Volume CIRS (24). Published April, 01, 2018
www.jnsciences.org
E-ISSN 2286-5314
JOURNAL OF NEW SCIENCES
WWW.JNSCIENCES.ORG

Study of the growth and development of *Acacia Cyanophylla* on different substrates from municipal solid waste.

Étude de la croissance et du développement d'*Acacia Cyanophylla* sur différents substrats provenant de déchets solides municipaux

W. OULED SEGHAIER[*1,2], C. ABBES[2], I. ZOUAOUI[2], N. JDIDI[2] AND B. HASNAOUI[2]

[1] Faculty of Science of Bizerte, Jarzouna (Bizerte) 7021
[2] Sylvo-Pastoral Institute of Tabarka, Jendouba University. BP. n ° 345, 8110 Tabarka (Jendouba), Tunisia

*Corresponding author: ouledseghaierwided@yahoo.fr

Abstract - Our work aims at the application of several biological and chemical parameters in order to determine the impact of variation in the levels of heavy metals and mineral elements in the soil for the growth of a forest plant *Acacia cyanophylla* since plants are good indicators of environmental pollution. We studied the impact of heavy metals on the germination and growth of *Acacia cyanophylla* cultivated in the soil of the three-controlled municipal solid waste of the Northwest of Tunisia and compared with plants of the same species cultivated in non-contaminated soil and soil receiving a mixing of a contaminated soil and a nursery soil.
Morphological Parameters (stem height, root length, leaf area, the ratio of the stem / root and biomass) was measured.
In the different substrates used, the morphological parameters vary significantly for the different criteria studied.

Keywords: municipal solid waste, heavy metals, morphological parameters, forest.

1. Introduction

Actually, the most important problem of pollution is directly related to human activity. Indeed, with the improvement of the standard of living and the development of the technology arises the problem of the disposal of the solid waste. In Tunisia, the installation of controlled landfills remains the most economical method used to reduce the environmental impact of this waste. In these landfills, bad odors resulting from the process of fermentation of organic matter, as well as high levels of heavy metals (cadmium, copper, zinc, manganese) can create poisoning problems in the environment or Human health.
The problem of the collection and treatment of solid household, agricultural, or industrial waste, is relatively recent in Tunisia, which was a country essentially concerned about its economic development. But, With the help of the European Union, of the World Bank and bilateral funds, the Tunisia implements for some years one program coherent and progressive control, removal and recycling of waste (Casalino 2005).
The different landfills studied, in the North-West of the Tunisia, are managed by municipalities, and considered as controlled. These landfills contain all kinds of garbage that can be even dangerous waste. These wastes are stacked and abandoned, uncovered, without any maintenance or monitoring.
They didn't ensure the protection of the groundwater or leachate recovery and there is no waste treatment system.
However, the ever-increasing load of solid waste had many effects on the environment and public health, such as explosions resulting from fuel gas generation: CO_2 and CH_4 (Johannessen 1999 ; EPA 2000), which also contribute to the greenhouse effect, as well as the contamination of groundwater (Micó et *al*.

2006), soil and plants (Özer et *al*. 2006) by heavy metals. Birds and other herbivorous domestic animals (sheep, goats, and cows) are attracted by these landfills, as they are used by the inhabitants for grazing. Thus, besides the fact of exposure to contamination, these animals can become vectors of contamination and diseases to their consumers (Campan 2007).
Far from the limit of landfill leachate migration and their release into the environment present serious environmental hazards (Adriano 2001), including vegetation as well as for those of surface and groundwater (Djikstra et al. 2004).
The aim of our study was to determine the impact of soil polluted by heavy metals impact on the growth of *Acacia cyanophylla* growth (seed germination and morphological parameters)

2. Materials and Methods

2.1. Setting in culture

Plantings having made in perforated polyethylene bags, to avoid the irrigation water stagnation, 20 cm depth and 12 cm in diameter, filled with different types of substrates: three municipal solid wastes (MSW1: Jendouba municipal solid waste, MSW2: Beja municipal solid waste, MSW3: Tabarka municipal solid waste), S1: 50% soil from municipal solid waste of Jendouba + 50% control soil, S2: 50% soil from municipal solid waste of Beja + 50% control soil, S3: 50% soil from municipal solid waste of Tabarka + 50% control soil and P: control soil (Table 1). So we have for one specie seven substrates. The bags implementation approved in the nursery at a rate of 90 bags / treatment / specie: (30 bags / species / repetition).
Analyses of substrates realized in the laboratory of the Sylvo - Pastoral Institute of Tabarka (Tunisia).

2.2. Plant Material

The wood species used for this study was *Acacia cyanophylla*.

2.2.1. Presentation of the species used

The genus *Acacia cyanophylla* is originating in southwestern Australia (Hopper and Maslin 1978). It has been introduced in North Africa for more than a century to serve, first, to ornamentation. Currently, it is used for various purposes, such as against silting and erosion as well as for the production of fodder and wood, particularly in semi-arid regions (Nasr et al. 1999).
Acacia cyanophylla can adapt to all types of soil. In addition, through its ability to fix atmospheric nitrogen symbiotically, it helps to regenerate fertility of the soil. Analysis of this species shows that it has a certain forage interest (HAMROUNI And SARSON 1974). In Tunisia, the so-called "strategic" plantations of *Acacia cyanophylla* cover an Area of 65,000 ha. They are used as standing stock, Mainly during drought periods and can be exploited by direct grazing or by Size of folding. The foliage and young twigs of this species constitute a food well-received by small ruminants. This type of forage is rich in protein, but Relatively low in energy. In addition, this species improves the soil, protects the environment against erosion and produces wood multi-purpose.
Acacia cyanophylla can reach 6 to 8 m, its root system, of the pivoting type, characterized by the presence of nodules (Lange 1959), harboring bacteria, capable of binding Symbiosis of nitrogen (Nakos 1977 ; Nasr and Diem 1987).
The rooting of this plant is very powerful both laterally and deeply ; for this reason. Acacia was adopted as a species of lands conservation and rehabilitation. This shrub prefers environments, receiving more than 250 mm of rainfall annual average. The edaphic requirements of *Acacia cyanophylla* become strictly above 400 mm.
It was much preferred by farmers because it provides a feed supplement to livestock. The Acacia trees are considered a reserve (Foliage and pods), mainly for the safeguarding of livestock during the winter seasons where the grass is rare and during drought years (PAF
2009).

2.2.2. Soil analysis

In the different substrates studied, the highest conductivity values were recorded in the substrates taken from landfill sites with a maximum at the soil level of the Jendouba landfill (Table 1).
Organic matter levels recorded in the three municipal solid wastes were 17 %, 20 %, and 23.49 % for Jendouba (MSW1), Beja (MSW2), and Tabarka (MSW3), respectively, and did not exceed 9 % in the

nursery substrate used as a control soil (soil pilot). The highest C / N ratio is obtained for substrates containing a mixture of nursery soil and soil from the Tabarka landfill (20,32), a C / N ratio of 16,38 is recorded at the substrate level of The municipal solid waste of Beja. Significant concentrations of Zinc and Manganese, Magnesium, Copper and Cadmium are detected in municipal solid waste of Beja and Tabarka and Jendouba compared to the values determined in the rest of the substrates used.

Table 1. Summary of the compared chemical parameters of different soil types used for the culture

Substrate	Jendouba MSW1	Mixture S1	Beja MSW2	Mixture S2	Tabarka MSW3	Mixture S3	Soil Pilot
pH	7,3	7,52	7,9	7,9	7,6	7,9	8,23
Conductivity (Micro Siemens)	2853,33	1055	409	225	2283,66	750	145
C%	17,2	13,76	20,64	10,13	23,49	30,49	8,6
Nitrogen N%	3,78	2,10	1,26	1,1	3,50	1,5	0,98
Ratio C/N	4,55	6,55	16,38	9,20	6,71	20,32	8,77
Olsen Phosphorus P (mg/Kg)	8,24	3	15,24	12,55	9,88	2,5	0,85
Zinc Zn (mg/Kg)	15,53	45	102,21	15,7	135,49	17,5	15
Manganese Mn (mg/Kg)	1479,26	600	1458,27	550,2	1534,23	545	270
Magnesium Mg (mg/Kg)	52,84	50	98,17	45	130,23	48	126
Copper (mg/Kg)	311.83	40	194.44	12	198.55	17	30
Cobalt CO (mg/Kg)	1,85	0,99	1,650	0,2	1,917	0,5	0,99
Sodium Na (mg/Kg)	1172,61	560,23	279.77	90 ,10	942,10	120,32	75
Cd (mg/Kg)	31,73	6,5	40.76	4	42.910	3,5	2

2.3. Determination of Morphological Parameters of plant seed germination

2.3.1. Plant seed germination

We determined the rate of germination of the studied species when we noted a homogeneous seedling emergence.

2.3.2. Measurement of Leaf Area

Leaf area determined using Mesurim software and scanned images. It was measured on a sample of 10 leaves taken randomly in the middle of the plant (median sheets). The value estimated in mm^2.

2.3.3. Collar Diameter

It corresponds to the diameter of principal stem, at the contact zone between the aerial part and the root part. It is important as the height of the stem factor.

2.3.4. Height of the Stem

The height of the stem measured starting from the collar to the base of the bud terminal (Thompson 1985).

2.3.5. Root Architecture

The root length of the seedlings was estimated by a grid of 1 cm * 1 cm by counting the number of the intersections of the root with the position of each square of 1 cm * 1 cm. The roots of each plant fixed on the grid to help with counting. Once counting finished, the root will be cut off so that it does not recounted.

The total number of intersections for each order converted into length according the relation modified of Tennant (1976).
Root length = (11/14) * Number of intersections * the grid unit
So for a grid of 1 cm x 1 cm, we have:
Root length = 0.7857 * Numbers of intersections (cm)
Ratio Aerial Root Biomass
The use of the ratio BA / BR (based on dry weight) is one of the most morphological criteria used. It is considered as a balance measurement between the perspiring surface and the absorbent surface of a plant. According to Russel (1977) a value of 1, 4 corresponds to better balanced seedlings. However, its use is limited because of its variation depending on the size of the plant (Ledit et *al*. 1970).

3. Results

3.1. Seed germination

Data in fig1 shows that the germination rates of *Acacia cyanophylla* vary from one treatment to another. The highest germination rate was recorded on a substrate containing a mixture of soil from municipal solid waste (station Jendouba and Beja) and control substrate (98%).
The germination rate in different treatments used to be over than 70%.

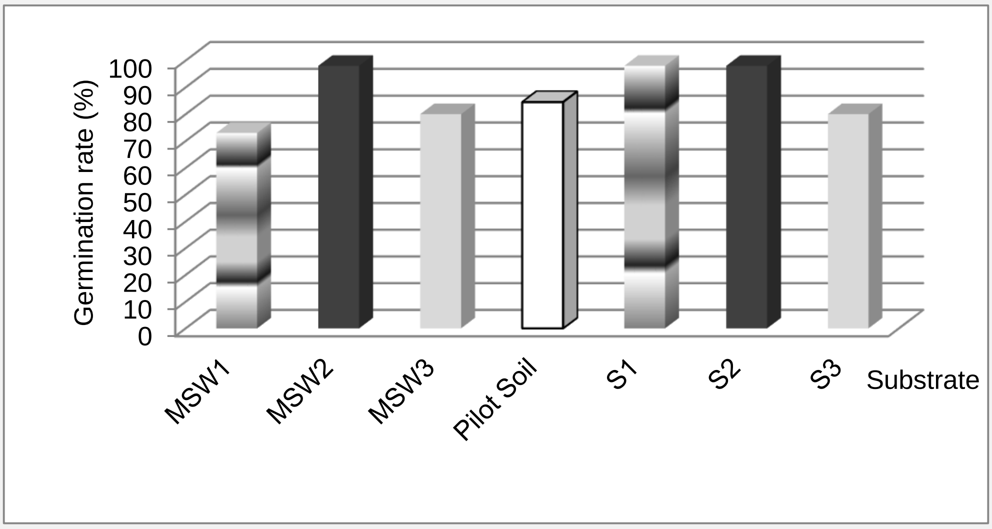

Figure 1. The rate of germination after 60 days of the date of sowing.

(S1: MSW Jendouba, S1 + P: 50% control soil + 50% soil MSW Jendouba, S2: MSW Beja, S2+ P: 50% control soil + 50% soil MSW Beja, S3: MSW Tabarka, S3 + P: 50% control soil + 50% soil MSW Tabarka, Pilot soil).

3.2. Leaf area

The results of the variance analysis of the *Acacia cyanophylla* leaf area in the different substrates (soil taken from the different municipal solid waste on the North west of Tunisia, a soil containing a mixture of soil from landfill And nursery soil compared to a nursery soil considered as control soil) show that the station factor and the site factor have a significant effect on variation in leaf area of plants. The site station interaction is not significant.
Figure 2 shows the variation of leaf area in *Acacia cyanophylla* according to the substrates, which was in the range of 2500 to 4500 mm^2.
The leaf area highest values are recorded in plant introduced in a soil having undergone a deposit of household waste and it was around 4800 mm2 (MSW1, MSW2) and decrease in plants taken from a soil containing a mixture of soil from landfill and nursery soil (4305 mm2).

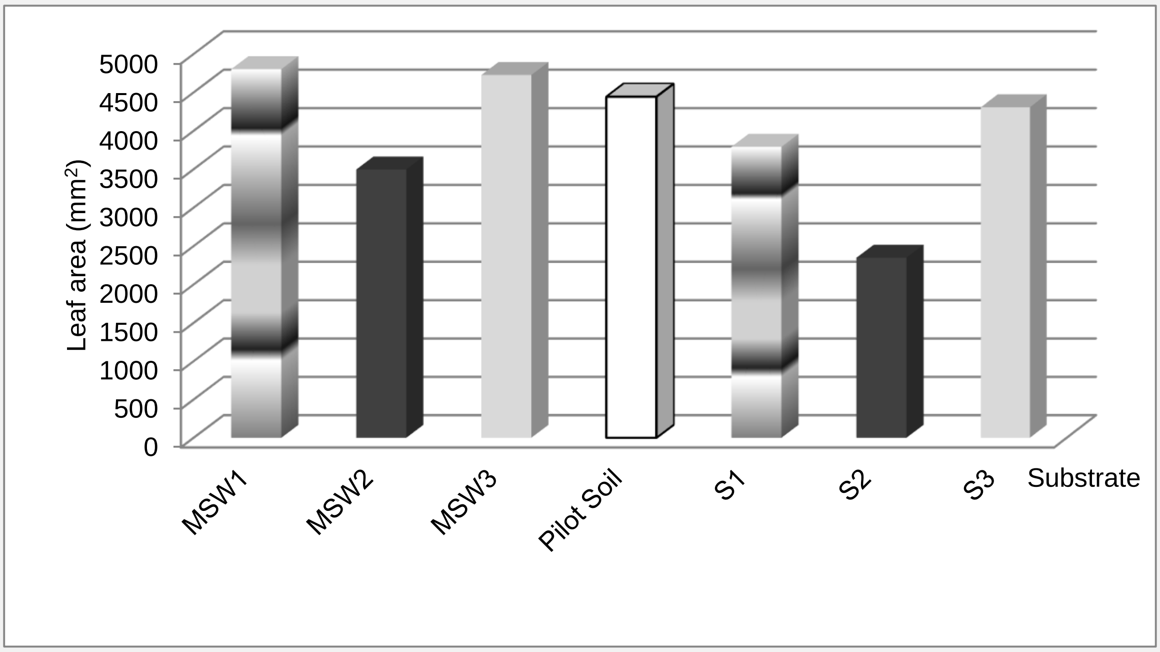

Figure 2. Variation of leaf area in *Acacia cyanophylla* according to the substrates.

3.3. Collar Diameter

The results of the analysis of the collar diameter variance in different substrates show that the site factor exerts a significant effect ($P < 0.05$). While the variation of the station and the site station interaction have no significant effect.

The collar diameter increases considerably in soils receiving a mixture of soil from landfills and soil from the nursery.

The highest value is observed in soil taken from the landfill of Beja (11,8 mm).

Acacia plants grown on soil containing 50% soil from landfills have larger diameters than those on control soil, up to 50%.

At the level of the landfill substrates, the maximum collar diameter is obtained for the Tabarka substrate (7,83 mm).

In the control substrate, the average collar diameter for *Acacia cyanophylla* is 6,63 mm.

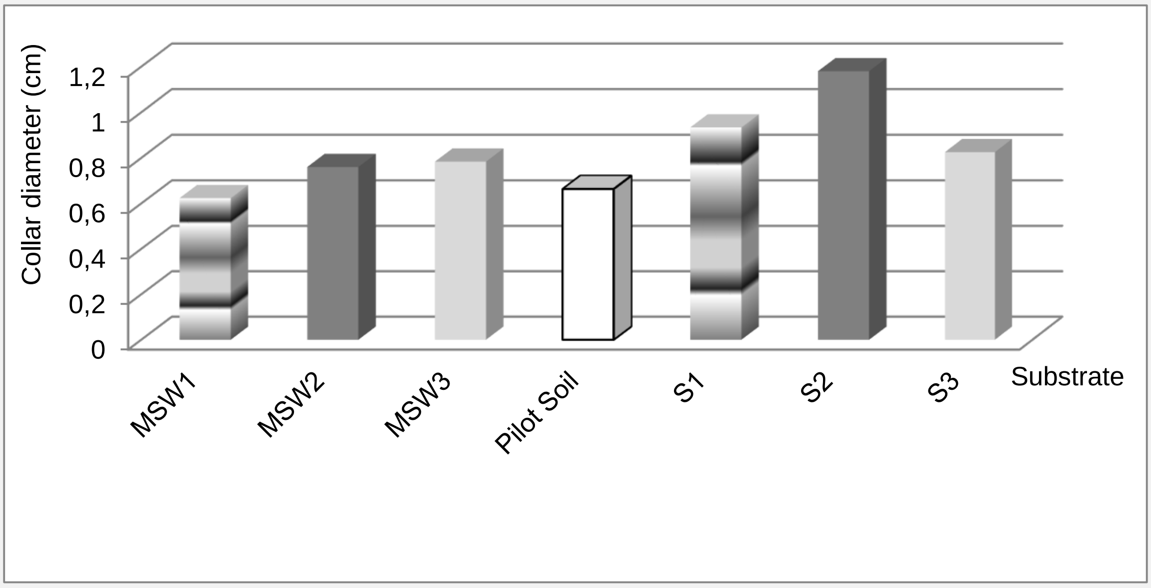

Figure 3. Variation in collar diameter in *Acacia cyanophylla*

3.4. Height of the Stem

The results of the analysis of the variance of the *Acacia cyanophylla* in the different substrates show that the height of the stem varies significantly by locality (P <0,01). The station factors and the interaction stations and sites are not significant. For *Acacia cyanophylla*, the length of the stem increases considerably in soils receiving a mixture of soil from landfills and soil from the nursery (Figure 4). The highest value is observed in the substrate S1 (140 cm). On the soil of municipal solid waste, the length of the stem is maximized on the Jendouba and Tabarka (88 cm). On the control substrate, the average length of the *Acacia cyanophylla* stem recorded was 124 cm.

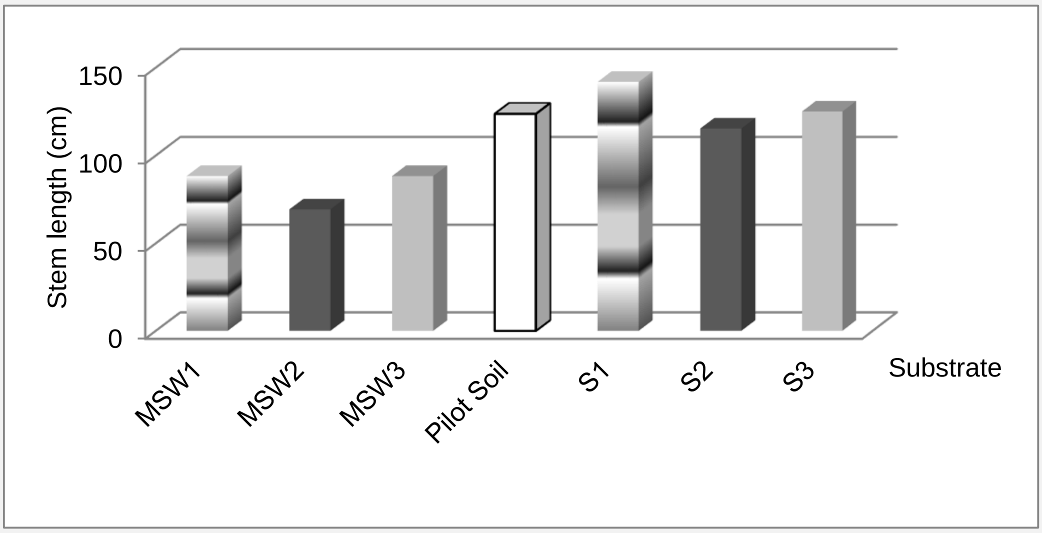

Figure 4. Variation of the stem length in *Acacia cyanophylla*.

3.5. Ratio Height Diameter

The results of the analysis of the variance show that the substrate factor exert a highly significant effect (P <0,001).

The most important ratio was 17. It was recorded for the plants raised on uncontaminated soil (nursery soil) (Figure 5).

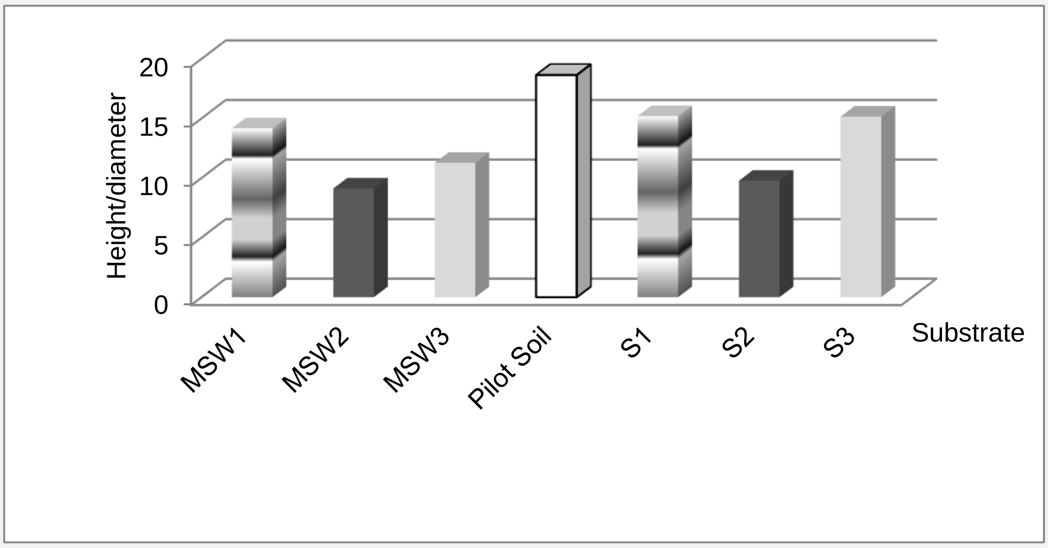

Figure 5. Variety of H/D ratio in *Acacia cyanophylla* depending on the substrates.

3.6. Root length

The results of the root growth variance analysis show that both station and site factors exert a highly significant effect (P <0,001). The interaction station * site had a significant effect on total root length growth (P <0,05). *Acacia cyanophylla* root growth was greater on the control soil (800 cm) than on the soil from the landfill, where it decreased by half (400 cm) after 12 months of rearing.

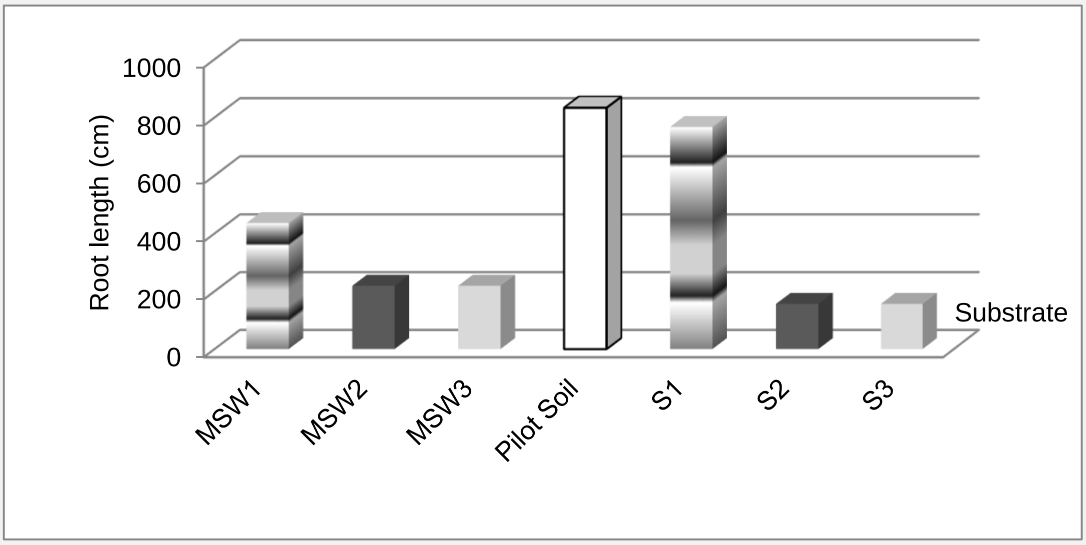

Figure 6. Variation of the root length in *Acacia cyanophylla*

3.7. Ratio Height Stems/ Root length

For *Acacia cyanophylla* introduced in different substrates the analysis of variance shows that the substrate factor exert a highly significant effect on the variation of this ratio (P <0,001)

The ratio, height Stems/ root length increases considerably on a soil containing a mixture of contaminated soil and nursery soil (this is the case for the Beja and Tabarka stations) (0,8) (Figure 7). In the different municipal solid waste used, the ratio determined is most important at the landfills of Tabarka and Beja (0,5) and is equal to 0,2 at the level of the Jendouba landfill.

At the pilot soil, the ratio obtained is equal to 0,14.

In the various substrates studied, the ratio H / R is always less than 1.

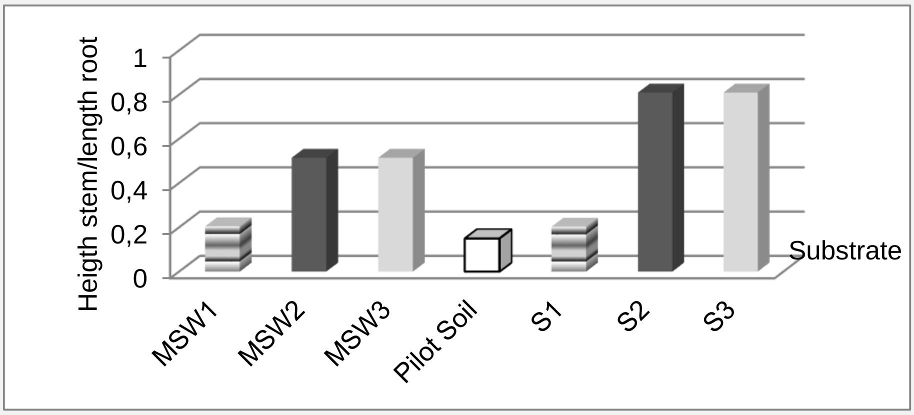

Figure 7. Variation of the height stem, length Root ratio in *Acacia cyanophylla* in different substrate.

3.8. Ratio Stem, Root Biomass

The analysis of variance shows that the substrate factor exert a highly significant effect (P <0,001) on the ratio A/R (aerial biomass / root biomass).

The results for the variation of the BA / BR ratio illustrated in figure. 8 which shows that the most important ratio is recorded in the substrate from the landfill of Tabarka (BA/BR is 4). This ratio is equal to 1,19 for the plants raised on the substrate taken from the landfill of Jendouba and decreased to 0,69 on the substrate taken from the landfill of Beja. In substrates containing a mixture of municipal solid waste and nursery soil, the highest ratio is recorded in the Jendouba substrate (4,14). At the pilot soil the ratio BA / BR obtained is 2,78.

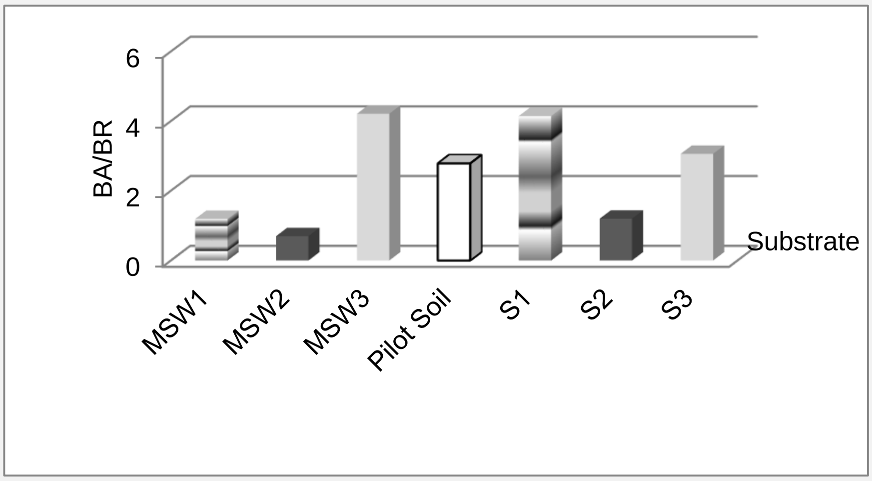

Figure 8. Variation of BA / BR ratio in *Acacia cyanophylla* according to the substrates

3.9. Discussion

Germination study shows that for the plants of *Acacia cyanophylla* planted on a municipal soil waste of Beja or soil containing a mixture of landfills and nursery (case of Jendouba and Beja) are in agreement with those of CEMAGREF (1983), which considers that the germination rate is satisfactory from 85%. Our results are confirmed by Ouanouki and Igoud (1993), who worked on two species: the Maritime pine and Acacia, they found after 6 months of planting, a high survival rate reaching its maximum of 87,5% for Acacia through better nutrition in mineral elements.

Study the variation of the height of the stem of the *Acacia cyanophylla* shows that values standards are obtained on soils having received a mixture of soil subjected to the deposit of waste and soil of nurseries. Our result coincides with the results of Tamgholt (2005), which shows an increase of the length of the stem of Cedar of the Atlas cultivated on substrates made from sludge of 11,79 %.

Benbrahim and al. (2003), results also showed a beneficial effect of the application of liquid sludge on the total height of maritime pine trees. Growth at the level of three studied landfills is inhibited; their rate of reduction is of the order of 60% by report to the pilot soil. This is the probably to the toxicity of the environment by heavy metals, and Salt stress affecting the growth of the stem. This is confirmed by the results of Cicak (2002).

The diameter of the collar is most important on a soil containing a mixture of substrate. The similar results were observed for *Quercus suber* plans amended with sludge liquids that show an increase of 22% of the trunk as the trees of the plots pilot soil (Karoune 2007).

The H/D ratio is a key indicator to evaluate the effect of the competition for light According to Jobidon (2000) and that in this sense, it reflects the growth potential of seedling in a plantation.

According to the standards cited by Lamhamedi et al. (1997), the ratio of robustness: height diameter expressed in (cm/mm) should be less than 7. In the different substrates studied, the report of strength is higher than 7.

The ratio height stem / root length is higher in the different substrates used compared to the pilot soil, this may explain the high rate in fertilizer items containing these substrates compared to the control soil.

This is confirmed by Villeneuve (1999), who found that, excess nitrogen promotes the elongation of stems to the loss of the maturation and the root growth which may lead to inadequate supply of water and in mineral elements. Similar results were observed for the oak plants collected on waste sludge (Karoune 2007)

The height / length root ratio T/R of *Acacia cyanophylla* on different substrates is less than 1.

Brower (1983) reported that the growth of air and root systems was following a strict harmony and the relationship between these two systems is predictable on a wide variety of circles. However, variations of this report are sometimes frequent. According to Ferchichi (1990), rich and well fed soil water can promote the growth of aviation; in

However, a soil poor and little wet, at least at the level of the superficial horizons, is underground development.

The A/R ratio is considered to be an index of equilibrium between the surface of sweating (leaves) and the surface of absorption of a plant (roots) (Lamhamdi et al. 1997).

A weak report indicates that the roots are abundant compared to leaf biomass, and that can tolerate and survive in drought after planting conditions.

On the other hand, a high ratio means that the roots are not abundant and that this type of plant is going to be more sensitive to water stress especially in semi-arid areas or in sites where demand evaporation is high (Lamhamdi et al. 1997).

Biomass PA/PR report has been tested by many authors as a stress marker (Kaufman 1977)

According to Kelly et al, (1979), the reduction of the biomass of the root would have implications obvious as for air production and the survival of the whole plant, these comments raise the issues of the impact of heavy metal on forest regeneration in areas where heavy metals are introduced.

This report varies between 0,5 and 4 for the Acacia for different substrates and reached it maximum for soil from municipal solid waste of Tabarka. This report expresses the plasticity of the tree and capacity of resistance to salt (Duke1983).

4. Conclusion

The morphological parameters vary significantly in different substrates used for the criteria studied.

Indeed, we noted in the foliar surface that the maximum value is saved in the soils taken from the landfills.

For the length of the stem, the diameter of the collar. It is at the level of substrate pilot soil that it has registered the lowest value. So, the length of the root's maximum on a substrate that is uncontaminated.

It was also noted that there is a gain of aerial and root biomass at the level of the plants subject to a deposit of garbage.

The use of a ground from the landfill in whole or in mixture with a nursery ground has changed significantly the morphological parameters for *Acacia cyanophylla.*

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