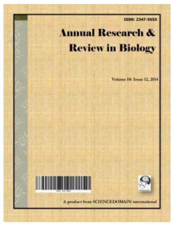

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Effect of Water on the Growth of Different Types of Propagation Stocks of *Lippia multiflora* Moldenke (Verbenaceae) Grown on Nursery in Toumodi, Central Region of Côte d'ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author MPH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EFA and EBBB managed the analyses of the study. Author JBDE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Lippia multiflora is used for its therapeutic properties in spite of this it remains a wild plant and therefore runs a risk of extinction which would cause an imbalance of the ecosystem. For the effective domestication of this plant and becoming perennial, a better knowledge of its water needs is necessary. A study of a 5-level hydration treatment of water (I_1, I_2, I_3, I_4 and I_5) on seeds (roots cutting (S_1), stem cuttings (S_2) and seeds (S_3) of *Lippia multiflora* in nurseries, was conducted under shade for 2 months. The study aimed at evaluating the growth of this plant in central Côte d'Ivoire, precisely in Blé, 6 km from Toumodi, 180 km from Abidjan. The soil is of Ferralsol type, on a slope. The experimental design was a factorial block in 3 replications. Seed collection was carried out by plowing *L. multiflora* wild plants. Mature seeds were harvested from the plants. The nurseries were

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established by seeding in plastic bags. The germinated seedlings were split, one seedling per bag. The water needed was applied manually and by sprinkling every 2 days before 9 am, for 60 DAP. Plant growth parameters (growth and survival rates, number of rejections, plant height, number of leaves produced, and leaf surface area) were assessed using either a meter rule, or numerically. The results obtained show that the water supply has a significant influence on the growth of *Lippia multiflora*. The growth was observed for three days after planting (DAP) but this growth was effected between the seventh day and at the 30th DAP, whatever the water application. The seeds (S_3) and roots cutting (S_1) have a high evolution rate and survival (about 100 p.c.) with well-developed growth in all water intakes. Cuttings are lagging behind others as regard to evolution. Seeds can be ranked according to their death resistance and optimal growth as follows roots cutting (S_1) - seeds (S_3) - cuttings (S_2).

Roots cutting (S_1) or seeds (S_3) are the main choice for planting *Lippia multiflora*. Cuttings require further investigation to complement our results. The optimal irrigation doses I_3 (50 p.c. UR) and I_4 (70 p.c. UR), which are very close to the easily usable soil water reserve (EUR), permit optimal growth for stumps S_1 and S_3 seeds of *L. multiflora.*

Keywords: Lippia multiflora; nursery; seed; water supply; Toumodi; Côte d'Ivoire.

1. INTRODUCTION

Lippia multiflora is a grassy plant or shrub that can reach more than 3 m height [1]. It is an aromatic and natural plant that grows preferably in tropical savannas [2]. It is used by local populations for its socio-economic [3] and therapeutic properties [4,5]. The leaves are the most used (fresh, dry). They are used as an infusion, decoction and often in combination with other elements to treat certain diseases such as febrile abscesses, bronchial diseases, nasopharyngitis, conjunctivitis, jaundice and hypertension [4,5,6]. The works of Kerharo and Adam, [7] showed that the infusion of leaves heals stomach ache and the steam shower cure cough and cold. The essential oil, extracted from the leaves, has antipyretic and anti-inflammatory effects, according to the work of Abena et al. [8]. The warm drink of the leaves as tea in the evening fights against insomnia [8, 9,10]. Its many potentials have finally convinced the scientific world to pay attention to it with the aim of highlighting its usefulness and create awareness. The marketing of the leaves of this plant can also procure a substantial income for modest families. In the dry season, bush fire ravages the savanna forest, but at the beginning of the rainy seasons, new leaves of *Lippia multiflora* appear on the plants, they are collected by the country women and then sold in the market. In general, the leaves collected are transported to the city of Abidjan where they are processed before being exported to foreign markets [11]. This marketing takes place in all regions of Côte d'Ivoire and even in the sub-region. A recent economic study showed through a survey in Côte d'Ivoire that a bag of 12 kg was estimated at 2000 francs for wholesalers and in supermarkets one gram of dry bagged leaves are sold for 500 francs [11,12]. Despite the socio-economic importance of *Lippia multiflora*, its production remains at the picking stage and is affected by bush fires and seasonal climatic changes. It becomes necessary to guarantee its durability. Sustaining the cultivation and production of *Lippia multiflora* leaves requires a better understanding of the initial stage of development of the plant in relation to its water requirement. Indeed, previous work has shown that the seedling in nurseries after a normal evolution, gradually dried-up after heavy rain. Thus, the management of water in the growth of this plant in the early stages of development appears as the key factor in its optimal growth. The study aimed at evaluating the effect of different water intakes on growth parameters of different types of planting seeds in nurseries for 2 months under shade in Toumodi.

2. MATERIALS AND METHODS

2.1 Study Site

The study site is located in Toumodi about 180 km from Abidjan in the central region of Côte d'Ivoire (Fig. 1). The Blé village (6 ° 00 to 6°30 N and 4°30 to 5°00 W) located 6 km from the city, along the Toumodi-Abidjan expressway has been the experiment site at the bottom of a slope. The average rainfall is 1200 mm and bimodal with 2 peaks in June (219 mm) and October (154 mm). The average temperature is 27.80°C and changes in the opposite direction to rainfall. The graph in Fig. 2 shows a heavy rainy season from February to July with a peak in June and a small season in October and a large dry season from November to January and a small season from August to September. The average humidity is 72.65 p.c. and the insolation of 70.37 nbhj^{-1}.

The vegetation of the study area is a savanna forest, shrub and woody type [13], with little wood cover. This dominant herbaceous stratum in the study area supports the strong water imbalance associated with edaphic conditions. The soils of Toumodi are ferralsols or cambisols developed on volcano-sedimentary complexes of the Birimien. The area of Toumodi is dominated by hills and is surrounded by mountain ranges. The geology of Toumodi is part of the furrow of Fętekro-Toumodi-Hiré. Lastly, the river system is characterized by the presence of the main river called "Kouetchê" which flows into the River Kan. All these rivers flow into the N'zi.

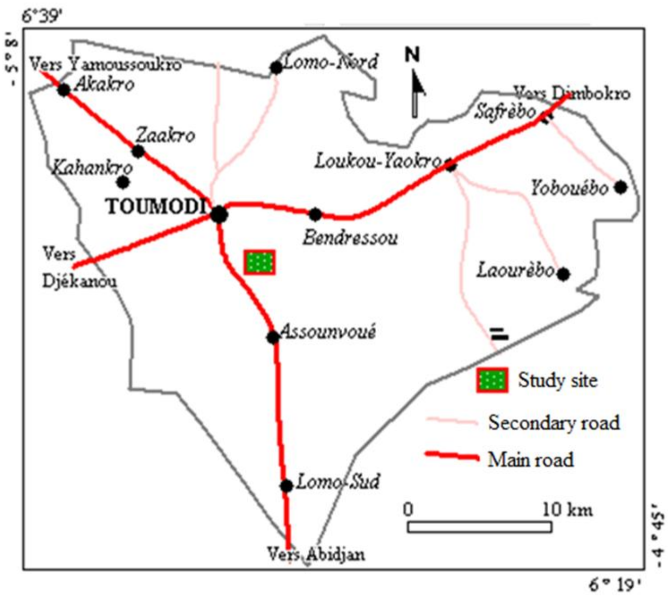

Fig. 1. Location of the study site

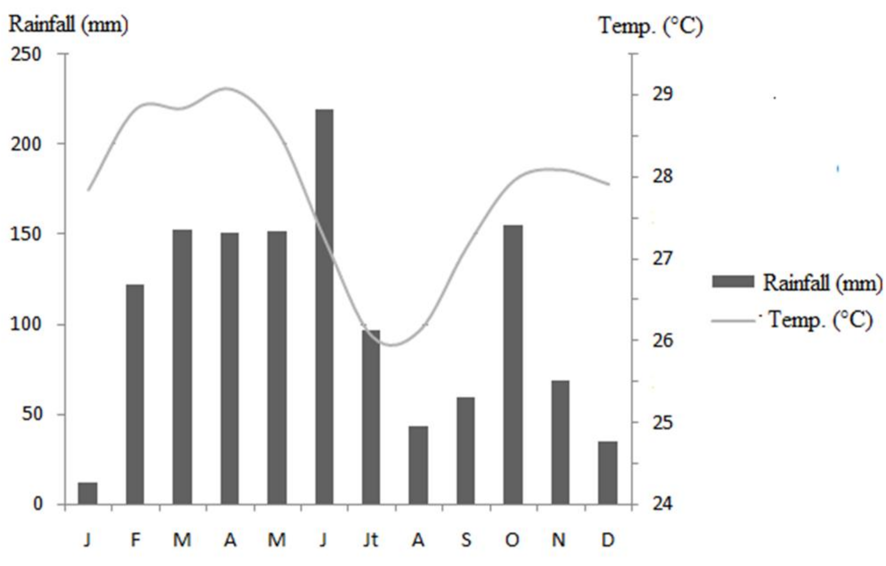

Fig. 2. Umbrothermal curve of the Toumodi region from 2009 to 2011

2.2 Material

The plant material consisted of three types of propagation stocks, roots cutting (S_1), stem cuttings (S_2) and seeds (S_3) collected in the vicinity of the study site. The soil is described using the usual equipment (shovels, picks, knives, folding meters, Munsell code, water). Hoes and cutlasses were used to clean the plots. A bait covered with transparent plastic sheltered the nurseries. The nursery bags are completely filled with soil. A 70-litre drum is used for the storage of water. Graduated buckets were used as measuring instruments.

2.3 Soil Characterization by Soil Profiles along a Toposequence

The morphopedological description was made. The parameters were described following the method as described by the Office of the Scientific Research and Overseas territory (ORSTOM) as reported by Boulet et al. [14], detailed by Yao-Kouamé [15]. Sampling of soil samples from the depth to the surface to avoid contamination was carried out on profiles of 0-20 cm and 20-40 cm for physicochemical and hydrodynamic analyzes in the laboratory.

2.4 Condition of Cultivation

The setting up was carried out in November 2010. The experimental setting was a block factorial. The main factor is the three propagation stocks (S_1 roots cutting, S_2 cuttings and S_3 seeds) and the water supply constituted the treatment and the secondary factor (I_1, I_2, I_3, I_4 and I_5) with 3 repetitions. Planting of the seeds was done randomly on a germinator in September 2010 and plants are transplanted at the stage of 6 leaves with cuttings and stumps collected from natural plants. The plot (12 m x 8 m) consisted of 45 elementary plots of 30 nursery bags 1.5 x 1 m in size and 0.6 m apart (Fig. 3).

Water application is estimated as in relation to the useful water reserve (UR) of the site, which is about 70 mm. Water intakes were 15 p.c. (I_1), 30 p.c. (I_2), 50 p.c. (I_3), 70 p.c. (I_4) and 100 p.c. (I_5) of UR applied every 2 days for 2 months using a graduated buckets. These values correspond to I_1 = 10 mm, I_2 = 20 mm, I_3 = 35 mm, I_4 = 50 mm, I_5 = 70 mm.

2.5 Monitoring and Measurement of Growth Parameters

Measurements and observations were done on a monthly basis. The variables measured are the number of rejections on each plant by counting, the height of the plants by measuring tape in cm, the number of leaves count on the whole plant, the length and width of the leaves with the measuring tape in cm which allowed calculating the foliar surface area (L x l x 0.75) in cm^2 and the percentage survival rate by calculating with the formula TS (p.c.) = b / n x100 where b is the number of growing plants and n is the total number of plants from the sub - plot of 30.

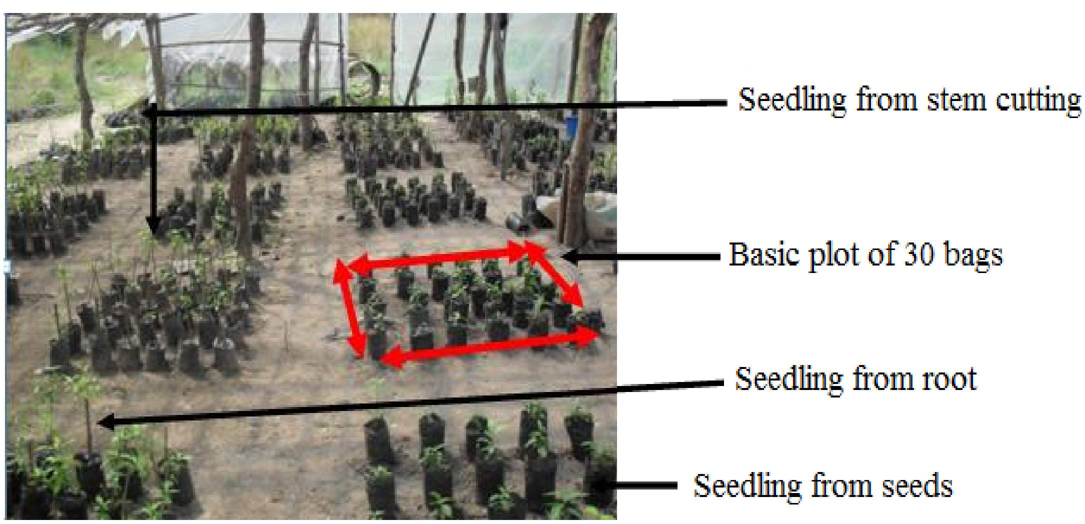

Fig. 3. Experimental set-up: *Lippia multiflora* nursery under shade

2.6 Statistical Analysis

The data obtained are processed using Excel and analyzed by Statistica 7.1 and XLSTAT. Results were considered significant at the probability threshold $p \leq 0.05$, highly significant for $p \leq 0.01$ and very highly significant for $p \leq 0.001$. Student Newmann Keuls tests were used to identify sample means that are significantly different.

3. RESULTS

3.1 Characteristics of the Soil in the Study Site

Table 1 presents the results of the soil description. The soil is deep (about 120 cm), with five (5) type A horizons they are A_{11}, A_{12} (g), A_{131} (g), A_{132} (g), A_{133} (g). The texture is sandy-clayey with little surface silt with a massive structure with a particular tendency, both on the surface and in the depth. The soil is coarse sandy with a content of more than 80 gkg^{-1}. The clay content is less than 10 gkg^{-1}. The transitions of the horizons are progressive. Many roots are found on the surface and reduced in depth. The soil is fresh to moist and porous. The appearance of a water table is observed at 1.20 m. Drainage may be stressful, expressed as brown beige colorations (7.5YR 6/8, 7.5YR 7/6), spotted ocher rust (10YR 6/4, 7.5YR 5/6), brownish-gray to brown. The soil type is a Hyperdystric Pseudogleyic Ferralsol. The bulk density is 1.64 gcm^{-3} to 1.63 gcm^{-3}.The study site is acidic (5.93) to slightly acidic (6.02). The total carbon content is from 1.48 to 1.42 gkg^{-1} and the total nitrogen is 0.29 and 0.15 gkg^{-1}. The soil is rich in organic matter with values of 2.54 to 2.44 gkg^{-1}. The volumetric moisture content at the soil field capacity is16.06 and 15.2 p.c. and the wilting point is 4.21 to 3.65 p.c.The useful water reserve is 38.87 mm on the surface and 37.49 mm.

3.2 Germination rate

The germination was observed 3 DAP. From 7 to 14 days, the germination reached 100 p.c. The germination of the roots cutting observed at 14 DAP varied according to the quantity of water received, 60 p.c. when the water quantity I_5 was given and 80 p.c. for the water quantity I_2. Stem cuttings had a very low germination rate compared to that of stumps. For seed, germination started by sprouting of a seedling with a high percentage germination rate when sown immediately after harvesting in November, December and January. Germination of *L. multiflora* seeds showed the highest rate up to 100 p.c. followed by that of stumps.

Table 1. Physical characteristics,chemical physico-chemical and hydrodynamic of the study site

	0 - 20 cm	20-40 cm
Coarse sand (gkg^{-1})	60,33± 2,06a	62,75± 2,14a
Fine sand (gkg^{-1})	22,83± 0,77a	21,92± 0,75a
Coarsemud (gkg^{-1})	5,38± 0,37a	4,52± 0,31a
Fine mud (gkg^{-1})	4,02± 0,23a	4,66± 0,26a
Clay (gkg^{-1})	7,03± 1,01a	8,79± 1,27a
Bulky density (gcm^{-3})	1,64	1,63
pH water	5,93 ± 0,06a	6,02 ± 0,02a
C (gkg^{-1})	1,48 ± 0,15a	1,42 ± 0,15a
N (gkg^{-1})	0,29 ± 0,09a	0,15 ± 0,003b
MO (gkg^{-1})	2,54±0,51a	2,44±0,45a
C/N	6,45 ± 1,06a	9,19 ± 1,66a
P (gkg^{-1})	0,49± 0,48a	0,40± 0,16a
Ca ($cmolkg^{-1}$)	1,21±0,22a	0,97± 0,17a
Mg ($cmolkg^{-1}$)	1,27± 0,03a	1,01± 0,02a
K ($cmolkg^{-1}$)	0,11± 0,01b	0,96± 0,01a
CEC ($cmolkg^{-1}$)	14,45± 0,54a	11,56±0,43a
S ($cmolkg^{-1}$)	2,61± 0,23a	2,10± 0,18a
V (%)	18,01±1,13a	18,07± ,13a
pF 2,5 (%)	16,06a	15,2a
pF 4,2 (%)	4,21a	3,65a
UR (mm)	38,87a	37,49a
EUR (mm)	25,91a	24,99a

3.3 Survival Rate

The survival and the germination rate of plants varies, for roots cutting as propagation stock from 93.33 p.c., when applying I_1 quantity to 98.89 p.c. with I_3 water quantity (Table 2). In fact, the survival rate increased with the quantity of water I_3 (98.89 pc), then decreased with I4 (96.5 p.c.) and with I5 decreased further to 94.80 p.c. (Table 2). Using stem cuttings as propagation, the survival rate depends on the amount of water applied. The survival rate was 85.55 p.c for I_1 quantity up to I_3 with the highest value (94.44 p.c.). From this quantity, the survival rate decreased with I_5 quantity to (83.66 p.c.). For seed as propagation stock, the observed germination and survival rate is 100 p.c. at 60 DAP. The survival rate of *L. multiflora*, 60 DAP, showed a difference, and according to the water quantity applied (Table 2).

3.4 Number of Rejections

The quantity of water applied produces a significant difference in the number of rejections in plants grown from the stumps. The quantity of water I_1, I_2 and I_5 presented different outcome compared to those of I_3 and I_4 (Table 2). Plants grown from stem cuttings showed a higher number of rejections compared to other propagation stock in all quantities of water. The plants grown from the seeds started with 1 rejection, and then ramifications were seen over time. Water quantity I_3 presented the lowest number of rejections (1.5) and differed from other quantity of water. Plants grown from the seeds gave the lowest number of rejections of the 3 propagation stock types (Table 2).

3.5 Height of the Plants

In general, the height of *L. multiflora* plants varied from 8.67 cm to 59.20 cm. For plants grown from roots cutting, the height increased based on the quantity of water applied. The higher the water quantity, the higher the height of the plant, up to 34.27 cm by the water quantity I_3. Above this quantity, the height of the observed plants was decreasing to the height of 25.67 cm at I_5 level. Plants grown from stem cuttings showed lower height growth and showed no difference in water quantity (Table 2). Plants grown from the seeds showed the lowest heights, but differed according to the quantity of water received. And the plants which received the quantity of water I_3 (11.87 cm) and I_4 (10.87 cm) gave the highest heights. The results showed that the plants grown from the roots cutting presented the highest heights (Table 2). Plants grown from stem cuttings and seeds produced heights of 2 and 3 times lower respectively than those from the stumps. Thus, the height of the plants grown from the stumps is almost twice that of the stem cuttings and 3 times that of the seeds according to the quantity of water applied (Table 2).

3.6 Average Leaf Production

The number of leaves of these plants increases with the increasing quantity of water applied. For plants grown from roots cutting, water quantity I_4 and I_5 produced the highest number of leaves, 60 and 59 respectively (Table 2).

For plants grown from stem cuttings, no differences were reported for the quantity of water applied. The average number of leaves produced remained low, from 44 to 48 compared to the leaves produced by the plants grown from the stumps (Table 2).

The production of the leaves of the plants grown from the seeds was from 25 to 41 leaves. Increasing the amount of water intake resulted in a decrease in leaf production for plants grown from seeds. Thus, the lowest quantity of water brought in higher number of leaves (Table 2).

The production of leaves showed a contrary effect in the plants grown from the roots cutting and those grown from the seeds. The number of leaves increased with the quantity of water applied for those grown from the stumps and decreased for those grown from the seeds.

3.7 The Leaf Surface Area

For the leaf surface area, a significant difference was observed between the quantity water applied and the propagation stocks. For the plants grown from roots cutting, the I3 water quantity induced the highest leaf surface area with 8.8 cm^2. Water quantity I_1 and I_4 differed from quantity I_2, I_3 and I_5 with leaf surface area values ranging from 4 cm² to 6 cm² (Table 2). As for plants grown from seeds, the water quantity showed no difference in the leaf surface area.

4. DISCUSSION

A significant difference was observed on the growth parameters based on the quantity of water intake by the 3 types of germination stocks studied. The growth parameter's value increased by the quantity of water applied up to I_3 and / or I_4 before dropping at, I_5. Plants grown from seeds had the highest survival rate in all water intakes. Regarding the roots cutting, the survival rate was 98.89 p.c. with water intake quantity I_3, which was justified by a high rate of germination. The average number of rejections produced has evolved differently. The plants grown from the roots cutting gave more rejections with low water intake as well as those grown from the cuttings. Aside from the number of rejections, plants grown from cuttings showed the lower value of growth parameters in response to different quantity of water compared to other propagation stocks. This could be explained by the implantation of the less dense, hairy-shaped root system than other types of propagation stocks. The cuttings are simply stems of the *L. multiflora* plant, cut into a bevel and buried in the soil to obtain a plant-son. This method is very risky, because the plant first feeds on its own reserves and has to draw, in the long run, nutrients by its roots that it has emitted. Indeed, the plants grown from seeds with low quantities of water showed the highest values for plant growth parameters. Plants grown from stumps produced the highest number of leaves. The length and width were different from one leaf to another. Thus, the leaves obtained showed several morphologies (thin and small, thin and large, large and small, large and large ...) that will allow geneticists to differentiate *L. multiflora* [16]. The average length of leaves values on plants, 60 DAP, are closer to those of Adou et al. [16] for a 4-months leaves grown from roots cutting (between 12 to 17 cm in length, and 4 to 13 cm in width). These results are consistent with those of [17] who reported less extensive leaves in *L. graveolens*, of the order of 2 to 4 cm. Quantity of water affected (P = 0.05) the lengths and widths of leaves of *L. multiflora*, as well as the leaf surface area. These variables increased in value with the applied water level and reached a maximum value with I_3 and / or I_4 before falling at I_5. The larger sizes of the leaves were produced when water quantity I_3 or I_4 were applied to the plants grown from the roots cutting. These quantities can therefore be considered as the quantity to be applied to *L. multiflora* plants in the nursery stage. Water being a limiting factor of growth, however, the 5 doses of water applied has more or less favored the growth of the plant. These results showed that *L. multiflora* could tolerate low water inputs for its optimal growth, therefore agrees with the work of [18] which showed that this plant is not water-demanding. Lamhamedi et al. [19] obtained similar results for the cultivation of black spruce (Pinaceae), known as spruce. At the young stage, plants can easily control early water shortage by closing their stomata [20]. The gap between the 5 doses of water applied to the plants, and the 15, 20 and 30 p.c. of the usable reserve UR, showed differences in the plant growth parameters, as shown by the work of [21], on white spruce, under tunnel crops, with water regimen with a gap of 15 p.c. [22], of 3 water regimen, a gap of 20 p.c. did not produce any differences in the height of the potato (Convolvulaceae).Similar results were obtained for the yield in alfa-alfa plant (*Medicago sativa, Fabaceae*), with 4 doses of water, a gap of 20 pc of the soil water content [23]. A 30 mm gap of the evaporation from three water regimen from 70 to 130 mm did not induce any difference in the yield values of the rape (*Brassicaceae*) in summer and winter, except for summer cover [24]. On the other hand, Mirabad et al. [25] observed the influence of difference in irrigation on the growth of melon (*Cucurbitaceae*) with a difference of 20 p.c.

Lamhamedi et al. [19] obtained differences in the growth parameters of a few plants with gap in water regimen of 40 mm from the ETM., which showed a positive effect of irrigation on yield, but the influence observed was based on time, as indicated by the work of [11] on the planting density of *L. multiflora*. The results observed do not correspond to those of [26], where irrigation did not influence growth and yield on rice (*Poaceae*). Our experiments showed that the different quantity of water applied to the plant induced differences in the growth parameters value. The water dose I_4 gave maximum values of the growth parameters. This could be due to the type of soil, because the soil is a Hyperdystric Pseudogleyic Ferralsol, which is filtering [27]. The germinating rate of plants from roots cutting and seeds generally exceeded 90 pc in Toumodi. The 5 doses of water applied, generally allowed growth of the 3 types of planting stock with the survival and germinating rate ranging from 83.66 to 100 p.c.. Indeed, the water quantity I_3 and I_4 are water levels that are within the easily usable reserve (EUR), whose plants made use for their growth. The quantity of water I_5 appeared to be asphyxiating to plants.

Table 2. Effect of water treatment on growth parameters of different types of seed of *Lippia multiflora*

Seeds	**S_1**					**S_2**					**S_3**					***P***
Water supply	**I_1**	**I_2**	**I_3**	**I_4**	**I_5**	**I_1**	**I_2**	**I_3**	**I_4**	**I_5**	**I_1**	**I_2**	**I_3**	**I_4**	**I_5**	
Growth parameters																
SR (p.c.)	93,33b	94,80b	98,89ab	96,5ab	94,80b	85,55c	90,1bc	94,44b	86,33c	83,66	100a	100a	100a	100a	100a	<.0001
NbRej	3b	3ab	4a	3ab	4a	3b	3ab	3ab	4a	3b	2b	2b	2b	2b	2a	<.0001
Hp (cm)	26,5b	32a	34,27a	31.5a	25,67b	13,5a	12,5a	13a	13,60a	12,1a	10,5a	9,3b	9,87b	10,87a	9,7	<.0001
ALPr	50ab	52ab	55ab	60a	59a	45b	43b	44b	43b	45b	40bc	39bc	35c	33c	29d	<.0001
LSA (cm^2)	8b	8,4ab	8,8a	8b	7,8b	4,3d	5,8bc	5c	4,1d	5c	6bc	5,5bc	5c	5,5bc	5c	<.0001
P			<.0001					<.0001					<.0001			

S_1: Seedling from cutting root, S_2 : Seedling from cutting stem, S_3 : Seedling from seed; I_1 : 15 p.c., I_2: 30 p.c., I_3 : 50 p.c., I_4 : 70 p.c. and I_5 : 100 p.c.; SR: Survival Rate, NbRej : Number of Rejections, Hp: Heigh of plants, AlPr: Average leaf Production, LSA: Leaf Surface Area.

5. CONCLUSION

Soil characteristics showed a Hyperdystric Pseudogleyic Ferralsol. The site had a predominance of sand and no clay. Nutrient levels are relatively poor but high in surface horizons except for phosphorus. The soil is acidic, with pHs ranging from 5.5 to 6.02.

An effect of water intake on the growth of the nursery plant was significant on the variables studied, and for each type of propagating stock.

On a Pseudogleyic Ferralsol Hyperdystric, with a high sand content, the I_3 and I_4 watering doses favored optimal nursery growth, 60 DAP. These results showed the importance of soil texture on the water intake of young *L. multiflora* seedlings in nurseries.

Optimal watering doses I_3 (50 pc UR) and I_4 (70 pc UR) for *L. multiflora* plants were found to be very close to the easily usable soil water reserve (UR) by *L. multiflora* plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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