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Banat's Journal of Biotechnology

2014, V(10)

BIODIESEL SOLID WASTE AND URBAN SLUDGE SEWAGE AS A SOIL AMENDMENT FOR THE SEEDLINGS PRODUCTION OF *Eucalyptus Camaldulensis* AND *Morus Alba*

DOI: 10.7904/2068–4738–V(10)–77

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Abstract. We examine in this study a possibility of use the biodiesel industry waste as a source of nutrients for the production and development of seedlings seems like a very good option. We added the diatomaceous earth residues of Biodiesel with sludge from urban wastewater treatment or manure to stimulate the activity of soil bacterial. The Dickson Quality Index (DQI) values *Eucalyptus Camaladulensis* and *Morus Alba* seedlings growing in test substrates with sewage sludge and diatomaceous earth are all higher than the recommended minimum value of 0.2. The treatments with manure and diatomaceous earth do not meet the desired quality standard. The *Eucalyptus Camaladulensis* and *Morus Alba* seedlings have good growth in substrates containing sewage sludge and diatomaceous earth than only the commercial substrate. They can use up to 50% by volume of the substrate without compromising the seedlings quality with a reduction of cost of seedlings production.

Keyword: diatomaceous earth, Dickson Quality Index, *Eucalyptus Camaladulensis* and *Morus Alba*.

Introduction

Recently, with the growing demand for biodiesel in the world, several plants for biofuel production, mainly from vegetable oils, are being installed in Brazil. For the elimination of various impurities, both the first and itself biodiesel material, these industries use filters of various types, particularly those using diatomaceous earth (D.E.) as a filter element. The organic compounds formed from industrial waste can be used as sources of organic matter and nutrients in a substrate Drill [WENDLING and GATTO, 2002].

They increase the water retention capacity by improving aeration of the roots of plants and they increase the availability of nutrients from the growth of beneficial microorganisms. Can interfere with the increase of pH and levels of exchangeable cations of substrates.

To avoid environmental problems associated with deposits of residues biodiesel industry, we can use as a source of nutrients for the production and development of plants seems like a very good option. The residue D.E. has specific physical properties that can improve the soil properties. The D.E. is a naturally occurring, soft, siliceous sedimentary rock, consisting of shells or frustules seaweed, which has many properties. It is a lightweight material with low–density porous structure with low thermal conductivity and non–toxic [MEISINER, 1981; SOUZA *et al.*, 2003].

At the end of the production, process of biodiesel, it is as a residue impregnated of organic material. We added the DE residues of Biodiesel with sludge from urban wastewater treatment or manure to stimulate the activity of soil bacterial [JOSEPH *et al*, 2009].

The advantage of this operation is to eliminate both wastes, residues Biodiesel industry and sludge treatment plants. Given the economic and environmental importance of reusing these resources considered as waste, this study aims to evaluate substrate test (DE and sludge sewage) as an alternative for improving the composition of soils for plant production.

Material and methods

The study was conducted in the period May–August 2014 at the Institute of Agricultural Sciences–ICA, of the Federal University of Minas Gerais–UFMG, Montes Claros, located in northern Minas Gerais state, latitude 16°51'38" S and longitude 44°55'00' W and 647 m of altitude. The climate of the region is semi–arid, hot and dry tropical.

The rainy season is concentrated between Octobers to March. The average annual rainfall is 1060 mm and uneven [ELOI, 2001]. The experimental substrate was prepared from different proportions of commercial substrate control (Bioplant), DE, sludge or manure (table 1).

Manure from breeding ICA–UFMG.

The sewage sludge from the treatment plant wastewater Montes Claros–MG.

Diatomaceous earth from the disposition of Petrobras biodiesel plant in Montes Claros, after use as a filtering agent of vegetable oils (soybean).

Table 1.

The materials used and their proportions by volume for each substrate tests

Substrate tests (T)
T1 = 100% substrate control (Bioplant)
T2 = 75% substrate control + 12.5% sludge + 12.5% Diatomaceous earth
T3 = 75% substrate control + 8.3% sludge + 16.7% Diatomaceous earth
T4 = 50% substrate control + 25% sludge + 25% Diatomaceous earth
T5 = 50% substrate control + 16.7% sludge + 33.3% Diatomaceous earth
T6 = 75% substrate control + 12.5% manure + 12.5% Diatomaceous earth
T7 = 75% substrate control + 8.3% manure + 16.7% Diatomaceous earth
T8 = 50% substrate control + 25% manure + 25% Diatomaceous earth
T9 = 50% substrate control + 16.7% manure + 33.3% Diatomaceous earth

Diatomaceous earth, before use, was submitted to the combustion process for the removal of residual oil.

Chemical and physical characterization of the substrates is shown in Tables 2 and 3.

Table 2.

The chemical characteristics of substrate tests and substrate control (T1)

Substrate tests	pH	CE	P	K	Ca	Mg	H + Al	SB	t	T	V	MO	C
		dS m^{-1}	- mg dm^{-3} --		------------------------ cmol$_c$ dm^{-3} -------------						%	--- dag kg^{-1} --	
T1	6.4	1.54	900	249	7.3	5.4	0.91	13.34	13.34	14.25	94	18.57	10.79
T2	6.4	1.72	1.020	497	8.2	3.4	2.32	12.88	12.88	15.2	85	38.44	22.34
T3	6.4	1.68	980	298	9.3	3.0	2.32	13.07	13.07	15.38	85	18.19	10.57
T4	6.4	1.64	960	497	9.6	3.5	1.38	14.38	14.38	15.75	91	13.6	7.9
T5	6.7	1.71	880	895	8.0	3.0	1.39	13.30	13.3	14.69	91	14.6	8.48
T6	6.9	1.82	510	970	7.0	3.6	1.55	13.09	13.09	14.64	89	16.34	9.5
T7	6.8	1.78	960	990	6.0	4.0	1.55	12.54	12.54	14.09	89	16.7	9.7
T8	7.6	1.65	960	846	4.0	4.8	0.85	10.97	10.97	11.82	93	16.34	9.5
T9	7.5	1.35	700	647	4.6	2.8	0.85	9.06	9.06	9.91	91	13.6	7.9

The pots had a volume of 55 cm3 and the species studied were *Camaladulensis Eucalyptus* and white mulberry (*Morus Alba* Yu vc–62).

The seeds of *Eucalyptus Camaladulensis* forum obtained from the ICA / UFMG and seeds of white mulberry *Morus Alba* vcYu–62 were CIA / Cuba.

At the time of sowing three seeds were placed in each pot and 21 days after sowing took place clarified leaving only one plant per pot.

The experiment consisted of two tests, one for each species.

The experimental design was a randomized complete block design with three replications and four pots per experimental unit.

The compositions of the pots were made on April 30 and May 1, 2014.

The plants were sown on May 2.

A shade sail was used with a rate 30% and 50% shade and was placed 1.5 m above the ground, oriented east–west to provide shade from the experimental unit. This shade sail was removed 30 days after the lifting of plant.

Both treatments were irrigated three times daily.

Physical and chemical analyzes of different substrates tests and control substrates (commercial substrate Bioplant) were performed at the Laboratory of Solid Waste UFMG following the methodology of Embrapa [EMBRAPA, 1997].

Were determined pH, electrical conductivity (EC), density (ρ), matter (OM), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and potential acidity (H + Al), the density of the particles and the water holding capacity (WHC).

The diameter and height of the stem of the plants were measured from the 49th day to the 118th day. Starting at thirty days, we calculated the rate of emergence of two cultures.

Where the species reached a height greater than 10 cm and 42 days after planting, we add the ammonium sulphate fertilizer.

The Speed Emergence Index (SEI) was calculated based on the following formula of Maguire [MAGUIRE, 1962]:

$$SEI=E1/N1+E2/N2+\cdots+En/Nn,$$

Where E1, E2, En = are number of seeds germinated on first, second and nth day, respectively and N1, N2, Nn=are number of days from sowing to first, second and nth count, respectively.

Dickson [DICKSON *et al.* 1960], we determine total dry matter TDM (g), shoot height H (cm), collar diameter D (mm), dry biomass of the above ground part GPDM (g) and root dry matter RDM (g) following this formula:

$$DQI = TDM / ((H/D) + (GPDM/RDM)).$$

The number of leaves per plant was calculated. Fresh weight of shoots, leaves and roots were measured by weighing.

Table 3.
Water retention capacity (WHC), density (ρ) and total porosity (TP) of substrate tests and substrate control (T1).

Substrate tests	WHC	ρ	TP
	mL g^{-1}	g cm^{3}	%
T1	0.56	0.35	64.73
T2	0.49	0.46	73.38
T3	0.42	0.47	62.16
T4	0.38	0.55	58.40
T5	0.43	0.55	56.30
T6	0.60	0.43	63.51
T7	0.53	0.43	64.28
T8	0.57	0.46	64.44
T9	0.44	0.50	62.60

After drying in an oven at 65°C for 72 hours to constant weight, the dry weight matter was weighed.

We evaluated the relationship between shoot dry weight and root dry weight.

All data were subjected to analysis of variance and means were compared by the Scott–Knott test at 5% probability.

Results

The pH in the control substrate Commercial T1 chosen as substrates tests mixtures of T2 to T4 is the same 6.4; it passes to 6.7 in the mixture T5 (33% of DE).

The pH was higher in mixtures with manure between 6.9 and 7.6.

The electric conductivity (1.64 to 1.82 dS / m) in the mixtures T2 to T8 is higher, compared with control substrate (1.54 dS/m).

Except for the case T9, manure mixture, is very low 1.35 dS/m. K is high 880 mg/dm^3 in the mixture T5. K remains high in the mixtures Manure (647–990 mg/dm^3) compared with the control substrate (249 mg/dm^3).

Table 4.

Means of the variables total height (**H**), collar diameter (**D**), number of branches (**NB**), number of leaves (**NL**), root volume (**RV**), fresh biomass of the above ground part, (**GPFM**), root fresh matter (**RFM**), dry biomass of the above ground part (**GPDM**) and root dry matter (**RDM**) seedlings of *Eucalyptus Camaldulensis* from different substrates tests and control substrate.

Substrates tests	H	D	NB	NL	RV	GPFM	RFM	GPDM	RDM
	Cm	mm	n^o /seedling		Cm^3	g			
T1	22.03 A	2.43 A	1.62	5.12	3.69	2.59 B	1.14 A	0.86 A	0.45 A
T2	24.51 A	2.95 A	1.60	5.85	5.42	3.96 A	1.57 A	1.50 A	0.54 A
T3	23.25 A	2.65 A	1.75	5.49	4.92	3.83 A	1.39 A	1.41 A	0.54 A
T4	20.34 A	2.75 A	1.85	6.08	4.53	4.24 A	1.58 A	1.52 A	0.65 A
T5	21.98 A	2.89 A	1.79	5.97	2.72	4.15 A	1.43 A	1.36 A	0.61 A
T6	16.55 B	1.78 B	1.65	4.78	2.83	1.89 B	0.65 B	0.55 B	0.27 B
T7	16.97 B	2.02 B	1.55	4.57	3.00	1.86 B	0.80 B	0.55 B	0.26 B
T8	13.49 B	1.85 B	1.62	5.05	2.44	1.93 B	0.69 B	0.61 B	0.27 B
T9	15.36 B	1.59 B	1.66	5.03	1.69	1.57 B	0.58 B	0.39 B	0.20 B

The organic matter (OM), total porosity, water holding capacity (WHC) and density decrease significantly in mixtures T4, T5 and T9 (table 2 and 3).

Eucalyptus Camaladulensis

The main observation is that the mixture with manure (T6–T9) show characteristics (Table 4–5) very different from the development of *Eucalyptus* relative to the mixture with D.E. and Sludge sewage (T2–T5).

The volume of the root is very low in the T5 (2.5 cm^3) compared to the control substrate T1 (3.69 cm^3) or T2, T3 e T4 (Table 4).

Table 5.

Means Speed Emergency Index (SEI), GPDM/RDM ratio, H index (H/D) and Dickson Quality Index (DQI) seedlings of *Eucalyptus Camaldulensis* from different substrates tests and control substrate.

Substrates tests	SEI	GPDM/RDM	H INDEX	DQI
T1	4.58 A	1.94	9.44	0.12 B
T2	4.61 A	2.96	8.33	0.18 A
T3	3.09 B	2.68	8.96	0.17 A
T4	3.32 B	2.38	7.45	0.23 A
T5	3.13 B	2.25	7.66	0.20 A
T6	2.22 B	2.05	9.28	0.07 B
T7	3.32 B	2.17	8.70	0.08 B
T8	2.65 B	2.75	7.72	0.09 B
T9	4.85A	2.26	9.63	0.05 B

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Table 6.

Means of the variables total height (**H**), collar diameter (**D**), number of branches (**NB**), number of leaves (**NL**), root volume (**RV**), fresh biomass of the above ground part, (**GPFM**), root fresh matter (**RFM**), dry biomass of the above ground part (**GPDM**) and root dry matter (**RDM**) seedlings of *Morus Alba* from different substrates tests and control substrate.

Substrates tests	H	D	NL	RV	GPFM	RFM	GPDM	RDM
	cm	mm	-- n^o /seedling		Cm^3	g		
T1	7.49 C	3.04 C	2.75 A	1.00 B	1.00 C	1.45 B	0.35 B	0.43 C
T2	7.96 A	3.83 B	2.47 B	3.28 A	1.61 B	2.01 A	0.57 B	0.63 B
T3	10.10 A	3.78 B	2.67 B	1.50 B	1.47 B	1.75 B	0.48 B	0.56 B
T4	12.38 A	4.46 A	2.48 B	2.92 A	2.27 A	2.51 A	0.83 A	0.78 A
T5	11.11 A	4.76 A	2.54 B	3.11 A	2.19 A	2.90 A	0.82 A	0.94 A
T6	9.06 B	3.53 B	2.84 A	1.67 B	1.28 B	1.55 B	0.37 B	0.43 C
T7	7.96 C	2.90 C	2.86 A	1.25 B	1.03 C	0.99 C	0.29 B	0.23 D
T8	8.98 B	3.49 B	2.91 A	1.67 B	1.31 B	1.35 B	0.38 B	0.30 D
T9	9.24 B	2.99 C	3.06 A	0.64 B	1.12 C	0.92 C	0.28 B	0.20 D

Means without letters or followed by the same letter are not statistically different according to the Scott-Knott test at 5% probability

***Morus Alba* Yu vc–62**

The heights of rods, the diameter, number of leaves and the root volume are higher in all the substrates tests than in the control substrate (Table 6).

However, all the morphological characteristics and weight biomass of the seedling in substrates with manure are less than those seedlings growing in substrates tests with sewage sludge and diatomaceous earth.

Seedlings treatments T4 and T5 have the largest diameter 4.46 mm and 4.76 mm respectively (Table 6). All other values are higher than the minimum required (2.0 to 2.5 mm). DQI seedlings *Morus Alba* is high (Table 7) in the treatment with sewage sludge and diatomaceous earth T4 (0.42) and T5 (0.55) and very low in treatment with manure T7 (0.13), T8 (0.18) and T9 (0.11). Results of the yield of cut up pieces and the meat quality in Table 3.

Table 7.

Means Speed Emergency Index (SEI), GPDM/RDM ratio, H index (H/D) and Dickson Quality Index (DQI) seedlings of *Morus Alba* from different substrates tests and control substrate.

Substrates tests	SEI	GPDM/RDM	H index	DQI
T1	2.97	0.80	2.53	0.24 B
T2	2.21	0.91	2.74	0.33 B
T3	2.62	0.85	2.68	0.29 B
T4	2.73	1.09	2.83	0.42 A
T5	2.38	0.89	2.35	0.55 A
T6	2.73	0.85	2.77	0.23 B
T7	1.84	1.40	2.81	0.13 C
T8	2.19	1.32	2.65	0.18 C
T9	1.95	1.35	3.12	0.11 C

The broilers growth performance of the experimental group are characterized by a significant increase of body weight at slaughter age (+8.5%, P=0.02). Marl improves the feed conversion ratio by a decrease of about 5%.

Discussion and conclusions

Eucalyptus Camaladulensis

The observed values of the plant height, diameter of the stem and the fresh and dry weight of roots and shoots in the treatment with a manure and diatomaceous earth were statistically lower, compared with the control substrate (fig. 1).

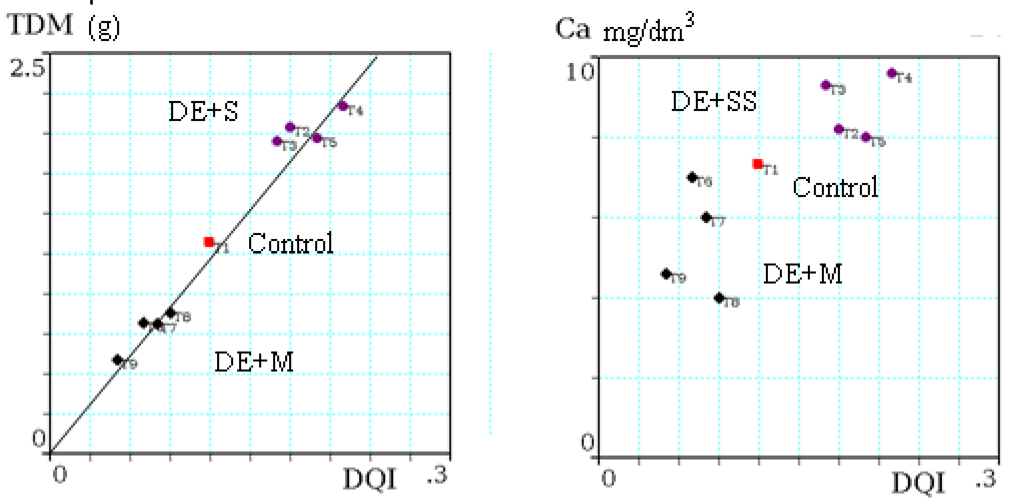

Figure 1. Dickson Quality Index (DQI) versus total dry matter (g) of seedlings of *Eucalyptus Camaldulensis* and Ca from different substrates tests and control substrate.

Whereas the physical and chemical properties of the substrates were similar in all treatments (Table 2 and 3), with the exception of pH (fig. 2), the effects observed may be attributed to the higher pH (higher than 6.5) of these substrates.

In more alkaline pH conditions, there may be nutrients and micronutrients are not available which will cause a physiological disequilibrium plants. The pH should be in the range of pH 5.5 to 6.5 to obtain the recommended quality of seedlings. According to Mello and Vitti [MELLO and VITTI 2002] and Miyazawa [MIYAZAWA *et al.* 2000], the precipitation of the solution of aluminum ground causes, among other effects, the increase of pH.

Moreover, the application of manure may cause complexing Al by organic acids released by these materials. The high acidity of the soil caused by the sludge can be associated with the nitrification of ammonium nitrogen and the oxidation of sulphites and feasible production of organic acids during the degradation by microorganisms of the residue [SIMONETE *et al*, 2003]. If we consider that seedlings good quality must have a minimum stem diameter of 2.0 to 2.5 mm [LOPES et al., 2007, WENDLING and DUTRA, 2010], the treatment with the substrate containing sludge sewage and diatomaceous earth corresponds to the desired quality (fig. 2).

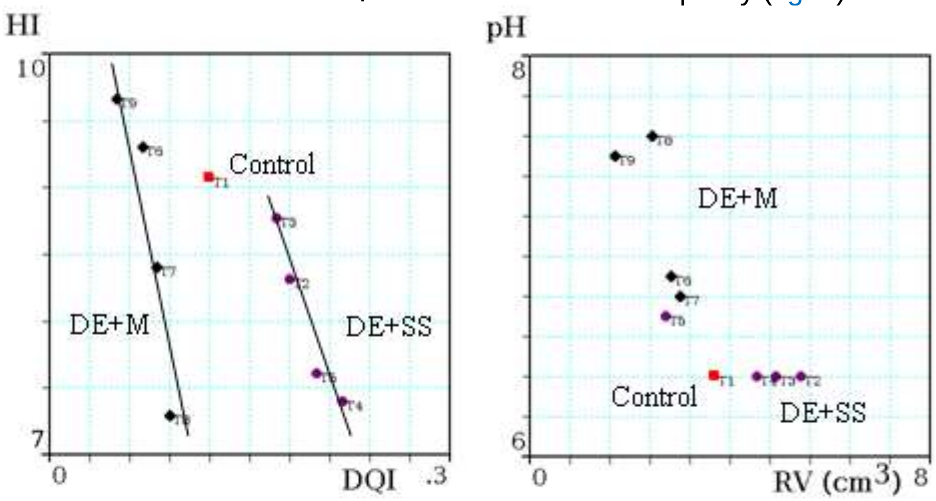

Figure 2. Dickson Quality Index (DQI) versus H Index (H/D) of seedlings of *Eucalyptus Camaldulensis* and pH versus RV root volume of seedlings from different substrates tests and control substrate.

The seedlings may be placed on the field after 120 days of growth, with an average diameter stem from 2.65 to 2.95 mm. The emergency speed index of T2 and T9 is statistically equivalent to treatment with substrate control (Table 5). This result differs from that observed by Oliveira [OLIVEIRA JUNIOR *et al.* 2011]; these authors studied the value of speed emergency index based on mixtures of the substrate with rice husk, cow manure, powdered coconut and fine vermiculite.

Different mixtures had no effect on the speed emergency index of *Eucalyptus urophylla*. It should be noted that the decline in the rate of seedling emergence is an undesirable feature, because most of the time in the early stages of growth, it makes plants more vulnerable to adverse environmental conditions [MARTINS *et al.,* 1999]. The faster root development is important, the better the vegetative development and stability of the plant [SOARES *et al.*, 2008].

The GPDM/RDM ratio showed no statistical difference between the different substrates (Table 5).

However, DQI is more important in the substrate test mixture of sludge sewage and DE (0.17–0.23), whereas it is very low in substrates based on manure and DE. (0.05–0.09) and in the order of 0.12 in the control substrate (fig. 1); we have a good correlation with total dray matter TDM and DQI.

This links up the observations of Caldeira [CALDEIRA JUNIOR *et al.,* 2009 and CALDEIRA *et al.,* 2013] found that on the field the best results in the survival of eucalyptus seedling are those substrates containing sewage sludge. These authors also noted that 2/1 of GPDM/RDM is a good balance for the growth of plants.

In our case, the values are slightly higher but still reasonable for the different substrates tests. We find that the ratio between the height and diameter of the plant did not differ significantly between the substrates tests studied (H in Table 5). Greater the value of this ratio is, the better the ability to survival and establishment of seedlings in the field [CARNEIRO, 1983]. However, this ratio is influenced by the volume pots experience Gomes [GOMES *et al.* 2003].

The best treatments are those with sewage sludge and diatomaceous earth. The DQI is above the recommended minimum by Gomes [GOMES *et al.* 2002]. These authors argue that DQI should be greater than 0.2. DQI than, the higher the quality standards seedlings. The study of seedlings of *Eucalyptus urophylla* by Oliveira [OLIVEIRA JÚNIOR *et al.* 2011] showed higher values for the DQI treatments with manure than what we got.

Morus Alba Yu vc–62

Tests substrates with sewage sludge and diatoms favor the growth of white mulberry (*Morus Alba*) seedlings (fig. 3). They are usually better than the control substrate (Table 6).

For all treatments the chemical and physical properties were similar except for pH (Tables 2 and 3), we can consider that sewage sludge played an important role in nutrient level and diatoms have improved physical properties including porosity, water capacity and density of retention substrates tested (fig. 4).

These observations are consistent with the Maia [MAIA, 1999] found that sewage sludge should not be used pure, despite its relative fertility [CALDEIRA et al., 2012], sewage sludge cause compression of the substrate. Diatomaceous earth is an important addition to the growth of seedlings.

All treatments showed levels of potassium (Table 2) very good according to the classification of Alvarez (1999), which explains the high values of stem diameter of seedlings. Valeri and Corradini [VALERI and CORRADINI 2005] showed that the potassium regulates the opening of the stomata and promotes the thickening of the stem of the seedling.

According to Daniel [DANIEL *et al.* 1997], the diameter of the stem is the most appropriate to evaluate the ability of seedling survival. It is also the most widely used to help determine the doses of fertilizer must be applied in the production of seedlings.

The speed emergence index of *Morus Alba* shows (Table 7) no statistical difference between treatments.

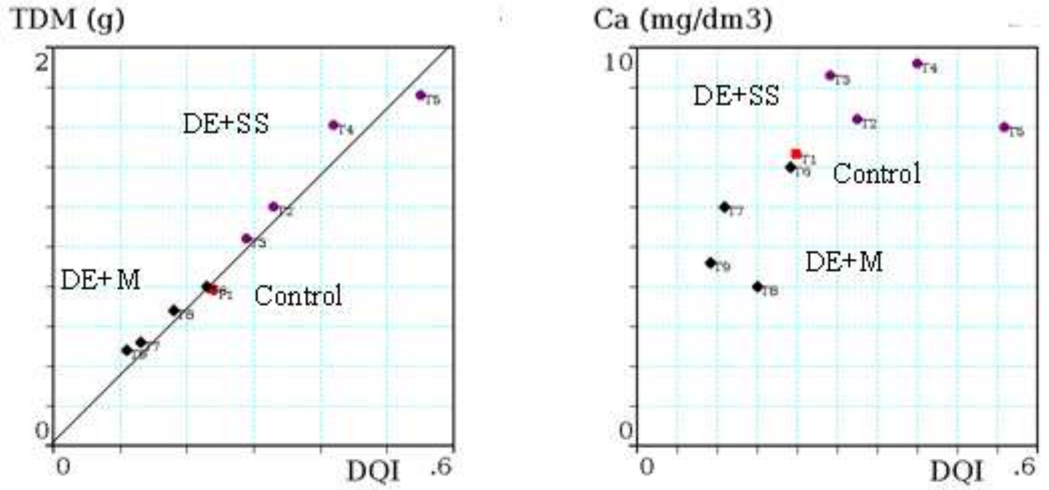

Figure 3. Dickson Quality Index (DQI) versus total dry matter (g) of seedlings of *Morus Alba* and versus Ca (mg/dm^3) from different substrates tests and control substrate.

At the stage of the germination and emergence of seeds, nutrients are not necessary for the reactions leading to the formation stem and root. Only hydration and aeration of the substrate and good porosity of the substrate allows a movement of the air and water favoring the quickest germination [SIMÃO, 1971].

The GPDM/RDM ratios of *Morus Alba* seedling (Table 7) are not statistically different for all treatments. They indicate that the seedlings have the same probability of survival.

The GPDM/RDM ratio and TDM (fig. 1 and 3) is directly related to the composition of substrate tests strongly influenced by the availability of water, which favors the flow of nutrients and seedling growth.

The HI ratio (stem height/stem base diameter) of seedlings *Morus Alba* (fig. 4) shows no statistically significant difference 2.35 to 3.12 (Table 7).

This ratio is less than that observed for the *Eucalyptus* (7.47 to 9.63) for the same experimental conditions.

The H ratio is likely to depend on the species of seedlings because in spite of the low values, seedlings showed good conditions for adaptation and resistance on the ground (fig. 4).

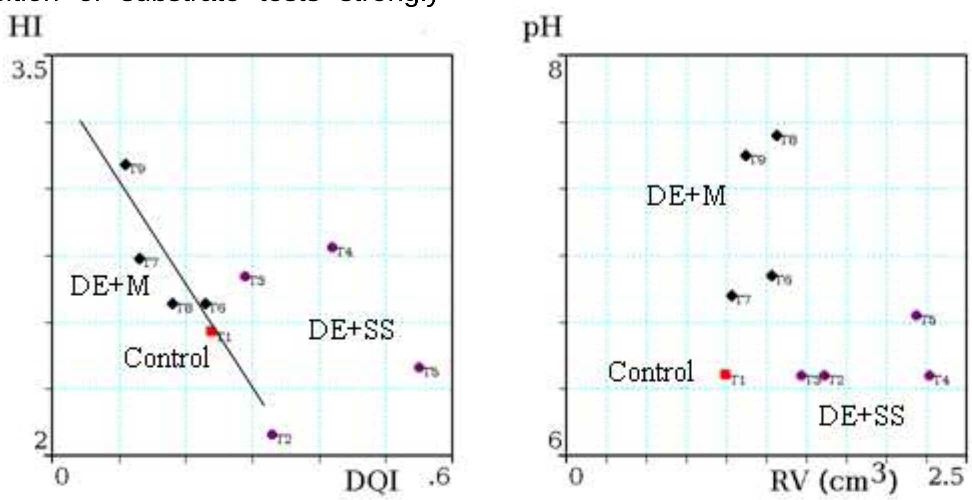

Figure 4. Dickson Quality Index (DQI) versus H Index (H/D) of *Morus Alba* seedlings and pH versus RV root volume of *Morus Alba* seedlings from different substrates tests and control substrate.

The DQI values *Morus Alba* seedlings growing in test substrates with sewage sludge and diatomaceous earth, are all higher than the recommended minimum value of 0.2, with high value for T4 and T5 rich substrate diatomaceous earth. Treatments with manure does not meet the desired quality standard (fig. 3).

The DQI is obvious that the morphological parameters' used to evaluate the quality of seedlings and morphological parameters should not be analyzed separately to determine the quality level.

The Eucalyptus and *Morus Alba* seedlings produced with test substrates (sewage sludge, diatomaceous earth and commercial substrate) showed similar results to those obtained with the use only commercial substrate.

The *Eucalyptus* and *Morus Alba* seedlings have good growth in substrates containing sewage sludge and diatomaceous earth. They show better results than using only the commercial substrate. They can be used up to 50% by volume of the substrate without compromising the seedlings quality with a reduction of cost of seedlings production.

Acknowledgements

This work supported by FAPEMIG (Fundação de Amparo a Pesquisa de Minas Gerais) and the Institute of Agricultural Sciences of the Federal University of Minas Gerais (UFMG), Regional Campus Montes Claros. We also thank the French Embassy and the Rectorate of UFMG for the grant of a visiting professor *Essaid Bilal* (ENSMSE, France) in the framework of the France–UFMG Academic Chair. The authors are grateful to Prof. *Monica Butnariu* for their assistance and for useful discussions.

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Received: October 29, 2014
Accepted: November 11, 2014
Accepted: Last modified on November, 25, 2014