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Improvement Tests on the Germination in *Lippia Multiflora*: Influence of Some Factors Related to Soil on Germination and Seedling Development

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Improvement Tests on the Germination in *Lippia Multiflora*: Influence of Some Factors Related to Soil on Germination and Seedling Development

Abstract

Studies on the germination and growth of seedlings of *Lippia multiflora* were conducted on several types of substrates. They consisted of germination tests and monitoring of seedling growth. The results showed that the best germination rates were recorded with the batch of seeds germinated on substrates made of a mixture of soil and manure (S2 = 82.67% and S3 = 65.33%). After 60 days of culture, the most significant heights of the plants of *Lippia multiflora* were recorded in culture media S2 (7.48 cm) and S3 (5.46 cm), with an average number of leaves 7; 12 and 6, respectively for the circles S1, S2 and S3. The root elongation is more important in substrates S2 (6.3 cm) and S3 (4.74 cm). The Pearson correlation tests carried out indicate that the contribution of organic matter in the substrates improves seedling development in *Lippia multiflora*.

Keywords: *Lippia multiflora*, Germination and Substrates

Introduction

Savannah tea (*Lippia multiflora*), aromatic species belonging to the family Verbenaceae, is found naturally in Côte d'Ivoire, from the forest-savanna contact area (Baoulé V) to the North (Yao-Kouamé *et al.*, 2009). The plant enters into the composition of some improved traditional medicines in Africa (*Malarial*® in Mali and *Tetra*® in Congo). It is used for the treatment of various diseases, such as hyperthermia and blood pressure disorder (Abena *et al.*, 2003; Etou-Ossibi *et al.*, 2005). Numerous studies indicate that the aqueous extract or essential oil of the plant have pharmacological (Hondi-Assah *et al.*, 2003), pesticides (Oladimeji *et al.*, 2000; Ossou *et al.*, 2008) and cosmetics properties (Kanko *et al.*, 2004). Today, this species is commercially available and is an export crop in Côte d'Ivoire (Yao-Kouamé *et al.*, 2009; N'Guessan *et al.*, 2010). However, like the savannas of sub-Saharan Africa, agro-ecological areas, where *Lippia multiflora* grows, belong to fragile ecosystems based on coexistence between herbaceous and woody stratum. They are dynamic systems whose evolution depends on

the intensity of environmental factors (such as drought, fires, farming, and culture). The plant is sometimes destroyed during fires and clearing for crops (Yao-Kouamé *et al.*, 2009). In addition, leaves-picking practices are very destructive. In fact, when the leaves are higher to be cut, the stems are often broken (Alui *et al.*, 2011). To avoid the loss of this plant, it is appropriate to think of his protection. That's why it is necessary to try to understand the techniques of multiplication in the case sexual germination. These studies once made will lead to the development of a new agricultural export produce for Côte d'Ivoire. The objective of this work is to identify opportunities to promote seed germination and seedling development of *Lippia multiflora* using different substrates that are within the reach of people.

In fact, this study was conducted in order to obtain a high and uniform germination, and good seedling development. For that, we compared the effect of substrates ensuring a better germination rate, good growth and healthy development of seedlings.

Materials and Methods

Materials

Plant Material

Lippia multiflora is a plant belonging to the family Verbenaceae that are plants consist of grasses and shrubs, often flavored with strong smell. The plant is biennial and is about 2-4 meters high (Eyog *et al.*, 2000). The flowers are small, whitish (Watson and Dallwitz, 1992; Ameyaw, 2009), 5 to 7 mm width and 5 mm to 2 cm length (Eyog *et al.*, 2000). At maturity, the ears bear seeds and have in this case, usually a spherical shape (Alui *et al.*, 2011). *Lippia multiflora* seeds are small and dry. Their observation to the microscope shows a spheroid shape, slightly flattened on the hilum side (Figure 1).

Source of *Lippia Multiflora* Seeds

Seeds used in the germination tests (*Lippia multiflora* seeds) were collected at their optimum morphological and physiological maturity during the year 2011, at the experimental site of Tiébissou, in the context of research activities conducted on *Lippia multiflora* by the Department of Soil Science, Faculty of STRM, University Felix Houphouet Boigny. Integrity seeds were selected and stored on cardboard in a well ventilated area until planting time.

Sources of Soil and Manure

Soils used were from the village of Bofia in the Department of Tiébissou, in middle of Côte d'Ivoire. Droppings were collected in the Coco services business located in Abobo and tertiary sand in Port-Bouet, in Abidjan District.

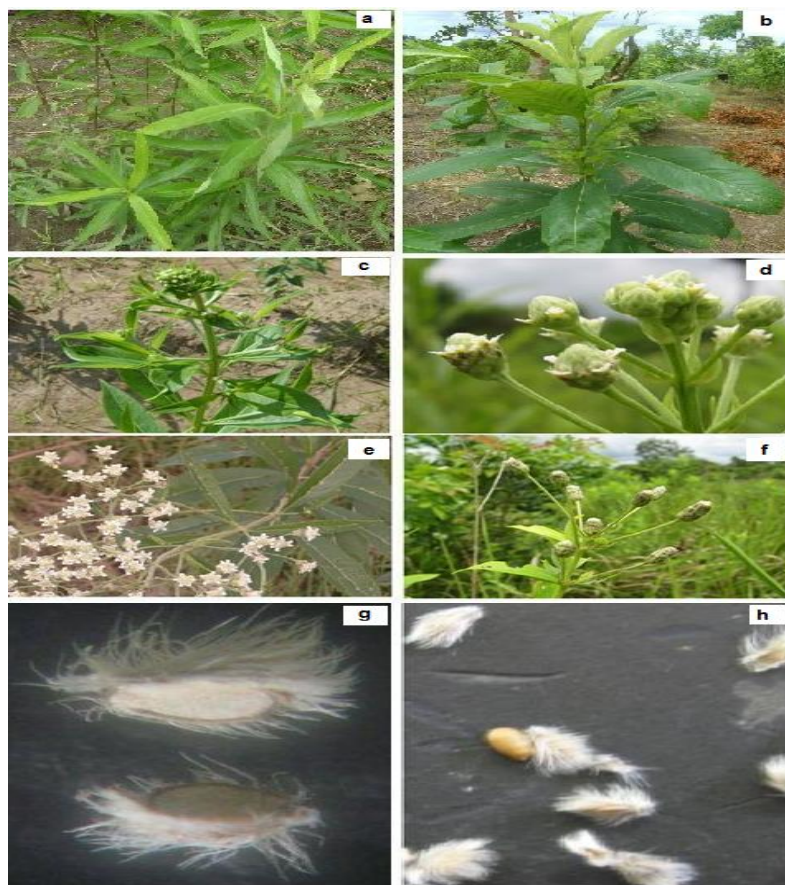


Figure 1: General Aspect of *Lippia multiflora*

a = long thin leaves and light green, b = broad leaves and long green sailor, c = cob formation, d = flower formation, e = end of flower development, f = cob maturity, g = seeds flattened side of the hilum (magnification $\times 3$) and h = seed without its envelope (magnification $\times 3$)

Methods

Obtaining Manure, Soil and Soil-manure Mixtures

Manure

Manure noted M in the experiment is composed of manure and litter of wood shavings, from breeding ground for broilers. The manure was collected and stored in the open air for 43 days because it cannot be incorporated in fresh state into the soil, because it contains pathogens (Aba *et al*, 2011).

Soil

Soil samples used for germination tests were collected in two distinct areas (Abidjan and Tiébissou). In Abidjan, samples were taken in areas intended for vegetable crops in the district of Port-Bouet. It is essentially tertiary sand noted Soil 1 in our experiment. At Tiébissou, ferralsols samples were collected from an area of natural population of *Lippia multiflora* (Soil 1) and another area where the plant was not observed (Soil 2). All these samples were carried out on a horizon band from 0 to 60 cm and packed in a plastic bag secured with a rubber band and labeled with references to the date and place of collection. To minimize the risk of tearing during

transport, the bags containing the samples were systematically doubled.

Soil-manure Mixture

The different samples obtained were from the mixture:

- manure + Soil1 (50% of M + 50% of Soil1), weighing 92.5 grams each for a total weight of 185 g;
- manure + Soil2 (50% of M + 50% of Soil2), weighing 92.5 grams each for a total weight of 185 g;
- manure + Soil3 (50% of M + 50% of Soil3), weighing 92.5 grams each for a total weight of 185 g;

Analyzes of Samples (manure, soil and soil-manure)

A set of 7 samples underwent standard laboratory soil analyzes in ESA (Agronomy High school), located at INP-HB of Yamoussoukro. These analyzes concern the water pH (pHe), organic carbon (C), exchangeable bases (Ca^{2+} , Mg^{2+} and K^+), the cation exchange capacity (CEC), total nitrogen (N), total phosphorus ($\text{P}_2\text{O}_5\text{t}$) and assimilable ($\text{P}_2\text{O}_5\text{ass}$), the particle size (A, Lf, Lg, Sf and Sg), iron (Fe) and manganese (Mn). The index of crusting (IC) was calculated (Table 1).

Table 1: Physico-chemical Characteristics of Manure, Soil and Substrates

Physico-chemical Characteristics		Treatments						
		Manure	Soils			Substrates		
		M	Soil ₁	Soil ₂	Soil ₃	S1	S2	S3
Particule size (g.kg ⁻¹)	A		7,5	13,7	14,11	7,11	15,2	13,88
	Lf		2,11	4,73	3,3	2,14	4,25	3,22
	Lg		3,8	4,8	4,64	3,72	4,15	4,67
	Sf		14,2	23,38	22,54	14	22,24	22,11
	Sg		72,2	53,38	55,41	72,89	54	55,32
index of crusting (IC)			0,46	0,49	0,55	0,44	0,57	0,55
Organic matter (g.kg ⁻¹), Nitrogen (g.kg ⁻¹) et Phosphorus	C	35,4	0,62	1,37	1,05	15,06	25,81	17,31
	MO	60,88	1,06	2,35	1,80	25,9	44,39	29,77
	N	1,56	0,04	0,22	0,25	0,45	0,67	0,81
	C/N	22,69	15,5	6,22	4,20	33,46	38,52	21,37

(mg.kg ⁻¹)	P ₂ O ₅ t	16,32	132,4	288,66	319	143,1	295,87	321
	P ₂ O ₅ ass	1,4	11,75	29,33	22,58	14,5	30,11	23,62
Acidity and cation complexes (cmol.kg ⁻¹) exchange	pH _{eau}	7,9	5,3	5,7	6,2	6,1	6,5	6,3
	pH _{kcl}	7,7	5	5,4	6	5,8	6,3	6,1
	Ca ²⁺	7,42	0,02	0,07	0,04	0,45	0,87	0,62
	K ⁺	2,77	0,03	0,06	0,09	0,11	0,15	0,17
	Mg ²⁺	2,51	0,11	0,31	0,83	0,47	0,97	0,84
	CEC	18	7,88	13,66	13,87	10,6	21,22	23,45

Filling, Weighing, Device and Sowing

Manure, soil and substrates were deposited each in aluminum tins (radius = 6 cm, height = 6 cm and weight = 10 g), carefully perforated to allow passage of water (**Figure 2a, 2b and 2c**). A sample of substrate weighting 195 g has been randomly chosen to conduct the

experiment. Total of 7 substrates repeated 3 times (21 tins) were obtained and arranged in blocks of Fischer in a space provided. The seeds were sown at a rate of 25 per container in an upright position, because of their small size. Seeds were then gently covered with a thin layer of each substrate (**Figure 2d**).



Figure 2: Experimental Device

a = aluminum cans perforated at the base, b = weight of the box (10 g) c = weight of the substrate made and d = indications of different treatments and complete block of Fischer.

Methods Expressing the Results and Analyzes Formulas

Germination Percentage

The counting of germinated seeds and the cumulative percentages of germination were performed every 3 days for 10 days. A seed is considered germinated when the radicle becomes visible (Mbaye *et al.*, 2002). Thus, the rate of germination (% G) at a given time is expressed by the ratio of the number of germinated seeds on the total number of seeds sown.

$$\% G = \frac{n}{N} \times 100$$

n = number of germinated seeds and N = total number of seeds sown

Speed of Germination

The germination rate can be expressed by the median germination (Scott *et al.*, 1984) or by the average time of germination (the time at which it reaches 50% of the seeds germinated) (Como, 1970). In our study, we used the mean germination time (TMG), which is calculated as follows:

$$TMG = \frac{\sum_{i=1}^{10} n_i t_i}{N}$$

With n_i = number of seeds germinated at time t_i (i ranging from 1 to 10) and N = number of seeds germinated at the end of the test.

Seedling Growth

The study of seedling growth is to characterize the speed of morphological development allowing the seedling to adapt to the actual conditions of the environment. The parameters studied are height growth, morphology and health status. Thus, using a carpenter tape measure, seedlings height (evolution of the stem) was monitored for 60 days. Also, simultaneously, a count of the number of functional leaves was it made. Regarding the morphological study, three randomly selected seedlings were extracted by treatment every 10 days for observations to assess the evolution of the root system.

Statistical Analyzes of Data

The results were statistically analyzed using the software Statistica 7.1 through analysis of variance (ANOVA). Whenever a significant difference was found, ANOVA is complemented by TUKEY test, which allows identify the variables significantly different of others. The averages of the variables were separated at the probability threshold $P < 0.005$ and $P < 0.001$. Correlations Test between variables were performed using the software Tanagra. Correlation measures the relationship between two or more variables. The correlation coefficient used is the Pearson r, also called linear correlation coefficient. The correlation coefficients were in the range from -1.00 to 1.00. Values -1.00 and +1.00 respectively represent a perfect negative or positive correlation and value of 0.00 represents a lack of correlation or independence between variables.

Results

Rate and Speed of Germination

Considering the criteria and germination parameters defined above, we find that 10 days after the sowing, germination rate (% G) of *Lippia multiflora* seeds differs statistical analysis, according to the type of treatment (FCAL = 25.72; ** PC = 0.00). Thus, the rate is 0% for manure (F), 18.67% 46.67% and 21.33% respectively for soil 1, soil 2 and soil 3. For substrates S1, S2, S3, germination rates are respectively 22.67%, 82.67% and 65.33%. The substrate S2 has the highest rate of germination. The germination rate is maximum at the 6th day for treatments Soil3, S1 and S3; 7th day for Soil1 and S1; 8th day for S2 (**Figure 3**). The mean germination time (TMG) registered shows a different of one day according to the culture medium. It is 7 days for seeds germinated in soil and 6 for those germinated in the substrates. The best results were recorded at the soil-manure treatments (S2 and S3) and soil 2.

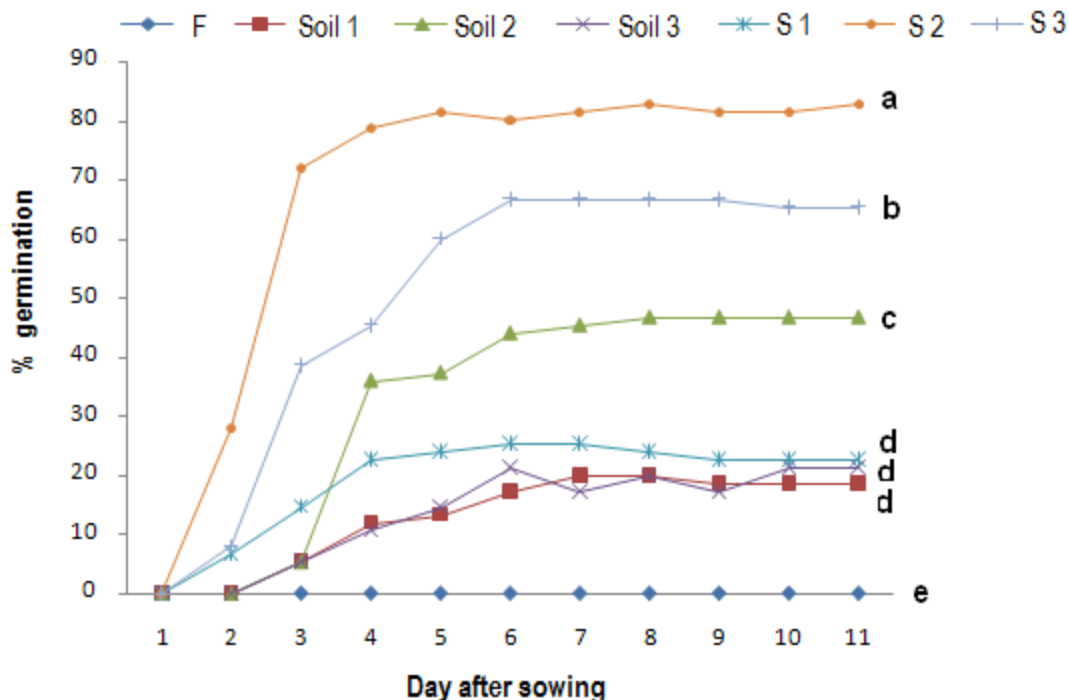


Figure 3: Evolution of Germination Rate in Different Treatments

(The same letters on different curves indicate that there is no significant different at the 1% level)

Growth Rate of Seedlings

Height of Stem

The data in Table 2 show the effect of treatment on the evolution of aerial growth of seedlings of *Lippia multiflora*. Examination of these data reveals that plants of *Lippia multiflora*, grow best on the mixture (S2). In fact, the length of the stem (7.48 cm) was significantly greater than those recorded with the other treatments tested (Figure 4).

Number of Leaves

Analysis of the results summarized in Table 2 shows that the number of leaves produced by seedlings was significantly affected by the type of treatment (Figure 4). Indeed, the best results were obtained in medium S1 (7 leaves) and S2 (12 leaves).

Root Growth

The average length of the primary root measured after 60 days of culture on the different treatments is given in Table 2. The most important root elongation (Figure 5) were recorded in plants grown on substrates S2 (6.3 cm) and soil 2 (5.14 cm).



Figure 4: Young Seedlings of *Lippia multiflora* After 60 Days of Sowing



Figure 5: Root System of *Lippia multiflora* Seedlings 60 Days after Sowing

Table 2: Change in Height, the Average Number of Leaves and the Average Length of the Main Root of *Lippia Multiflora* Seedlings After 60 Days of Culture on Different Treatments

Treatments		Seedling height (cm)	Number of leaves	Root mean length (cm)
Manure	M	0 ± 0.00 e	0 ± 0.00 e	0 ± 0.00 e
Soils	Soil 1	1 ± 0.15 d	2 ± 0.13 c	2.34 ± 0.15 c
	Soil 2	2.76 ± 0.11 c	5 ± 0.17 b	5.14 ± 0.11 b
	Soil 3	2.1 ± 0.15 c	4 ± 0.12 b	2.22 ± 0.08 c

Substrates	S1	2.5 ± 0.15 c	7 ± 0.2 b	2.68 ± 0.08 c
	S2	7.48 ± 0.23 a	12 ± 0.18 a	6.3 ± 0.15 a
	S3	5.46 ± 0.11 b	6 ± 0.27 b	4.74 ± 0.15 b
F _{cal}		148.16**	205.95**	165.17*
P _{cal}		0.00	0.00	0.021
P _{théor}		≤ 0.01	≤ 0.01	≤ 0.05

Means followed by the same letter in the same column are not significantly different at $\alpha < 0.05$, according to the method of TUKEY. ns = not significant, * = significant, ** = highly significant.

Relationship between Physico-Chemical Parameters of Different Treatments and the Growth Rate of *Lippia Multiflora* Seedlings

It is to make a connection (correlation) in a first time between physico-chemical parameters of substrates and evidence indicating the rate of growth of *Lippia multiflora* seedlings. The correlation is then sought between agronomic characteristics of seedlings. The syntheses of different correlation matrices were summarized in Table 3. Thus, at the Soil 1, the statistical analyzes show some strong correlations between: the height (H) plants and Sf (r = 0.95, Pr (> |t|) = 0.048), H - C (r = 0.98, Pr (> |t|) = 0.0113) and H - C/N (r = 0.98, Pr (> |t|) = 0.01). Agronomic parameter that interacts most with the characteristics of the soil was the height of the stems. Regarding the soil 2, pairs of variables that give the strongest correlations are: H - C (r = 0.99) H - N (r = 0.99), H - Nf (r = 0.99) and H - L root (r = 0.99). The lowest

among significant correlations was observed in the pair of variable % G - MO (0.95). Stem height is the parameter agronomic which interacts most with soil parameters. Pearson correlation test performed between the rate of seedling growth and elements of Soil 3 was used to select two pairs of variables that are statistically significant: Nf - P₂O₅ass (r = 0.99) and root L - N (r = 0.98). For different substrates, significant correlations obtained are governed at S1 through the pairs of variable % G - C (r = 0.97) and root L - C (r = 0.97). A significant and negative correlation H - Sg (r = - 0.96), has been observed in this treatment. As for treatments S2 and S3, the strongest correlations are respectively located at the root pair of variables L - C (r = 0.97) and Nf - C (r = 0.97). The stem length and the number of leaves respectively at treatments S2 and S3 are the agronomic characteristics which interact most with the physico-chemical parameters of the treatment.

Table 3: Correlation between the Parameters of Different Treatments and the Rate of Growth of *Lippia Multiflora* Seedlings

Treatments	Pair of variables	r	r ²	t	Pr (> t)	Pr _{théor}	Significativit y
Soil 1	H - Sf	0.956	0.9143	4.6188	0.0438	≤ 0.05	*
	H - C/N	0.9899	0.98	9.8995	0.0101	≤ 0.05	*
Soil 2	H - C	0.99	0.98	9.9046	0.01	≤ 0.05	*
	H - N	0.99	0.98	9.9029	0.01	≤ 0.05	*
	H - L root	0.99	0.98	9.9028	0.01	≤ 0.05	*
	% G - MO	0.958	0.9178	4.7261	0.042	≤ 0.05	*
Soil 3	Nf - P ₂ O ₅ ass	0.9969	0.9938	17.8494	0.0031	≤ 0.01	**
	L root - N	0.9827	0.9657	7.5041	0.0173	≤ 0.05	*
S1	H - Sg	-0.9642	0.9297	-5.1433	0.0358	≤ 0.05	*
	% G - C	0.9759	0.9525	6.3294	0.0241	≤ 0.05	*
	H - Mg ²⁺	0.9513	0.905	4.3643	0.0487	≤ 0.05	*
	L root - C	0.9759	0.9524	6.3246	0.0241	≤ 0.05	*
	% G - Sf	0.9526	0.9074	4.4272	0.0474	≤ 0.05	*

S2	H - Mg ²⁺	0.9513	0.905	4.3643	0.0487	≤ 0.05	*
	L root - C	0.9759	0.9524	6.3246	0.0241	≤ 0.05	*
	L root - P ₂ O ₅ _{sass}	0.9694	0.9397	5.5814	0.0306	≤ 0.05	*
	% G - IB	0.997	0.994	17.8591	0.0032	≤ 0.01	**
	% G - Sf	0.9526	0.9074	4.4272	0.0474	≤ 0.05	*
S3	H - MO	0.9926	0.9852	11.5471	0.0074	≤ 0.01	*
	Nf - C	0.9728	0.9464	5.9428	0.0272	≤ 0.05	*
	Nf - pH _{water}	0.9599	0.9215	4.8449	0.0401	≤ 0.05	*
	Nf - Ca ²⁺	0.9897	0.9795	9.7678	0.0103	≤ 0.05	*
	L root - K ⁺	0.9518	0.906	4.3894	0.0482	≤ 0.05	*
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	L root - K ⁺	0.9518	0.906	4.3894	0.0482	≤ 0.05	*

* = Significant, ** = highly significant, respectively Pr_{theor} ≤ 0.05 and ≤ 0.05 Pr_{theor}

Discussion

Testing and the results we have presented on the germination of *Lippia multiflora* seeds reveal that the germination rate obtained in substrates S2 (82.67%) and S3 (65.33%) are the highest. Establishing a relationship between the rate of seed germination in the soil and planted those sown in mixtures (soil + manure), we obtain a variation exceeding 1 that is said from 1.26 to 3.014, which shows that the highest rates of germination are obtained in mixtures. Correlation tests

performed on the overall results show that the rate of germination (% G) is correlated with sand (Sf), organic matter (OM) and the index of crusting (IC). Thus, the contents of Sf substrates S1 and S2, correlated with the rate of germination provide correlation coefficients in the range of r = 0.95. Regarding the relationship between organic matter and the rate of germination, we have at Soil 2, r = 0.95 and S1, r = 0.97. The pair of variable % G-IB at S2 gives a correlation coefficient in the range of r = 0.99. Indeed, the germination capacity of the seeds depends on factors

intrinsic and extrinsic to it (Ahoton *et al.*, 2009). But, assuming that *Lippia multiflora* seeds sown have practically the same intrinsic factors, as coming from the same area of production, changes in the germination capacity of seeds is due to extrinsic factors such as humidity, ventilation and some chemical characteristics of the substrates. However, the particle size of Soil 2, S2 and S3 is dominated by a relatively average sand content, characterized by a lack of hardpan allowing permeability resulting ventilation. Thus, the retention of water in the substrate allows the infiltration and increases the moisture necessary for germination (Benseighir-Boukari and Arillier, 2006). The germination capacity increases with increasing soil moisture. But extended moisture causes decay of some seeds, this is the case observed at soil 3 which have a low rate of germination and could not confirm the natural settlement of *Lippia multiflora* in that soil. Also, the temperature variation in treatment, which increases and decreases the day and the night, added to the humidity, causes cracking of the skin (removal of inhibition of the skin) and then the softening of the shell, allowing and the infiltration of water inside the seed. All these factors listed promote the absorption of water in sufficient quantities by living tissues; follows then removal of the inhibition of germination by the diffusion of oxygen to the embryo and consequently trigger the germination process (Come, 1970). The lowest rate of germination recorded on Soil 1 is due to the dominance of the latter coarse sand, characteristic of highly permeable soils, filtering and therefore lacking water at the sow horizon (Ahoton *et al.*, 2009). The germination rate correlated with organic matter could mean the action of humic acid on seed tissues. Indeed, in the early stages of development, humic acid increases the rate of germination with the increase of the enzymatic activity of seed tissues (Hartwigsen and Evans, 2000).

The statistical analyzes applied to the data of seedling development, indicating an influence of treatments on the growth rate. Indeed, seedlings grown on the soil and two different substrates perform best, especially the substrate S2. Crossing data (growth rate and physico-chemical treatments), indicates a

strong correlation between root length and organic matter. Thus, we have pairs of variables for Soil 1, H - C ($r = 0.98$), for Sol 2, H - C ($r = 0.99$), for S1 and S2, L root - C ($r = 0.97$); for S3 the pair of variables obtained is H - MO ($r = 0.99$). Indeed, the level of organic matter in the soil is one of the criteria for determining whether or not degradation. Aggregation and stability of soil structure increases with the carbon content of the soil. Consequences are direct on the dynamics of water (Kribaa *et al.*, 2001) and the oxygen in the substrate, both of which play a role in root growth of woody plants. Also, humic substances contained in organic matter, binding soil particles form aggregates that improve the structural stability. In these aggregates, the presence of micro-pores keeps an airy structure where water and air can circulate. In addition to aeration, surface gas exchange between the soil, the atmosphere and its water retention capacity is increased. There is thus improved biological activity by providing a substrate heterotrophic microorganisms energy and carbon (Balesdent, 1996). The activity of these organisms has a positive effect because it affects soil mineral reserves and improves the bioavailability of elements to plants by limiting their precipitation. Mineral nutrition of plants is thus facilitated. The lack of ventilation of certain substrates often leads to root mortality (Benseighir-Boukari and Arillier, 2006), or the development of the root system is a key factor in the difference in seedling survival, because it allows store reserves feeder. Correlations between root length and carbon could be under the influence of humic acid, because when present in growth medium, the responses result in an increase in the number and length of roots (Hartwigsen and Evans, 2000). Works of Groffman *et al.* (2001) demonstrated that humic substances promote the removal of minerals by plants. The absorption of macronutrients (N, P, K, Mg, Ca) and micronutrients (Cu, Fe, Zn.) increases in the presence of humic acids (Garcia-Gil *et al.*, 2000). The presence of humic substances in the soil affects fertility and mineral reserves by promoting the release and dissolution of macronutrients contained in the mineral components of soil. The transport of ions and their positioning in the form of complex

around the rhizosphere condition the absorption of minerals. The slowdown in the growth of seedlings in the soil, especially in the sand, could be explained by the relatively low content of organic matter. The contribution of organic matter in the substrate causes a resurgence of biological activities that affect the mineralization of organic matter provided, allowing an improvement of the soil structure (Sawadogo *et al.*, 2008). Hence the value of a contribution of organic matter if one wants to maintain the physico-chemical and biological soil that promotes rapid development of the root system of crops. Slow growth observed in seedling for treatment Soil 1 is influenced by the development of the root system.

Conclusion

Comparison of the rate of germination, seedling height, average number of leaves and the average length of the taproot, on different substrates, shows very highly significant differences. Manure had a significant effect on the physical and chemical properties of soils where it has been introduced. Therefore, the soil-manure mixture favored the growth rate of seedlings. The best germination was obtained on mixtures S2 (82.67%) and S3 (65.33%). 60 days of culture, the measurement of the height of plants of *Lippia multiflora*, the most significant were recorded in culture media S2 (7.48 cm) and S3 (5.46 cm), with an average number of 7, 12 and 6 leaves, respectively for S1, S2 and S3. The root elongation is more important in substrates S2 (6.3 cm) and S3 (4.74 cm). These experiences should be pursued by testing advanced stages of plant development to confirm the results found at germination and seedling stages.

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