves soil aeration, reduces erosion, and increases cation exchange capacity

(Bernhard-Reversa and Schwartz, 1997; Musvoto *et al.*, 2000). This action is also observed in the influence on the activity of soil microorganisms (Janssen, 1986) by a direct effect with the contribution of carbon substrate in soil-vegetation systems (Vance and Chapin, 2001). Soil microorganisms play a leading role in the transformation processes of organic matter including the mineralization of nitrogen essential for plant development. Dzwonko and Garwonski (2001) add that the removal of forest litter, a centuries-old practice, has the consequence of impoverishing forest soils and acidifying them. An experiment was conducted to account for this phenomenon. After 16 years of litter removal from a mixture of oak pines, the soil contained less Ca, P, and Mg, and had a reduced cation exchange capacity.

The comparison of the results between the effect of the three types of litter on the development of the plants has always shown that the treatment T3 (mixed litter) gave the best results for all the parameters studied indeed the old litter (under decomposition) represented by treatments T3 and T2 gave good results compared to fresh bedding treatment T1. The effect of decaying residues has been found to result in similar results in previous studies (Guisse, 1989; Thomsen and Christensen, 1996; Trinsoutrot *et al.*, 2000), in the study of seasonal variations in nitrogen mineralization, significant enrichment of soil mineral nitrogen (ammonium and nitrate) was observed under decomposing bedding (old litter). The effect of litter quality showed that mixed litter, that is, the mixture between the litter of the conifers and the litter of the deciduous trees (Aleppo pine and holm oak) gave very good results registered at the treatment T3 compared to the bedding of conifers (fresh or old Aleppo pine litter). Several studies have shown that the quality (biodegradability) and the amount of fresh organic matter arriving on the ground vary from one species to another. This results in equally varied effects on soil potential, the diversity, and effectiveness of decomposing organisms and microorganisms (Duchaufour, 1983, 2001; Binkley, 1995; Ponge, 2003; Albers *et al.*, 2004; Kelliher *et al.*, 2004). We generally distinguish the litters called 'improving', which are characterized by rapid degradation and turnover of the elements. These bedding are favorable to biological activity and offer great potential for plant regeneration. These are mainly hardwood species (birch, charm, alder, ash, oak, etc.). Other so-called 'acidifying' bedding, characterized by slower decomposition and a longer storage period, releases relatively large amounts of organic acids, lipids, and soluble phenolic compounds, modify the presence and/or activity of effective decomposing organisms and microorganisms, and, on the other hand, cause by acidification an intense alteration of minerals. This is often the case with coniferous litter (spruce, pine), (Toutain, 1987; Gallet and Lebreton, 1989; Ranger and Nys, 1992; Binkley, 1995; Rutigliano *et al.*, 1996). The bedding of

coniferous trees is generally less easily decomposable than those of deciduous trees, they tend to accumulate as the coniferous stands age this accumulation is accompanied by acidification and can cause, not only a loss of exchangeable bases, but also a premature alteration of reserve silicate minerals (Dzwonko and Garwonski, 2001; Albers *et al.*, 2004; Augusto *et al.*, 2002). The experiments conducted in this research allowed us to assess the response of Aleppo pine to certain bio-edaphic factors and underscored the notably advantageous effect of forest litter. The positive effect results in the better growth of the plants compared to controls without litter. Concerning the influence of different types of litter, we note particularly the notably beneficial of the mixed litter (Aleppo pine + holm oak) and, to a lesser degree for the old and fresh litters. Thus, under the effect of the mixed litter (Aleppo pine + holm oak) we find that the Aleppo pine seedlings were characterized by better growth of the aerial part, diameter of the collar, aerial and root plant biomass and a better germination rate. In practical terms, it appears that litter plays a significant role in improving the behavior of seedlings, while the presence of holm oak and Aleppo pine in a mixed setting can contribute to sustainability and the operation of the biogeochemical cycle involves essential mineral elements for the nutrition of forest species. Thus, there is a clear necessity to promote the establishment of mixed stands over monoculture reforestation.

Preserving leafy litter during silvicultural practices may necessary due to its beneficial effects on humus, which requires further research to examine the maintenance of diverse tree species mixes in plantation forestry to enhance our understanding of how each species contributes to the composition of forest humus.

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