Assessment of *Milinator Technologies* Fertilizer Formulations

Final report

La Cité collégiale in collaboration with *Milinator Technologies Inc.*

David Lemelin, Eric Berger, Camille Hébert-Martineau,
Rémy A Aubin, PhD and Michel Caron, Ph.D.

Confidential

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Objective

The objective of the project was to assess the fertilizing value of *Milinator Technologies Inc.* fertilizer obtained using a combination of natural and mechanical processes.

Introduction

The technology developed by Milinator Technology is based on the natural transformation of organic materials by fly larvae through a natural and mechanical assisted system. The technology available at this time is for the transformation by fly larvae (*Musca domestica*) of chicken manure produced by egg layers. A system of conveyer belts and air movement allows for the production in 3 to 4 days of a granular type dry organic fertilizer comprised only of chicken manure with no other additives.

Numerous trials were performed with the fertilizer resulting from the processing of raw chicken manure using fly larvae and the Milinator technology. Although most trials were unsupervised, they did give an indication of a commercial value for the fertilizer.

The trials conducted with tomato and spinach plants by the Centre for applied research in by-products of La Cité collégiale in Ottawa, were to obtain data on its mineral composition and to demonstrate the agronomic value of the fertilizer.

Methods

Plant fertilization schedule

Tomato plants fertilization

Week 1: Preparation of the cuttings in multicells from tomato plants, fertilize with Pro-mix & Stim-root#2 (*Premier tech*). Variety of tomatoes used: *Rhapsody (beef) Syngenta*
Week 2: Transfer of cuttings in 6'' pots. Fertilization: 10-52-10.
***Week 7*:** Transfer in 20 Liters pots - 4 kg and incorporation of the organic fertilizer *Milinator*
Week 8: The first chemical fertilization was applied

Table 1. Determination of the fertilization required for tomatoes cultivation in 4 kg pots based on the availability of the 3 major nutrients found in the *Milinator* fertilizer (based on the tomato fertilizer – **TF,** *see Table 2*)

Trt.	Material	Fertilization Rate			Quantity per pot of 4 kg		
		N *(mg/kg)*	P *(mg/kg)*	K *(mg/kg)*	N *(mg/pot)*	P *(mg/pot)*	K *(mg/pot)*
1	**Milinator**	220	81,6	59,4	880	326,4	237,7
2	**NPK**	220	81,6	59,4	880	326,4	237,7
3	**Milinator + NPK**	220	81,6	59,4	880	326,4	237,7
4	**Milinator + PK**	220	96,0	250,0	880	384,0	1000,0
5	**Milinator**	110	40,8	29,7	440	163,2	118,8
6	**NPK**	110	40,8	29,7	440	163,2	118,8
7	**Milinator + NPK**	110	40,8	29,7	440	163,2	118,8
8	**Milinator + PK**	110	48,0	125,0	440	192,0	500,0
9	**Milinator**	55	20,4	14,9	220	81,6	59,4
10	**No treatment**	-	-	-	-	-	-

Table 2. Composition of the fertilizers used for the fertilization of the tomato plants.

	Milinator ***(% as is)***	**Urea** ***(%)***	**DAP[1]** ***(%)***	**SOP[2]** ***(%)***
Nitrogen, N	5,01	47	21	0
Phosphorus, P	1,86	0	23	0
Potassium, K	1,35	0	0	41

[1]DAP = Diammonium phosphate, $(NH_4)_2HPO_4$

[2]SOP = Sulfate of potash, K_2SO_4

Table 3. Total quantity in *mg/pot* needed of each fertilizer used (*see Table 2 for a detailed composition*)

Treatments	***Milinator*** ***(mg/pot)***	**Urea** ***(mg/pot)***	**DAP** ***(mg/pot)***	**SOP** ***(mg/pot)***
Milinator 220	17550	-	-	-
NPK 220	-	1238	1419	580
Milinator +NPK 220	8775	302	1419	580
Milinator +PK 220	10558	-	1670	2439
Milinator 110	8775	-	-	-
NPK 110	-	619	710	290
Milinator +NPK 110	4388	151	710	290
Milinator +PK 110	5279	-	835	1220

Milinator 55	4388	-	-	-
No treatment	-	-	-	-

Table 4. Total quantity in *mg/pot* needed of each fertilizer used (*see Table 2 for a detailed composition*)

Treatments	*Milinator* *(mg/pot)*	Urea *(mg/pot)*	DAP *(mg/pot)*	SOP *(mg/pot)*
Milinator 220	17550	-	-	-
NPK 220	-	1238	1419	580
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Milinator + PK 220	10558	-	1670	2439
Milinator 110	8775	-	-	-
NPK 110	-	619	710	290
Milinator + NPK 110	4388	151	710	290
Milinator + PK 110	5279	-	835	1220
Milinator 55	4388	-	-	-
No treatment	-	-	-	-

Spinach plants fertilization

Table 5. Fertilization rates for the spinach trials.

Trt.	Material	Fertilization Rate			Quantity per pot of 0,5 Kg		
		N *(mg/kg)*	P *(mg/kg)*	K *(mg/kg)*	N *(mg/pot)*	P *(mg/pot)*	K *(mg/pot)*
1	**No treatment**	-	-	-	-	-	-
2	**Milinator (7 mg N/Kg)**	7	2,6	1,9	1,75	0,65	0,47
3	**Milinator (14 mg N/Kg)**	14	5,2	3,8	3,5	1,3	0,94
4	**Milinator (28 mg N/Kg)**	28	10,4	7,5	7,0	2,6	1,89
5	**Milinator (55 mg N/Kg)**	55	20,4	14,8	13,75	5,1	3,71
6	**Milinator (110 mg N/Kg)**	110	40,8	29,6	27,5	10,21	7,41
7	**Chemical (110 mg N/Kg)**	110	36,7	36,7	27,5	9,17	9,17
8	**Milinator (220 mg N/Kg)**	220	81,7	59,3	55	20,42	14,82

Table 6. Quantity of fertilizer used for the spinach trial
(7 pots per conditions)

Trt.	Conditions	Mass of fertilizer *(mg/pot)*	Application rate
1	**No treatment**	-	-
2	**Milinator (7 mg N/Kg)**	35	applied once
3	**Milinator (14 mg N/Kg)**	70	applied once
4	**Milinator (28 mg N/Kg)**	140	applied once
5	**Milinator (55 mg N/Kg)**	275	applied once
6	**Milinator (110 mg N/Kg)**	550	applied once
7	**Chemical (110 mg N/Kg)**	92	applied weekly
8	**Milinator (220 mg N/Kg)**	1100	applied once

Sample preparation

Moisture content

Procedure

- Place an aluminum dish in the oven at a temperature of 103-105°C and leave it for 2 hours.
- Let it cool down to room temperature in a desiccator. Weigh the empty dish (Empty dish weight = **A**).
- Weigh at least 10g of sample in the dish and weigh it again (Wet sample + dish weight = **B**).
- Place the dish with the sample at least 12 hours in the oven at 105°C. Then cool it down to room temperature in a desiccator and weigh again (Dry sample + dish weight = **C**).

Calculation

$$\text{Moisture content, M (\%)} = \frac{(B-C)\times 100}{(C-A)}$$

$$\text{Moisture correction factor (mcf)} = \frac{100+\text{M (\%)}}{100}$$

Tri-acid digestion

For the release of mineral elements we have the choice between two methods: *dry ashing* and *wet oxidation*. Dry ashing is usually carried out at an ignition temperature of 550°C followed by its extraction in diluted HCl or H_2SO_4. Ashing could lead to considerable volatilization loss of phosphorus and potassium. Wet oxidation employs oxidizing acids like HNO_3-H_2SO_4-$HClO_4$ in a tri-acid mixture. The use of the tri acids and especially the addition of $HClO_4$ avoid the volatilization loss of phosphorus and potassium and provide a clear solution. This is why this method was chosen.

Reagent preparation

- Mix all concentrate acids HNO_3, H_2SO_4 and $HClO_4$ in 10:1:4 ratio and let cool.

Procedure

- Transfer 1.0 g of dried and processed sample to a 250 ml conical flask.
- Add 5 ml of concentrated H_2SO_4.
- Keep a glass funnel on the flask, place it on a water bath and heat at 100°C for about 30 minutes.
- Cool and add 5 ml of tri-acid mixture.
- Heat at 180-200°C on hot plate, until the dense white fumes are dissipated and transparent white contents are left.
- Cool and add about 50 ml of double distilled water and filter (*Whatman #42- previously wash with ddH_2O*) into 100 ml volumetric flask, giving 3-4 washes. Finally bring the volume to 100 ml.
- Use the filtrate for analysis.
- The dilution factor is 100.

Dissolution test method

Procedure

- 3g of fertilizer were immersed into 200 mL of double distilled water in conical flasks.
- The flasks were placed on a shaker at room temperature for up to 48 hours.
- Each flask content is then filtrated on a *Whatman #42*.
- The analysis is performed on the liquid filtrate.
- Three (3) flasks were used for each time period.

Method for sample analysis

Flame Atomic Absorption (Ca, Cu, Fe, K, Mg, Mn, Na, Zn)

Apparatus: *Varian AA 240 atomic absorption spectrometer*

Calcium, Ca
Wavelength: 422.7 nm
Fuel: acetylene
Support: air

Copper, Cu
Wavelength: 324.7nm
Fuel: acetylene
Support: air

Iron, Fe
Wavelength: 372.0 nm
Fuel: acetylene
Support: air

Potassium, K
Wavelength: 766.5
Fuel: acetylene
Support: air
Control of interferences: Cesium Chloride

Magnesium, Mg
Wavelength: 202.6 nm
Fuel: acetylene
Support: air

Manganese, Mn
Wavelength: 279.5 nm
Fuel: acetylene
Support: air

Sodium, Na
Wavelength: 589.6 nm
Fuel: acetylene
Support: air

Zinc, Zn
Wavelength: 213.9 nm
Fuel: acetylene
Support: air

Sulfur content (S)

Source:
LECO CORPORATION. *Sulfur and carbon in cements, soils, rocks, ceramic and similar materials*, Application bulletin Form 203-601-222, January 2003

Principle

A certain mass of dried samples is put inside a crucible. The latter is then put inside a high temperature chamber where the sample combust in presence of oxygen to produce sulfur dioxide (SO_2). The gas is then passes in front of an infrared detector and analyzed automatically against a series of control samples.

Apparatus: *LECO TruSpec C*

Procedure

- Weight 0.2500 g of dried samples for each test to be done, and put inside a crucible.
- Weight also 0.2500 g of control samples
- Run a blank crucible to equilibrate the instrument
- After, insert each samples and control samples into the combustion chamber, following the instructions given by the instrument computer.

Calculation
The results are given automatically by the instrument in sulfur percentage, *S (%)*.

Total Kjeldahl Nitrogen (TKN)

Principle

Samples for nitrogen determination are digested in sulfuric acid and a catalyzer, copper sulfate, at a temperature of about 360-420°C. After digestion, the samples are cooled and steam distilled with NaOH. The distilled ammonia is collected in boric acid and titrated against standard acid.

Apparatus
Foss Digestion heater block and *Foss Keltec* steam distillation unit

Reagents

- Concentrate H_2SO_4.
- Catalyst mixture: K_2SO_4 and $CuSO_4$ (1000:32)
- 40 % Sodium hydroxide, NaOH
- 4 % Boric acid with mixed pH indicator methyl red and bromocresol green
- 0.01N and 0.1N HCl

Procedure

- Weigh 0.5 to 10.0 g of sample into digestion tube and moist with double distilled water.
- Add 10 ml of conc. H_2SO_4 and 1.0 to 10 g of catalyst and place the tube in the digestion unit.
- Turn the heating equipment to 420°C and install the tube in the heater block.
- Let the digestion occur for about one hour, or until the sample is transparent blue. Let cool at room temperature.
- Put the tube into the distillation unit (Program: 80 mL dH2O and 60 NaOH 40%)
- Collect about 200 mL of distillate in 25 ml of 4 % boric acid solution.
- Perform a titration of the distillate against a 0.01 N HCl solution until a pink lavender color starts to appear.
- Run a blank for each set of samples.

Calculation

$$\text{Available nitrogen in soil (mg/Kg)} = \frac{(\text{A-B}) \times \text{N} \times 14.007 \times 1000}{\text{Sample weight (g)}}$$

Where
A = volume of acid used against sample
B = volume of acids against blank
N = normality of acid

Ammonium Nitrogen (NH_4-N)

Source
Foss- Application Note 303 *Determination of Nitrogen according to Direct Distillation (DD) using steam distillation*

Principle

The samples steam distilled with NaOH and MgO. The distilled ammonia is collected in boric acid and titrated against standard acid.

Apparatus
Foss Keltec steam distillation unit

Reagents

- Magnesium oxide, MgO
- 40 % Sodium hydroxide, NaOH
- 4 % Boric acid with mixed pH indicator methyl red and bromocresol green
- 0.01N and 0.1N HCl

Procedure

- Weigh 3 to 10.0 g of sample into distillation tube
- Add approximately 2 g of MgO
- Put the tube into the distillation unit (Program: 80 mL dH2O, 60 NaOH 40%)
- Collect about 200 mL of distillate in 25 ml of 4 % boric acid solution.
- Perform a titration of the distillate against a 0.01 N HCl solution until a pink lavender color starts to appear.
- Run a blank for each set of samples.

Calculation

$$\text{NH4-N (\%)} = \frac{(\text{A-B}) \times \text{N} \times 1.4007}{\text{Sample weight (g)}}$$

Where
A = volume of acid used against sample
B = volume of acids against blank
N = normality of acid

Alkaline Available nitrogen (Subbiah and Asija 1956)

Source:
Subbiah, B.V. and G.L Asija. 1956. *A rapid procedure for determination of available nitrogen in soils*. Curr.Sci. 25:259-60.

Principle

The available nitrogen is estimated using alkaline $KMnO_4$, which oxidizes and hydrolyses the organic matter present in the soil. The liberated ammonia is condensed and absorbed in boric acid, which is titrated against standard acid.

Apparatus: *Foss Keltec* steam distillation unit

Reagents

- 0.32 % Potassium permanganate: Dissolve 3.2 g of $KMnO_4$ in distilled water and bring the volume to one liter.
- 2.5 % Sodium hydroxide: Dissolve 25 g of NaOH in distilled water and bring the volume to one liter.
- 4 % Boric acid with mixed pH indicator methyl red and bromocresol green
- 0.01N hydrochloric acid

Procedure

- Weigh 5 to 10 g of soil sample in 250 ml Kjeldhal tube.
- Add 50 ml of 0.32% KMnO4 solution and add 50 ml of 2.5% NaOH solution and distill immediately.
- Collect the distillate in 25 ml of 4 % boric acid solution.
- Perform a titration of the distillate against a 0.01 N HCl solution until a pink lavender color starts to appear.
- Run a blank without soil for each set of samples.

Calculation

$$\text{Available nitrogen in soil (mg/Kg)} = \frac{(A\text{-}B) \times N \times 14.007 \times 1000}{\text{Sample weight (g)}}$$

Where
A = volume of acid used against sample
B = volume of acids against blank
N = normality of acid

Phosphorus determination (P)

Apparatus: Spectrophotometer, 880 nm

Reagents

- Sodium hydroxide (1M)
- Sulfuric acid (4M)
- Ammonium molybdate (4%) store it in a polythene bottle in dark.
- Potassium antimony tartrate (0.275% w/v)
- Ascorbic acid (1.75% w/v) prepare fresh daily
- Mixed Reagent: Add successively, using a measuring cylinder, the following reagents to a 500 ml bottle; 50 ml 4M Sulfuric acid,15 ml 4% Ammonium molybdate, 30 ml 1.75% Ascorbic acid, 5 ml 0.275% Potassium antimony tartrate, 200 ml water. Mix well after each addition and prepare fresh daily.
- The standard series of Phosphorus is made with KH_2PO_4 and range from 1 to 4 ppm for linearity.

Procedure

- Pipette 5 ml of the standard series, sample or blank to a test tube and add 5 ml of mixed reagent.
- Shake and stand for one hour for the blue color development.
- Measure the concentration of the solution at 880 using a spectrophotometer.
- Initially standardize spectrophotometer with a series of known concentration after that determine phosphorus concentration in the sample.

Calculation

$$\text{Phosphorus (mg/Kg)} = \frac{(S\text{-}B) \times D \times [V + \{W - (W/mcf)\}]}{W\ (g)} \times mcf$$

Where

S = P concentration in sample (mg/l) read by spectrophotometer.
B = P concentration in blank (mg/l) read by spectrophotometer.
D = Dilution factor (standard 1 for undiluted samples).
W = Weight of sample.
mcf = Moisture correction factor.
V = Volume of extractant.

Crude Lipids (Soxhlet)

Samples for crude lipids determination are subject to multiple cycles of delipidation with an appropriate solvent (i.e. Hexane). After, the solvent is evaporated and the remaining lipids are weight on a balance.

Apparatus
Foss delipidation unit *Soxtec*

Reagents

- Hexane

Procedure

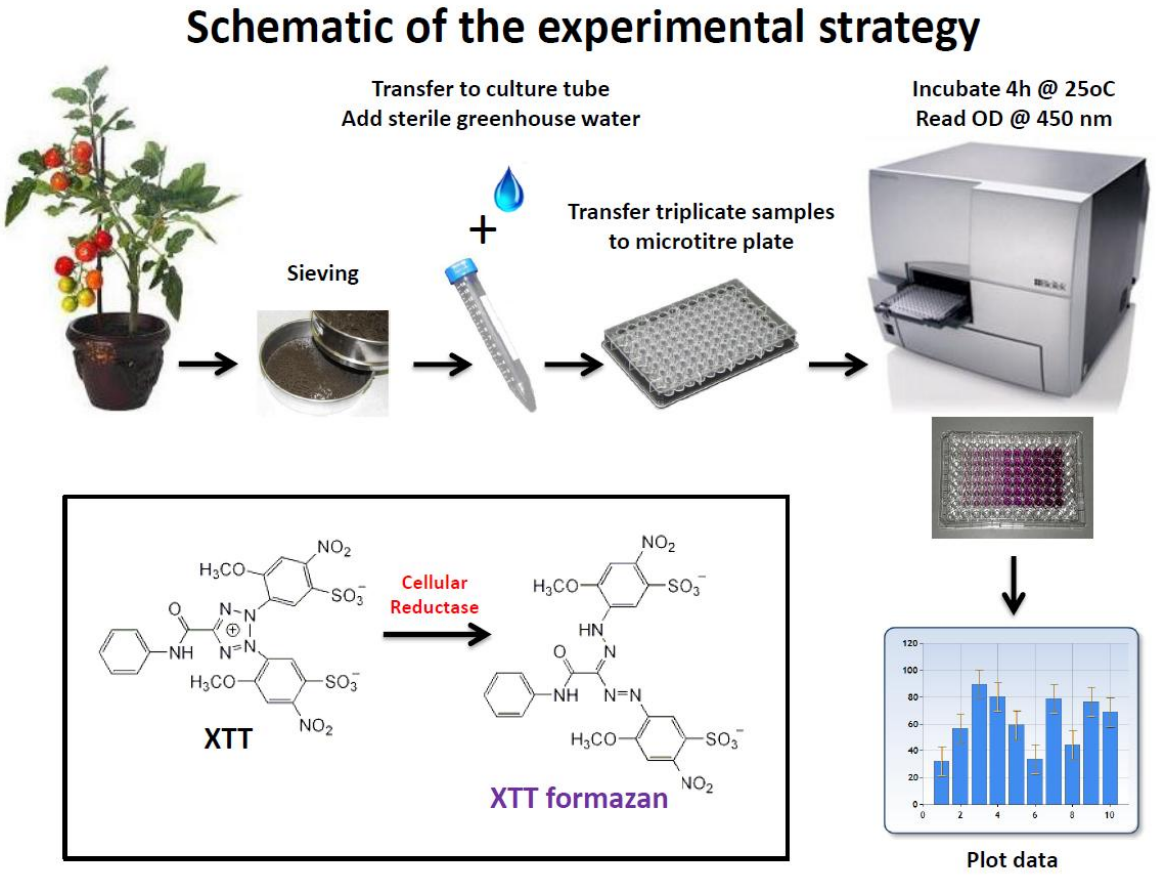
- Weigh 0.5 to 10.0 g of dry sample into the extraction thimbles
- Add 70 ml Hexane into the aluminum crucibles
- Turn the heating equipment on.
- Let the delipidation occur for about 2 hours
- Recuperate the Hexane by distillation (about 10 minutes).
- Let the crucible containing the oil cool in the desiccator before weighing

Calculation

$$\text{Crude Lipids (\%)} = \frac{(A - B)}{C} \times 100$$

Where
A = Mass of the lipids collected (g)
B = Mass of the crucible (g)
C = Sample weight (g)

Soil metabolic activity method



Results and discussion

Fertilizer analysis

The analyses of three different batches of the Milinator fertilizer produced at different times indicate some variations in their content (Table 7). The results indicate that the N-P-K content is variable with the time of production but also if the process is modified as shown by the results obtained with the darker fertilizer (DF) resulting from a modification of the process. The products identified as TF (the fertilizer used for the tomato experiment) and LF which is a light color fertilizer such as TF, show that variations can be obtained between batches under the same production conditions. These variations could be associated with the variations in the raw chicken manure content although no comparison analysis was done within this project.

To verify the impact of the raw material composition on the final product composition, analysis of the chicken manure used for various production batches will be required and then compared to their corresponding fertilizer production output.

Table 7. Composition of different production batches of *Milinator* fertilizers obtained from chicken manure.

Test	Samples			Unit
	TF[1]	DF[2]	LF[3]	
Humidity	6,7	19,8	16,1	**%**
Dry mass	93,3	80,2	83,9	**%**
Ash	40,6	49,6	39,8	**%**
Organic matter	59,4	50,4	60,2	**%**
Crude Lipids	0,71	-	-	**%**
Results on a dry matter basis				
Nitrogen, N	5,3	2,6	6,8	**%**
Phosphorus, P	2,0	1,1	1,2	**%**
Potassium, K	1,4	2,4	1,8	**%**
Carbon, C	28,9	24,5	29,1	**%**
Ratio C/N	5,4	9,3	4,3	
Nitrogen, NH4-N	1,768	0,984	1,665	**%**
Calcium, Ca	6,4	1,7	0,5	**%**
Copper, Cu	145,2	100,4	77,9	**ppm**
Iron, Fe	849,7	1368,3	2184,9	**ppm**
Magnesium, Mg	7317,5	627,5	630,1	**ppm**
Manganese, Mn	541,4	669,9	600,6	**ppm**
Sodium, Na	0,437	0,043	0,052	**%**
Sulfur, S	0,8	0,9	0,9	**%**
Zinc, Zn	971,3	35,5	48,3	**ppm**

[1]**TF** = Tomato fertilizer (used for the cultivation of tomato plants and spinach)

[2]**DF** = Dark fertilizer (refer to the dark color of the fertilizer)

[3]**LF** = Light fertilizer (refer to the light color of the fertilizer)

Fertilizer dissolution test results

Dissolution test performed in water over a 48 hour period using the fertilizer used for the tomato trials (TF), indicates that nitrogen (N) is initially slowly released in water as indicated by the small variation in N in the supernatant for the first 16 hours after an initial burst for the first hour and that solubility increases with time as shown by the higher level of N after 48 hours (Figure 1).

The results obtained following the analysis of the phosphorus (P) (Figure 2) and potassium (K) (Figure 3) of the supernatants indicate that very little P and K are readily soluble after the initial dissolution that occurs within the first hour.

A dissolution test comparing a light color fertilizer (LF) with the dark one (DF), shows that more N, P and K are found in the supernatant of DF after 16 hours than TF (Table 8). This could be an indication that the modification of the process (unknown to us) may have resulted in a lower contain in N but could allow for a greater solubility making this product potentially more suitable as a hydroponic fertilizer. This hypothesis will require to be confirmed by proper experimentation.

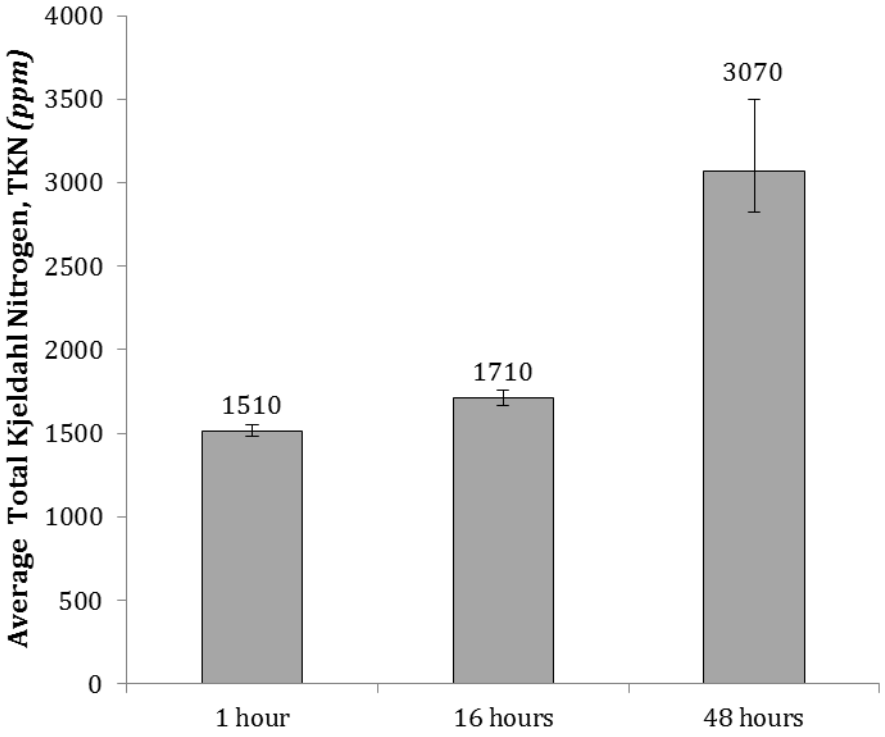


Figure 1. Evaluation of the average Total Kjeldahl Nitrogen found in the supernatant of the **tomato fertilizer – TF**, over different time period. The results are given in parts per million *(ppm)*. The error bars represent the highest and lowest values obtain from the 3 flasks used in that experiment.

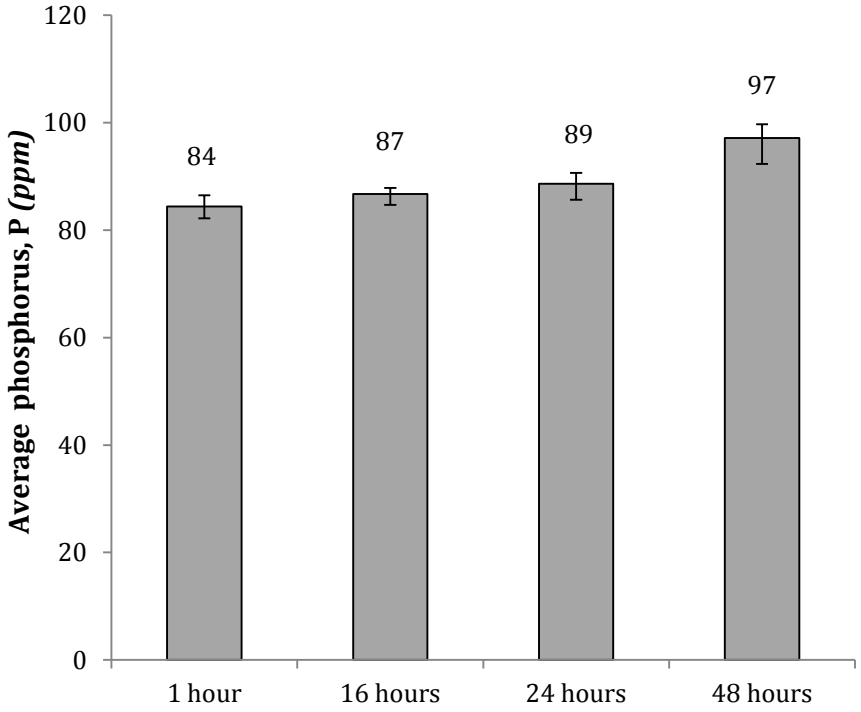


Figure 2: Analysis of the phosphorus found in the supernatant of the **tomato fertilizer – TF** over different time period. The results are given in parts per million *(ppm)*. The error bars represent the highest and lowest values obtain from the 3 flasks used in that experiment.

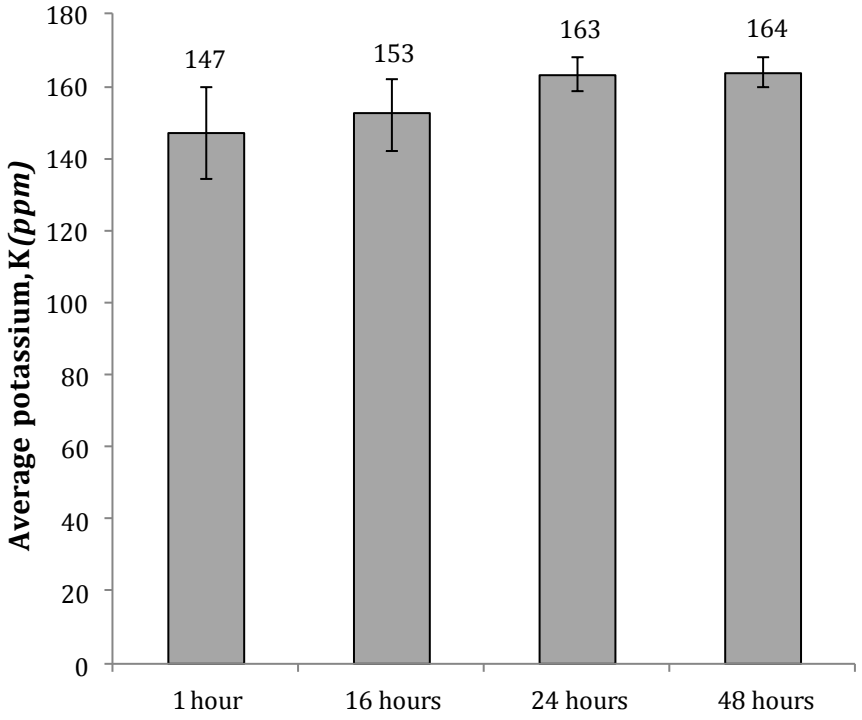


Figure 3: Analysis of the potassium found in the supernatant of the **tomato fertilizer – TF**, over different time period. The results are given in parts per million *(ppm)*. The error bars represent the highest and lowest values obtain from the 3 flasks used in that experiment.

Table 8. Average nitrogen, phosphorus and potassium composition of the filtrate supernatant of the dark fertilizer –DF and the light fertilizer – LF, after immersion in double distilled water (see *Dissolution test*, *method*)

	Nitrogen, TKN *(ppm)*		**Phosphorus, P *(ppm)***		**Potassium, K *(ppm)***	
	DF[1]	**LF[2]**	**DF**	**LF**	**DF**	**LF**
16 hours	1691	1113	61	48	193	163
48 Hours	2360	6333	66	50	222	165

[1]DF = dark fertilizer, [2]LF = light fertilizer

Tomato trial results

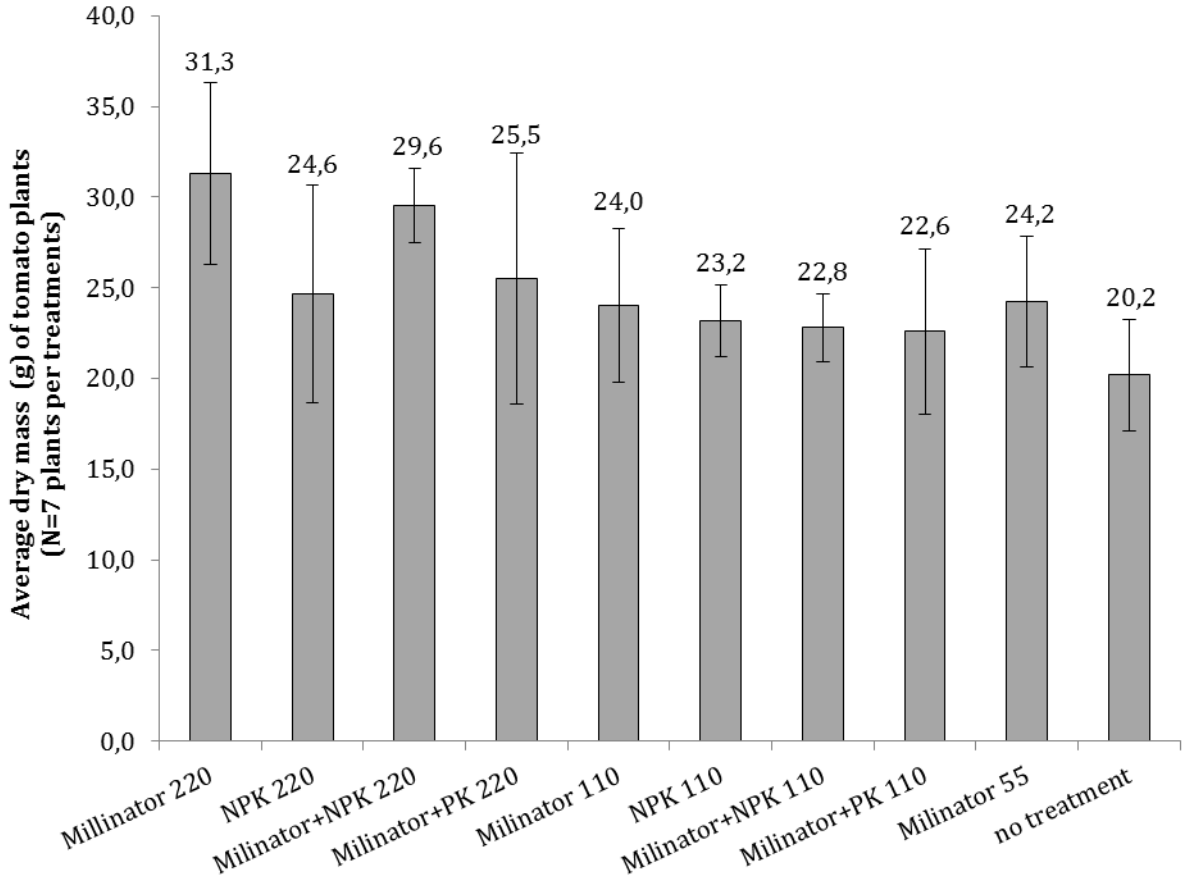


Figure 4: Tomato plants average dry mass (g) at the end of the experiment

The average dry mass (g) at the end of the experiment for tomato plants fertilized with Milinatior 220, is significantly different only from those fertilized with NPK 110 and Milinator+NPK 110 and also the no treatment plants (Figure 4). Meanwhile, plants treated with NPK 220 show no significant difference with any other treatment but plant treated with Milinator +NPK 220 have dry masses significantly different from those treated with NPK 110, Milinator +NPK 110, Milinator + PK 110, Milinator 55 and the no treatment.

These results demonstrate that the Milinator fertilizer can sustain plant growth as well as a synthetic fertilizer. The combination of the Milinator fertilizer and the chemical source of NPK, tends to indicate a positive effect on plant growth when comparing the results obtained for NPK 220 with Milinator + NPK 220.

At the same nitrogen rate of 220 mg per kilogram of soil, the plants grown with the Milinator natural organic fertilizer had a better overall development than the ones grown with a synthetic fertilizer (Figure 5).

Figure 5: Tomato plants treated with the Milinator fertilizer (left) and an N-P-K synthetic fertilizer (right) at the same rates of 220 mg of N per kg of substrate.

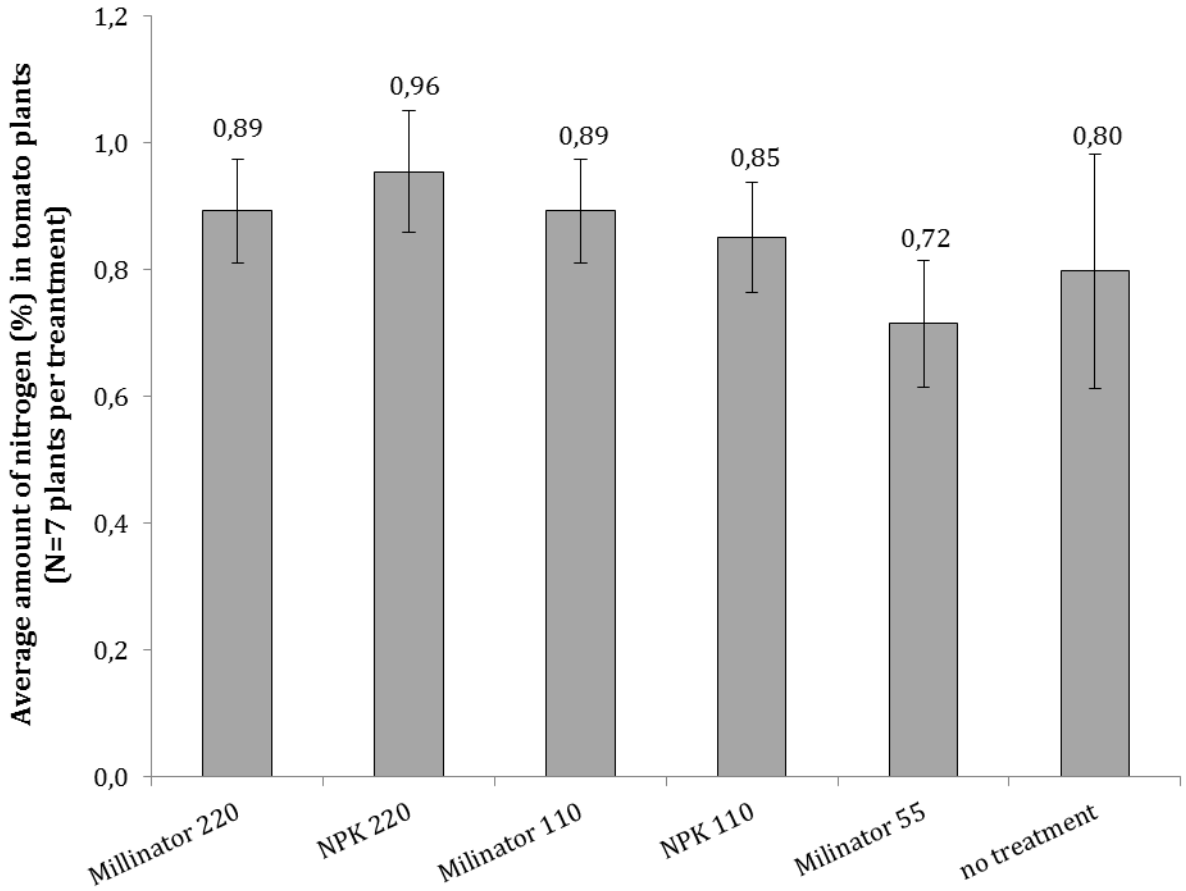


Figure 6: Tomato plants average nitrogen content (%)

No significant differences were observed in the plant nitrogen contents at the end of the experiment between Milinator 220, NPK 220, Milinator 110 and NPK 110 (Figure 6). We must also point out the high level of nitrogen content of the no fertilization treatment, which negates the observations made for the other treatments.

More fruits > 51 mm were obtained with Milinator 220 and that number was significantly different from the number obtained with NPK 220 but not from Milinator +NPK 220, showing again a improve results when both fertilizers are combined as seen with the plant dry weight results (Figure 5). The results with Milinator 220 were also not significantly different from those with Milinator + PK 220, Milinator + NPK 110 and Milinator + PK 110, indicating the same fertilizer combination positive responses tendency.

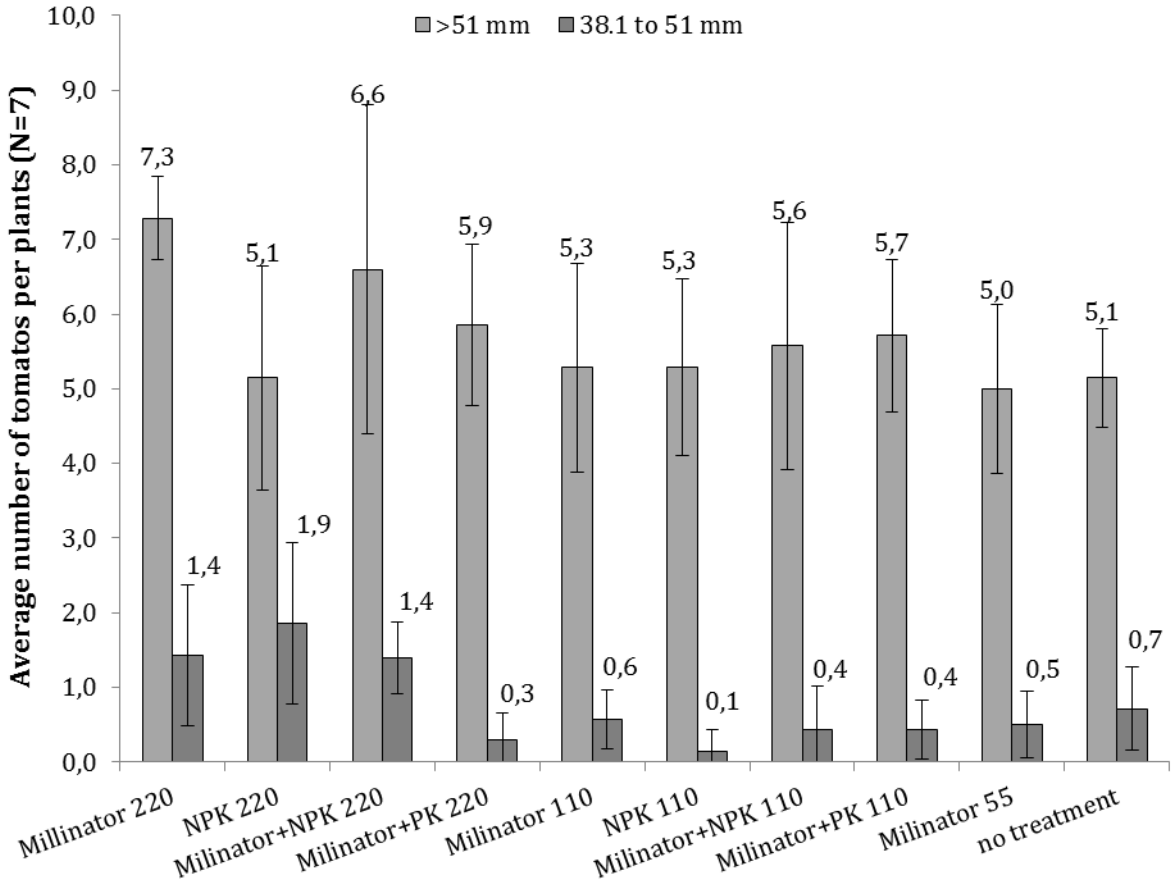


Figure 7: Tomato plants - number of fruits per size categories (>51mm and 38.1 to 51 mm).

Spinach trial results

A dose response experiment using the Milinator fertilizer in incremental amounts based on milligram of nitrogen per kilogram of soil (mg N/kg) and using 110 mg N/kg from a chemical source as a comparison point (Figure 8), shows that all treatments with a nitrogen content of 55 mg N/kg of soil and above, gave spinach plants with significantly greater average wet mass than those with N level below the 55 mg N/kg of soil.

For all treatments above the 55 mg N/kg of soil, only the Milinator treatment with 220 mg N/kg soil shows a significantly different average wet mass from the 55 mg N/kg of soil (Figure 8), but these results are no significantly different in average wet masses from the Milinator and the chemical treatments at 110 mg N/kg of soil.

The same differences are observed with the plants average dry mass between the treatments above and below the 55 mg N/kg of soil (Figure 9), but the increase observed with the wet masses does not translate is the same differences when comparing the average dry masses.

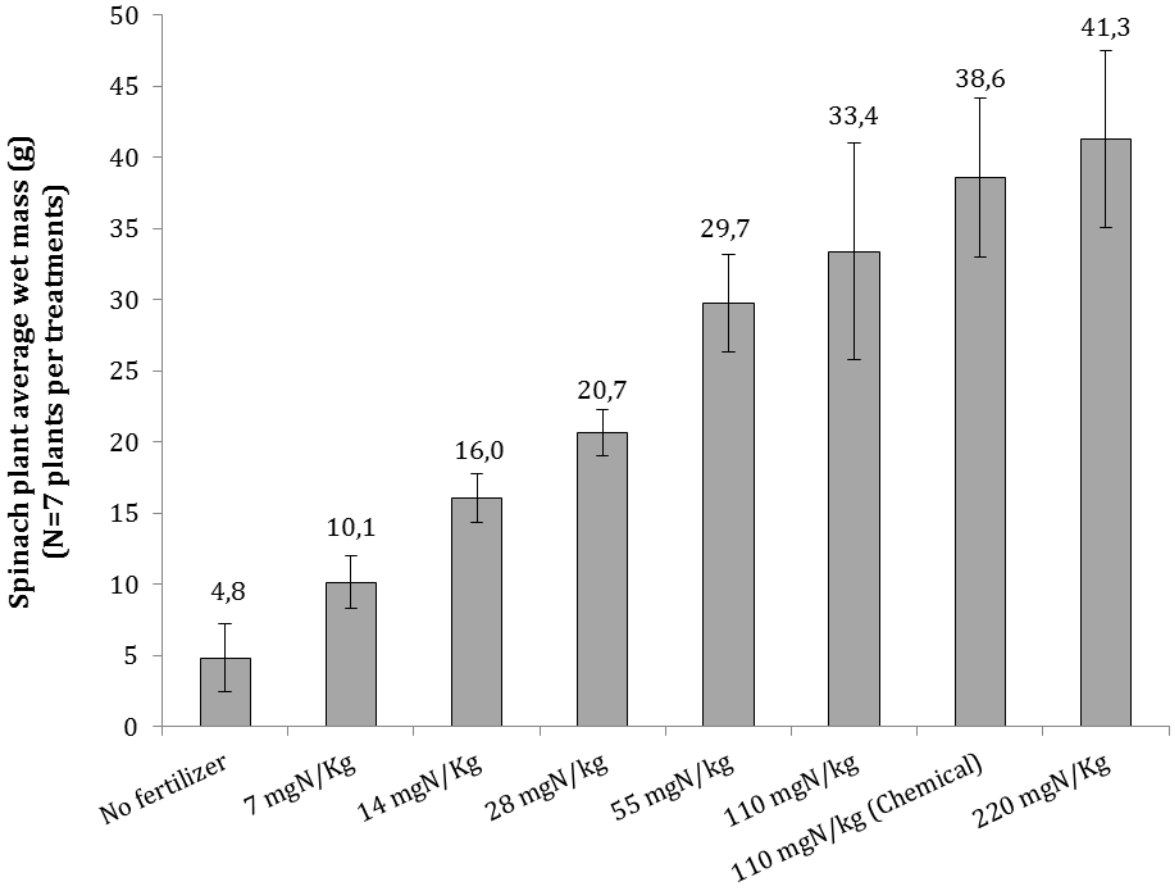


Figure 8: Spinach plants, dose response test – average wet mass (g)

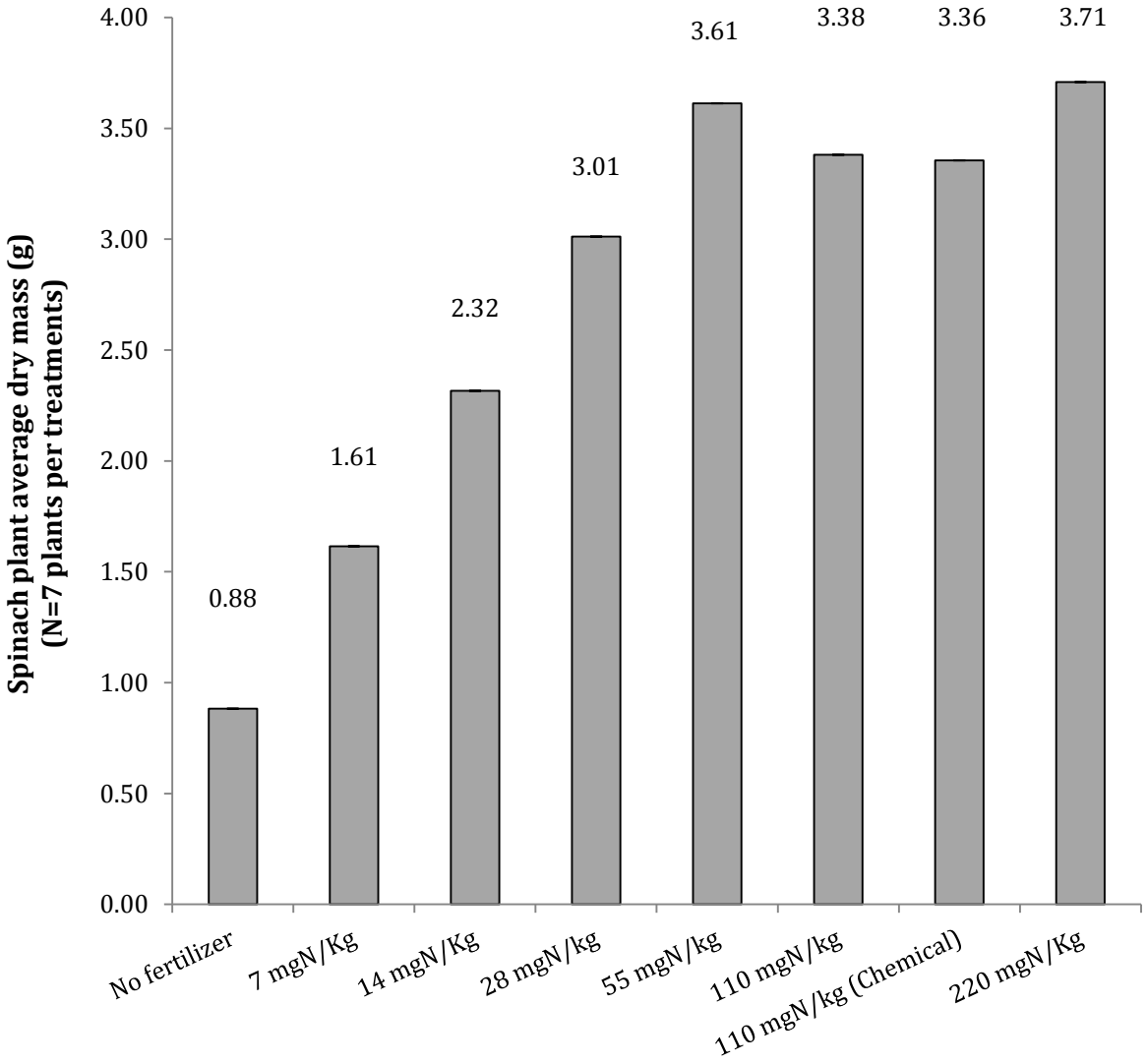


Figure 9 : Spinach plants, dose response test – average dry mass (g).

Soil metabolic activity results

No significant differences were observed in microbial metabolic activity between the tomato soils of the various treatments (Figure 10) or the sorghum soils of the various treatments (Figure 11). The only observed difference was with the sterile soil in both cases while the initial bulk soil did not show significant differences with the tomato treated soils but did for the sorghum soils. The tomato soil being a peat moss based organic soil that can sustain microbial activity, this could account for the lack of significant differences with the other soils.

Overall, the NPK and Millinator formulations appear to be equivalent in terms of their ability to support general microbial metabolic and, possibly, growth activities. The latter was not formally

assessed as it would have required knowledge of the microbial genera to be targeted in the assays. Based on the current study, Millinator formulations appear to provide a good substitute for currently available fertilization protocols. This being said, we must emphasize that the assay for microbial metabolic activity is biased in that it only takes into account readily extractable microbial populations. The assay could not, in its present design, factor in root symbionts or other closely associated commensal or mutualistic microbes. Thus, a more formal assessment of the Millinator formulation on relevant cultivar-associated microbial flora might be warranted.

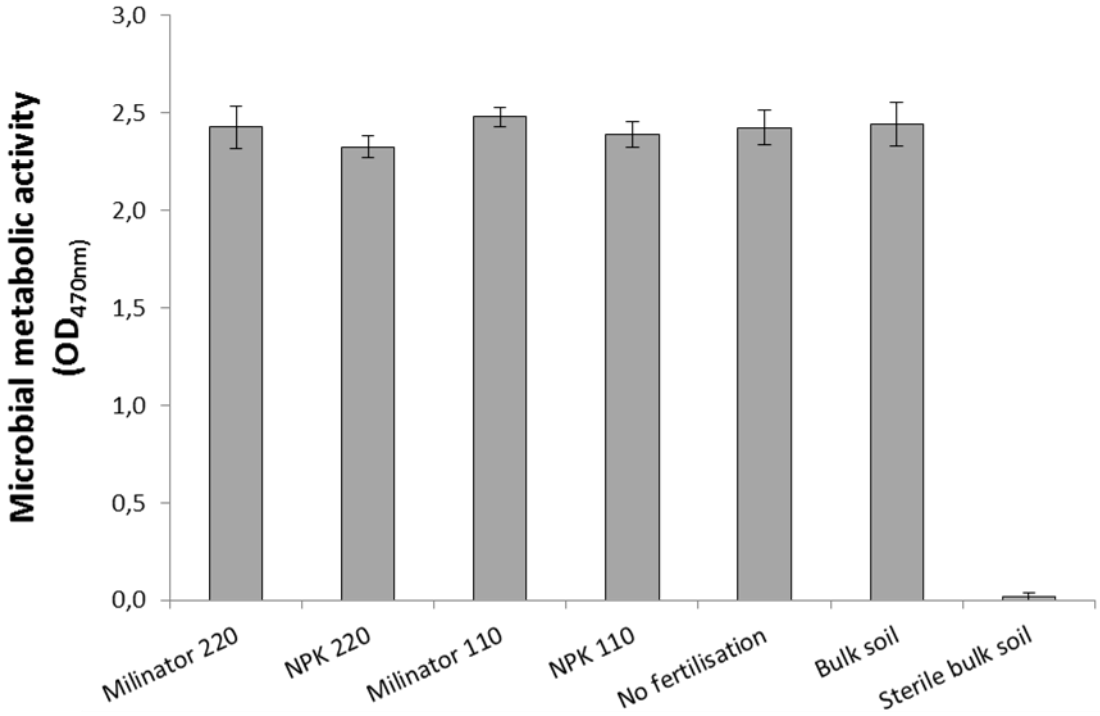


Fig 10: Determination of the tomato soil microbial activity as a function of fertilizer formulation using the Biolog™ MT2 microplates. The shaded bars represent the average maximum absorbance (λ=470nm) **Note**: a similar trend was observed at 590nm as per published in the peer reviewed literature. The extraction of soil bacteria was first performed using sterile distilled water followed by suspension in Nutrient broth. Thereafter, 150 µL of bacterial suspension was introduced into each well. Error bars represent confidence intervals at 95%.

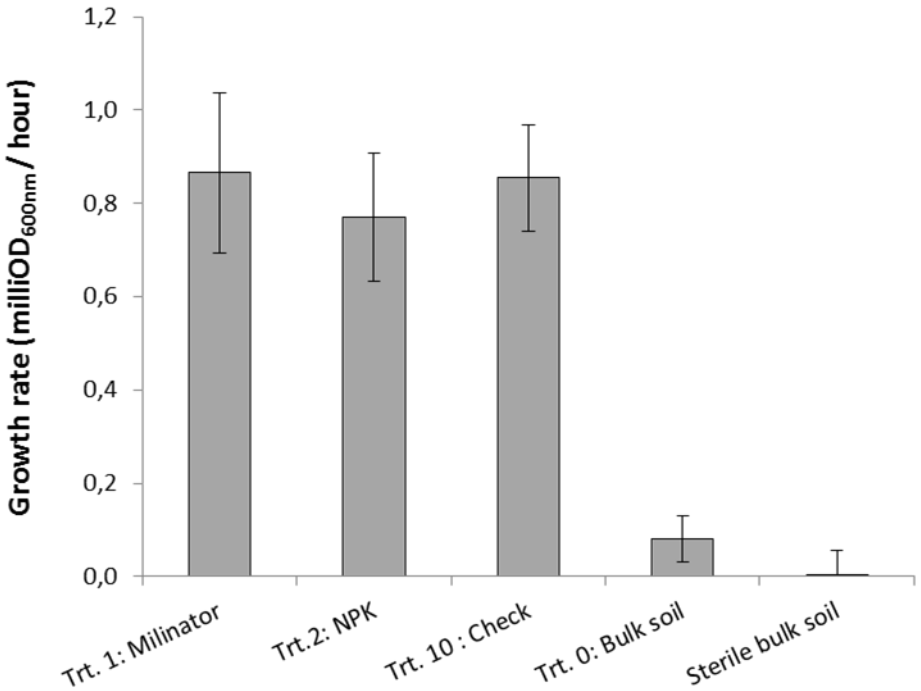


Fig 11: Comparison of bacterial growth rates in fertilized and control sorgho soils as calculated from log phase kinetic curves. Soil bacteria were recovered in sterile saline solution (NaCl 0.9%). Error bars represent confidence intervals at 95% . **Note**: The Δ max/min milliOD readings over a 32 h time course were found not to be significantly different (approx. 0,04) across soils samples.

Soil analysis

Significant differences in average total nitrogen content of the soil in which sorghum plants were grown (Figure 12) were observed only between the control treatment (Trt 10 Check) and the Milinator (Trt 1) and the NPK (Trt 2) treatments from trials conducted at the International Fertilizer Development Centre (IFDC) (www.ifdc.org) located in Muscle Shoals, Alabama, United States. No difference in soil average total nitrogen content was obtained between the bulk soil analysis and analysis of all treatments presented here.

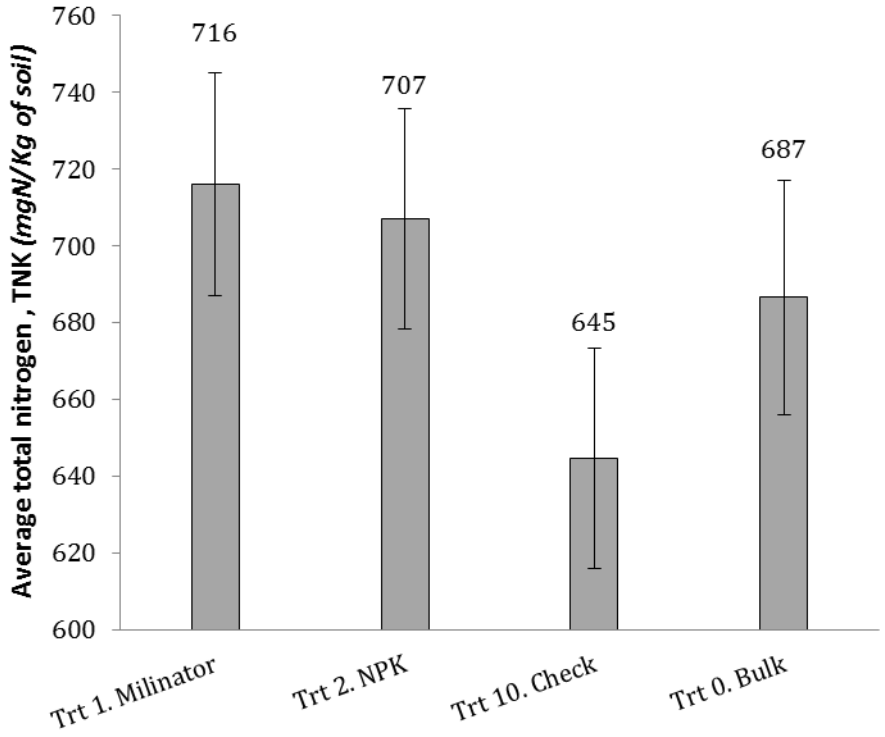


Figure 12: Average total nitrogen (mg N/kg of soil) found in the **soil** of **sorghum** plants. The results are given in mg of nitrogen/kg of soil. The error bars represent the confident interval at 95% where *n= 3* per treatments. Results are on a dry weight basis.

The treatment with the Milinator fertilizer (Trt 1) resulted in an average available nitrogen level in the sorghum soil, significantly different form all other treatments presented here (Figure 13). This result indicates that more nitrogen was still available when the sorghum experiment was terminated than for the treatment with NPK (Trt 2) which was in turn significantly different from the check and the bulk available nitrogen contents.

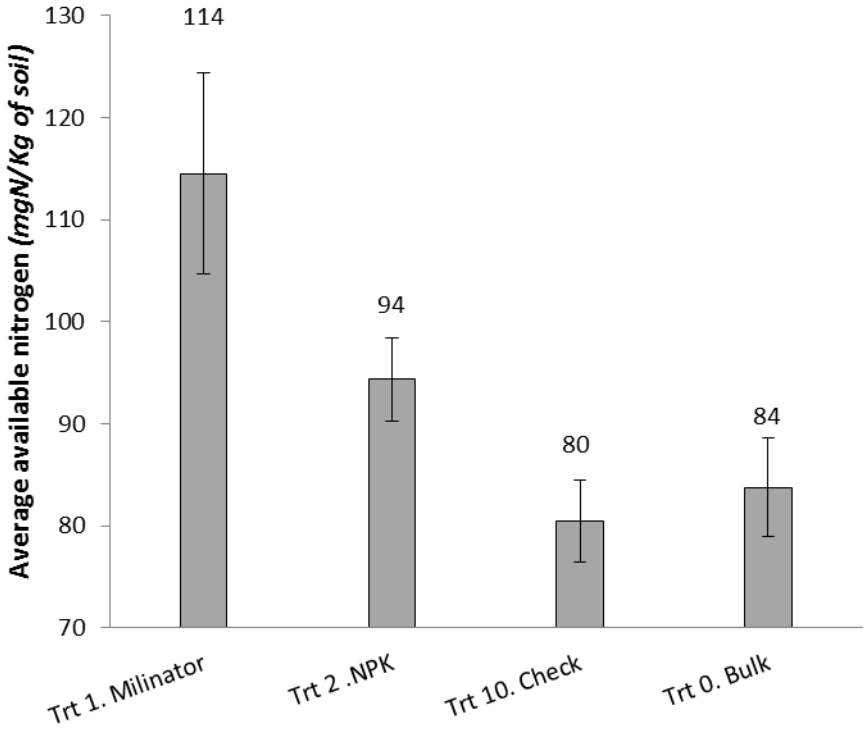


Figure 13 : Average available nitrogen (mg N/kg of soil) found in the **soil** of **sorghum** plants. The results are given in mg of nitrogen/kg of soil. The error bars represent the confident interval at 95% where *n= 3* per treatments. Results are on a dry weight basis.

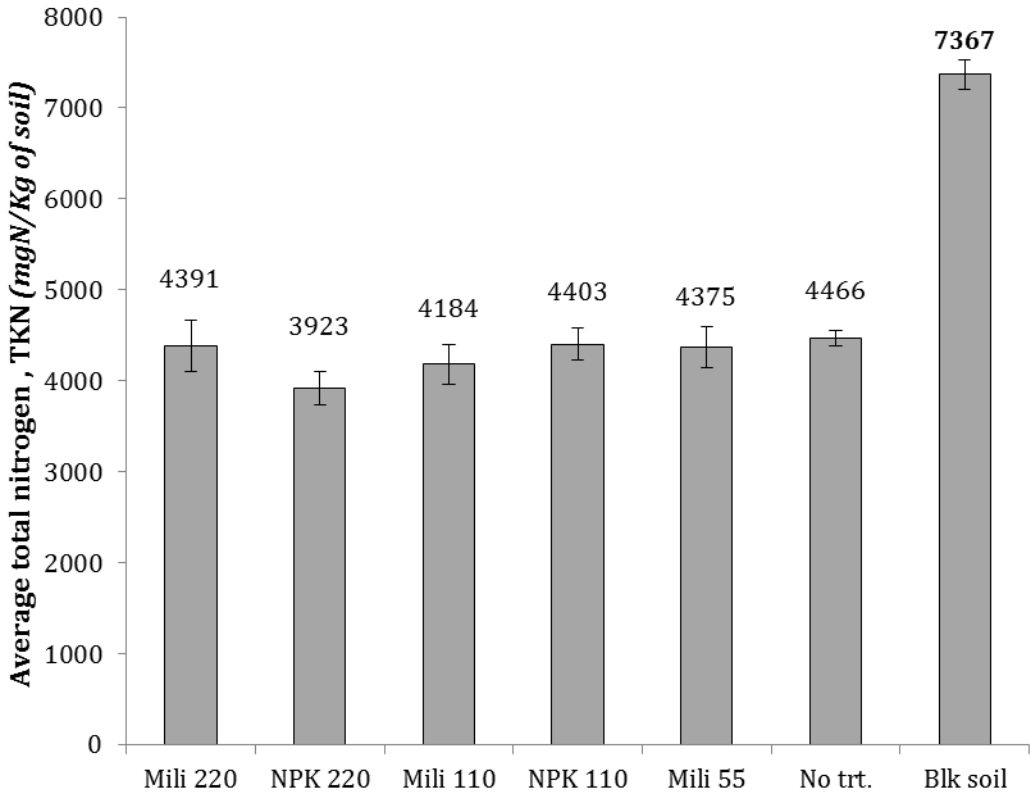


Figure 14: Average total nitrogen found in the **soil** of **tomato** plants. The results are given in mg of nitrogen/Kg of soil. The error bars represent the confident interval at 95% where *n= 3 or 7* per treatments. Results are on a dry weight basis.

The results obtained for both the average total nitrogen (Figure 14) and the average available nitrogen (Figure 15) in the tomato soils following the termination of the experiment, cannot be commented since the results obtained for all fertilizer treatments are corrupted by the results obtained with the analysis of both the control treatment and the bulk soil used for the experiment.

Results showed in Figure 16 compared the initial total nitrogen in the soils after treatments with the total nitrogen at the end of the experiment. Since the bulk soil was already high in total nitrogen content the difference in N content between the control treatment and the Milinator 220 or NPK 220 treatments was then only of 3%. Nitrogen could not be a limiting factor in the plant growth. This could have prevented us to observe larger differences between the various treatments.

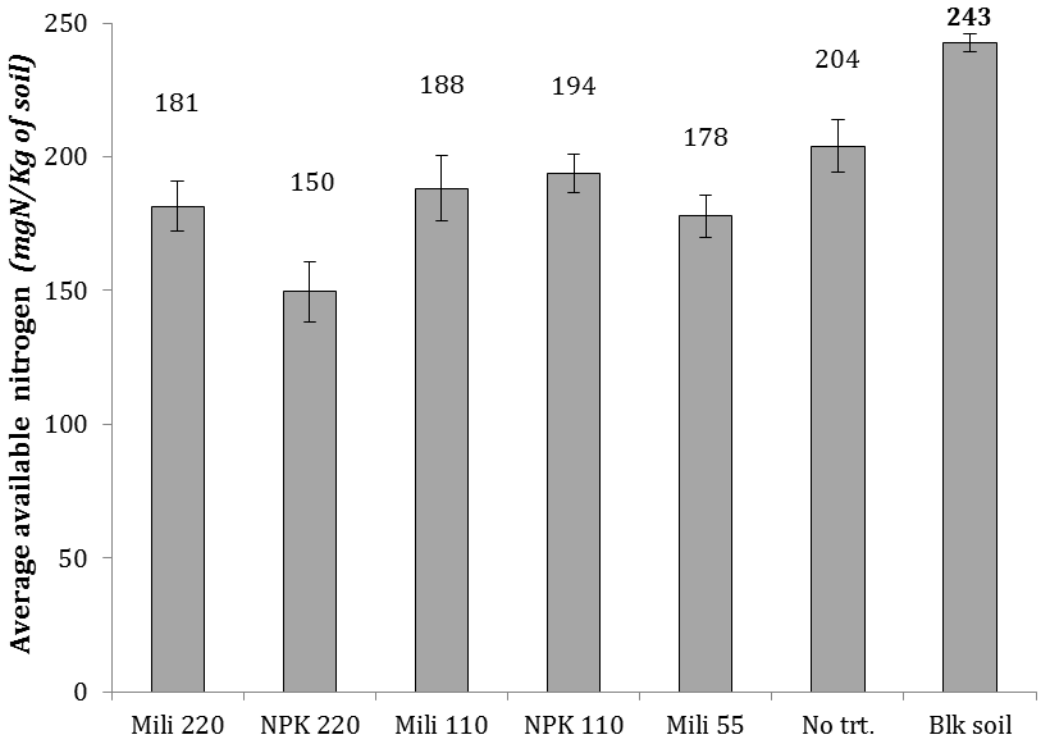


Figure 15: Average available nitrogen found in the **soil** of **tomato** plants. The results are given in mg of nitrogen/Kg of soil. The error bars represent the confident interval at 95% where *n= 3 or 7* per treatments. Results are on a dry weight basis.

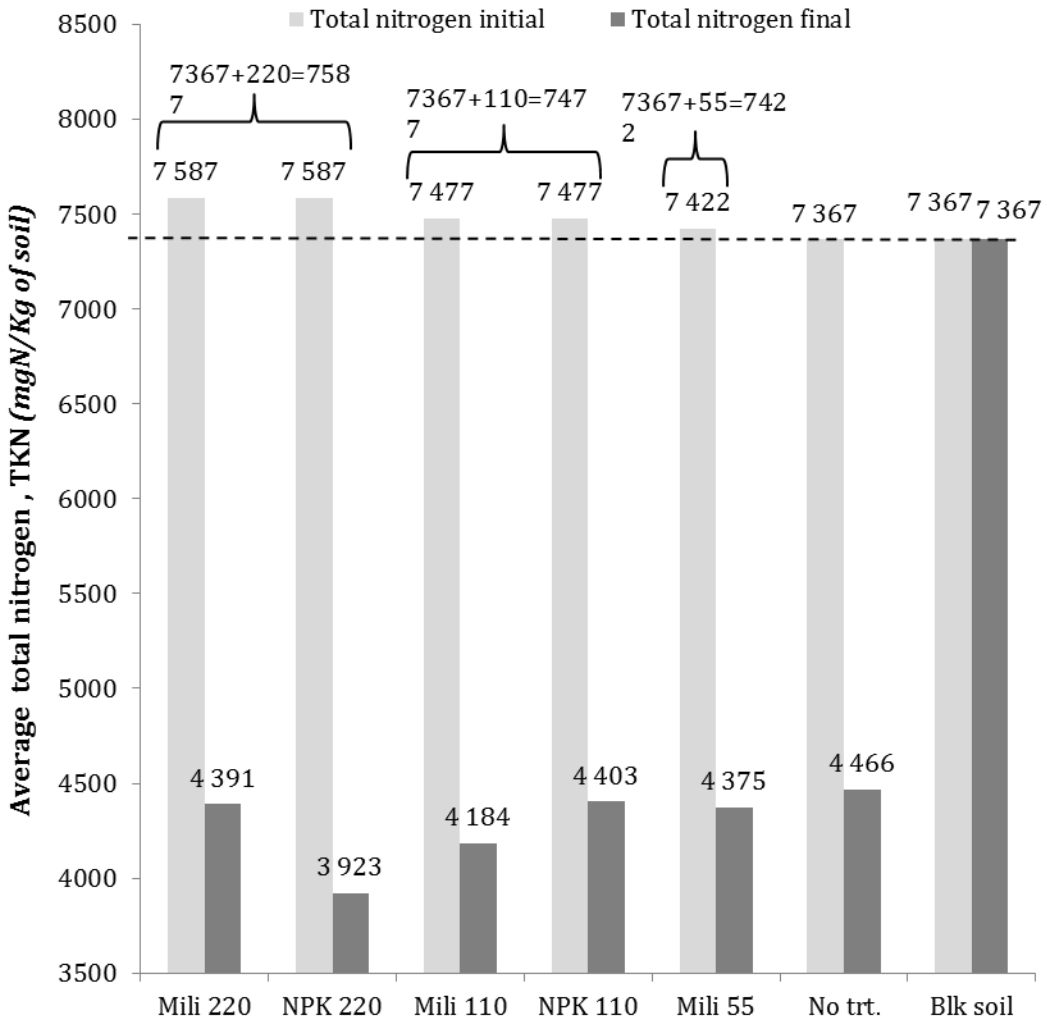


Figure 16 : Initial average total nitrogen and final average total nitrogen obtained from soil analysis at the beginning and the end of the tomato trials.

All soil pHs were close to neutrality at the end of the experiment compare to the initial pH of the soil which was more acid (6.29) (Figure 17). There was no significant difference between the pH values obtained with Milinator 110, Milinator 220, NPK 110 and NPK 220. The soils from the Milinator 110 and 220 and the NPK 220 all had a pH significantly higher than the no treatment soil, while NPK 110 had a pH not significantly different from the no treatment.

Uniformity in the pH values between treatments indicates that pH is not a limiting factor for the use of the Milinator fertilizer since its effect on the soil pH is comparable to what can be observed with a synthetic fertilizer.

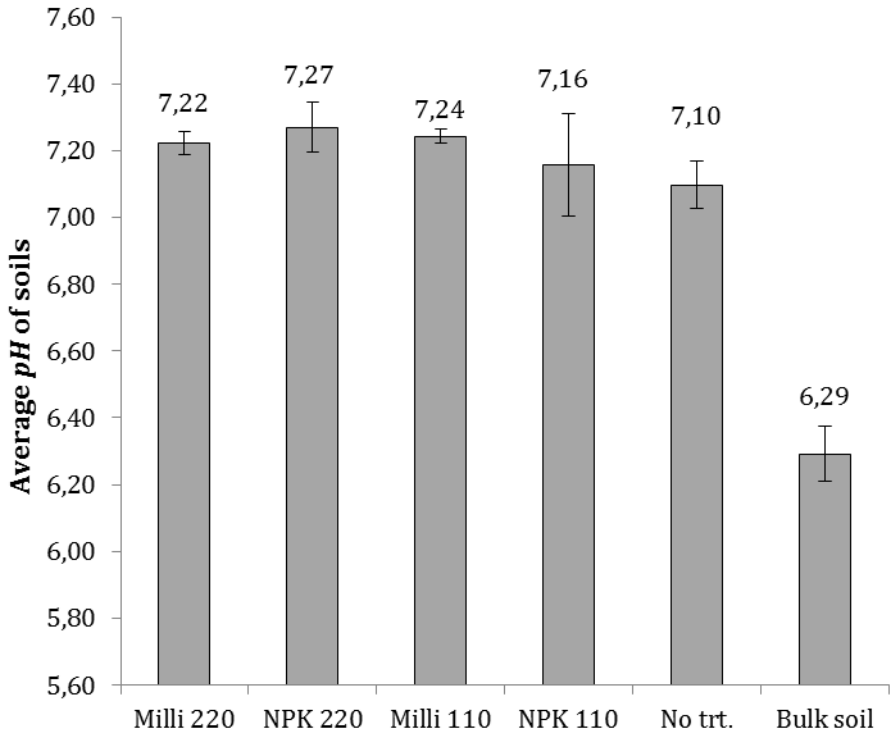


Figure 17: pH values of the soils at the end of the tomato plants growth season. The error bars represent the confident interval at 95% where *n= 4* per treatments.

Conclusion

The experiment conducted with the tomato plants under greenhouse conditions have demonstrated that the Milinator Technologies natural organic fertilizer can sustain plant growth as well as a synthetic fertilizer when applied with the same level on nitrogen per kilogram of soil. Initial observations indicate that tomato plants had a better stand and darker color when compare to tomato plants treated with a synthetic fertiliser at the same nitrogen level. Unfortunately, these differences were lost with time as the experiment reach a time of the year where greenhouse conditions were not conducive to tomato plant growth. Cooler greenhouse conditions and reduce light have resulted in growth reduction. It is suspected that these conditions and especially the cooler temperatures may have affected microbial activity which is expected to have a key role in the degradation of the organic fertilizer therefore rendering the nutriments it contains readily available to the plants. A reduction in microbial activity may account for a slower growth with the Milinator fertilizer, narrowing the gap initially observed between the growth of the plants treated with the Milinator fertiliser and those treated with the synthetic fertiliser.

Microbial activity of the soils tests did not show significant differences between soils for either the tomato trials or the sorghum trials. Since these tests were decided at the end of the trials and were not initially planned, the initial experimental design did not take into account that such measurements will be required. Specific experimentations need to be planned and properly designed in order to assess the impact of the Milinator fertilizer on the microbial activity of the soil in comparison to the same activity with a soil treated with synthetic fertilizers.

The analysis of various production batches of the Milinator fertilizer indicates that the process will need to be standardized in order to produce an organic fertilizer with a repeatable formulation in N-P-K. Results have shown that modifications in some aspects of the process can result in an important reduction in nitrogen content. It must also be keep into account that the source of chicken manure may result in some variations in the N-P-K content of the end product although these variations are far less important than the ones observed when the process itself is modified.

Although only anecdotal since no data were taken, an interesting observation was made during the experiment with tomatoes. Plants treated with the Milinator fertilizer seem to have less fungal gnats than those with no Milinator fertilizer. This observation will need to be validated with measurable data before a statement to the effect that the use of Milinator fertilizer has an impact on fungus gnats can be made. Meanwhile this observation remains interesting since some species of fungus gnats are pests of roots of potted plants in homes and greenhouses.

Trials performed with both tomatoes and sorghum, have shown the potential of the Milinator natural organic fertilizer but other experimentation are required to complete its evaluation and demonstrate its full potential. These experimentations shall include:

- Demonstration of the positive impact of the fertilizer on the soil microbial activity;
- Growth trials over a full growth cycle to evaluate the potential for the fertilizer to sustain growth following a single initial application of the fertilizer or to establish the frequency of applications required the case maybe;
- Determine the level of available nitrogen left at the end of a growing cycle to identify potential lack;
- Establish the potential synergy and its impact on plant growth of a combination of the Milinator fertilizer and synthetic fertilizer for large scale field application, since such synergy was observed with the tomato trials conducted.

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