

# Nitrogen Use Efficiency (NUE) in tomato (*Solanum lycopersicum*) seedlings in response to treatment with extract of *Cymbopogon citratus* and mineralization of *Tithonia diversifolia* leaves and cow dung

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**Abstract**— The aim of this work was to study the effect of *Cymbopogon citratus* extract on nitrogen metabolism in relation to the increase of Nitrogen Use efficiency (NUE) in tomato plants. The culture substrates ( $\delta$ ) were prepared with fertilizations of 15g N and 5g N following the formula:  $\delta$  + tomato plants + treatments (2%). Treatments included, Hydro Ethanol Extract (HEE) of *C. citratus* (2%), 2% Ridomil (R) and Control (C). The tomato seedlings were transplanted 32 days after sowing and ( $\delta$ ) sampled 12th, 24th, 36th and 48th days after transplanting and the following parameters determined: Total nitrogen, Electrical Conductivity (EC (dS/m)), Total Mineral content (TM (ppm)), pH water, nitrate ( $\text{NO}_3^-$  (ppm)), ammonium ( $\text{NH}_4^+$  (ppm)) and NUE ( $\text{kg}^{-1}$  DM), using these techniques: Kjeldahl, Electrochemistry, Spectrophotometry. The results from the dosage of N revealed that *Tithonia diversifolia* (Ti), Cow dung (Cd), soil/sand (2:1) mixture and NPK contained 3.32%, 2.13%, 0.23 %, and 23.00% of N respectively. The kinetics of mineralization in the  $\delta\text{Ti}$ ,  $\delta\text{Cd}$  showed a primary mineralization while that in the  $\delta\text{NPK}$  and  $\delta\text{C}$  showed a secondary mineralization. The values of  $\text{NUE}_{\text{NPKHEE}}$ ,  $\text{NUE}_{\text{NPKR}}$ ,  $\text{NUE}_{\text{TiHEE}}$ ,  $\text{NUE}_{\text{TiR}}$ ,  $\text{NUE}_{\text{CdHEE}}$ , and  $\text{NUE}_{\text{CdR}}$  increased by 38.49%; 37.45%; 27.74%; 52.07%; 93.93%; 70.52%, respectively.

The combination of plant spray with HEE of *C. citratus* and soil amendment with *T. diversifolia* or cow dung improved significantly the NUE of tomato plants confirming that *T. diversifolia* and cow dung are slow mineralization nitrogenous biofertilisers.

**Keywords**— Nitrogen use efficiency, Mineralization, Inputs from plants, Physicochemical soil parameters, Nitrogen release pathway.

## I. INTRODUCTION

From the second green revolution which is dedicated to increasing productivity while using sustainable farming methods and driven by significant advances in agricultural research and technology, was born the notion of Nitrogen Use efficiency (NUE) (Zeigler and Mohonty, 2010).

However, the last decades have been marked by a large use of nitrogen fertilisers, which has had a significant impact on agricultural yields, causing an increase in nitrogen fertiliser costs, and leading to growing needs in NUE (Mulvaney et al., 2009, Zeigler and Mohonty, 2010). Similarly, nitrogen fertilisers once applied to the soil are directly mineralized by beneficial soil microorganisms

(Sherlock and Goh, 1985). The nitrogenous minerals thus liberated will be used by the plant, of which it represents 2 to 5% of its dry weight and is the structural component of proteins, nucleic acids, cofactors and secondary metabolites (Miller and Cramer, 2005). The mineral nitrogen available to the plant is in the form of ammonium and nitrate (Herrera, 1999).

World consumption of fertilisers amounted to 196.4 million tons in 2016: 68.6% nitrogen, 19.2% phosphates and 12.3% potassium (Faostat, 2016). It is estimated that 50 to 70% of nitrogen fertiliser losses in the plant-soil-plant system recorded each year is caused by rapid fertiliser mineralization, soil type, plant species, and soil physico-chemical properties (Hogdes, 2002; Good et al., 2004). However, losses of these minerals have serious impacts on ecosystems, stratosphere, groundwater and agricultural yields. This leads to great economic losses for farmers (Peoples et al., 1995). The challenge is that of optimizing the use of nitrogenous minerals available in the soil by plants, while trying to optimize the nitrogen uptake provided by the mineralization of biofertilisers.

Chemical and engineering approaches have been used on nitrogen metabolism in plants and despite their limits and ethical issues among ecologists, they have shown effectiveness in raising the NUE in plants by 2% (Hongmei et al., 2008, Swain and Abhijita, 2013). However, it was stated that the NUE should be raised by 20% to save \$ 4.1 billion every year (Raun and Gordon, 1999). Kaho et al. (2011), evaluated the combined effects of *Tithonia diversifolia* (FTd) and inorganic fertilisers (NPK and Urea) on maize grain yields and soil properties. With the acceptability index (AI) of more than 2 in T4 (5 t/ha FTd, 0NPK and 0Urea) and T5 (2.5 t/ha FTd, 75 kg 20-10-10 and 75 kg of Urea), they found that *T. diversifolia* has a potential for improving plant nutrient availability in these soils for the cultivation of maize without chemical fertilisers. The leaves of *T. diversifolia* (Nuraini and Sukmawatie, 2014), and cow dung have fertilizing properties (Tanimu et al., 2013). Sakakibara (2002), stated that congestion of certain phytohormones communicates the availability of nitrogenous minerals to the root system of a plant, by demonstrating that algae extracts are rich in phytohormone that can stimulate absorption and the use of nitrogenous minerals in wheat plants. These previous research works constitute motivation of the present study.

The aim of this work was to study the effect of *Cymbopogon citratus* hydro ethanol extract on nitrogen metabolism in combination with *Tithonia diversifolia* (Ti), Cow dung (Cd) and an inorganic fertiliser (NPK) in relation to the increase of Nitrogen Use efficiency (NUE) in tomato plants.

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## II. MATERIAL AND METHODS

### 2.1. Experimental site

The study was carried out in a greenhouse at Institute of Agricultural Research and Development (IARD), Nkolbisson, Yaoundé, Cameroon at latitude 3° 51' North and longitude 11° 40' East, with an altitude of 759 m. The annual distribution of rains is bimodal with peaks in May and October. The average annual precipitation varies from 1134-2112 mm. Average temperature is around 24.7°C. Relative humidity varies between 50-80% in dry season and 70-90% in raining season.

### 2.2. Materials

#### 2.2.1. Biofungicide plant

Whole plants of *Cymbopogon citratus* (D.C.) Stapf were harvested at Nkolbisson, Yaoundé around households and identification of the plant species was conducted by the Cameroon National Herbarium in Yaoundé. The fresh plants were air dried at room temperature (25-27°C) for 10 to 14 days and milled into powder.

#### 2.2.2. Synthetic fungicide

Ridomil (4% w/w Metalaxyl-M or Mefenoxam, CGA329351; 60% w/w Copper hydroxide) used as synthetic fungicide was bought at the phytosanitary shop at Nfoundi market.

#### 2.2.3. Synthetic fertiliser

The synthetic fertilizer used, was NPK 23.10.5, bought in the market place.

#### 2.2.4. Biofertilisers

Fresh leaves of *Tithonia diversifolia* L. were harvested in the city at Nkolbisson, Yaoundé and air dried at room temperature (25-27°C) for 10 to 14 days and milled into powder.

Cow dung was collected at Institute of Agricultural Research and Development (IARD) Nkolbisson-Yaoundé farm, dried at open air and milled into powder.

#### 2.2.5. Tomato seeds' cultivar

Seeds of the tomato cultivar "Roma VF" bought at local shop at Nfoundi market, Yaoundé were used for the experiment.

### 2.3. Methods

#### 2.3.1. Preparation of Ridomil suspension

Twenty grams (20g) of mefexan powder and copper oxide (Ridomil) were weighed and introduced into one liter of distilled water and the mixture stirred for a few minutes.

#### 2.3.2. Preparation of the hydro ethanol extract of *C. citratus*

Dried plants were milled and freed from lipids by mixing 100 g of powder with 100 ml of hexane for 24h. After filtration, the residue was displayed for complete evaporation of the solvent. The hydroethanol extract was obtained by adding the residue to 100 ml of 70% ethanol and the mixture allowed standing for 24h. The supernatant was passed through Whatman n°1 filter paper. The ethanol was totally evaporated using a rotary evaporator and the water residue adjusted to 100 ml with distilled water and freeze dried for later use in tomato plants treatment.

#### 2.3.3. Extraction of the essential oil of *C. citratus*

The essential oil (EO) was extracted from dried plant material by hydrodistillation for five hours using a Clevenger-type apparatus. The EO collected was dried on anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) column and kept in the refrigerator at 4°C into airtight brown bottles. Yields of the oils were calculated as percent of dried plant material weight (% w/w).

#### 2.3.4. Experimental Design

Thirty two-day-old tomato plants raised in nursery in the greenhouse were used for the experiment. Three different nursery beds were fertilised as followed: to 5 Kg mixture of soil/sand (2: 1) were incorporated, 22 g of NPK in the first tray, 150 g of *T. diversifolia* powder in the second tray and 200 g of cow dung powder for the third tray; for a reasoning of the fertilization with 5 g of nitrogen. Tomato seeds were treated with 0.2% essential oil of *C. citratus* before seeding at the rate of 200 seeds per tray.

#### 2.3.5. Physicochemical parameters experimental design cultural and substrates

##### -Experimental design

The trial was conducted in a completely randomized blocks design. The main block was represented by seedlings sprayed with 2% hydroethanol extract of *C. citratus*, a positive control block was sprayed with 2% Ridomil and the negative control block was sprayed with water. Each block consisted of 12 pots, divided into 3 amendments with 3 repetitions each. The fertilisers were: *T. diversifolia* powder, NPK, cow dung. Unfertilized sand/soil was used as control.

##### -Cultural Substrates preparation

Amended soils were prepared according to the method described by Henrickson, 2005. To 5kg soil/sand mixture (2:1), was incorporated 67.5g of NPK, 600g cow dung powder and 450g of *T. diversifolia* powder, making a reasoning of fertilization of 15g of nitrogen. Amended soils distributed in pots of 50cm high and 30cm diameter included: cow dung potting soil ( $\delta\text{Cd}$ ), soil *T. diversifolia* ( $\delta\text{Ti}$ ), soil NPK ( $\delta\text{NPK}$ ), control potting soil ( $\delta\text{C}$ ). The 32-

day old tomato seedlings were transplanted into the previously prepared pots. The seedlings were treated once after transplantation with 250 ml of solution, by spraying with 2% Ridomil, 2% hydroethanol extract *C. citratus* and distilled water for the control. The plants were kept for an adaptation period of 20 days. After that, culture substrates were sampled periodically each 12<sup>th</sup> day after transplantation for 48 days. Part of substrates was dried for one week; the samples were re-milled and sieved with less than 2 mm mesh size. The upper fraction was used to determine  $\text{pH}_{\text{water}}$ , and the finest fraction used to measure the electro conductivity (EC), total minerals, nitrate and ammonium minerals. The results allowed to establish the kinetics of  $\text{pH}_{\text{water}}$ , EC; and Turnovers of TDS, nitrates and ammoniums concentration.

The pH of the culture substrates was measured by electrochemistry using HANNA pH meter instruments after preparing culture substrate suspensions in distilled water at a ratio of 1:5 (NF ISO 10390, 2005). Electro conductivity and total minerals concentrations of substrates were determined in cultural substrates suspension in distilled water at a ratio of 1:5 using an INOLAB brand conductivity meter (NFISO 11265, 2005). For the determination of nitrate minerals, the USDA formula was used by assigning to the EC determined in a ratio of 1:5 in  $\text{Ds/m}$  the coefficient 140 if and only if the water pH was below 7.2 (NF ISO 11265, 2005; USDA, 2014).

For the determination of ammonium minerals, samples of untreated cultural substrates were extracted with distilled water in a ratio 1:5. Four milliliters (4 ml) of the soil/water suspension of each sample were transferred to 40ml flasks; into which 1ml of the Nessler reagent was added and the orange-yellow colors are allowed to develop for 30 minutes. A standard was prepared using increasing concentration of ammonium minerals of 0, 1, 2, 3, 4, 5ppm; and 4ml of each solution were transferred into 40ml flasks to which 1ml of Nessler reagent was added and the orange-yellow colors were allowed to develop for 30 minutes. The optical density (OD) was determined spectrophotometrically at a  $\lambda$  of 410nm. Ammonium mineral concentrations were obtained graphically by referring to the calibration curves (NF ISO14256-2., 2007).

#### 2.3.6. Nitrogen Optimization experiment

##### -Experimental design

The trial was conducted in a completely randomized blocks design. The main block was represented by seedlings sprayed with 2% hydro ethanol extract of *C. citratus*, a positive control block was sprayed with 2% Ridomil and the negative control block was sprayed with water. Each block consisted of 12 pots, divided into 3 amendments with

3 repetitions each. The fertilisers were: *T. diversifolia* powder, cow dung and NPK. Unfertilized sand/soil was used as negative control.

#### -Cultural Substrates preparation

Amended soils were prepared according to the method described by Henrickson, 2005. To 5kg soil/sand mixture (2:1), is incorporated 25g of NPK, 200g cow dung powder and 145g of *T. diversifolia* powder, making a reasoning of fertilization of 5g of nitrogen. The 32-day-old tomato seedlings were transplanted into the previously prepared pots and were treated once with 250 ml of solution, by spraying with solution of 2% Ridomil, 2% hydroethanol extract *C. citratus* and distilled water for the control. The plants were kept for an adaptation period of 20 days and uprooted after 35 days. Fresh/dry weights were weighed using a METTLER PC 2200 precision scale. Nitrogen contents in dry tomato plants, cow dung powder, *T. diversifolia* powder, and the experimental soil were determined by digesting 0.1g of each sample into a mixture of  $H_2O_2$ , selenium,  $LiSO_4H_2O$  and 5ml of a 7N sulfuric acid solution. The mixtures are transferred to a digestion device which is programmed at a temperature of 400°C. for 12 hours. The nitrogen contents were determined spectrophotometrically at a  $\lambda$  of 655nm (NF ISO 11260 2005). NUE was expressed as the amount of dry matter produced by nitrogen content in the plant, as written by Masclaux-Daubresse et al., 2010 by the formula following:

$NUE = \frac{DM}{N} \div \frac{DM}{N}$  with DM (Dried Matter in mg); N (Nitrogen in mg/Kg or ppm)

#### 2.4. Statistical analyzes of the results

The results obtained were subjected to a descriptive analysis for the calculation of means, standard deviations and the search for significant differences using Statistical Package for Social Sciences (SPSS) software version 22.0. The Analysis of Variance (ANOVA) test coupled with the Newman Keuls t-Student test was used to evaluate the smallest significant difference at the 0.05 probability level. The graphs were built from the Microsoft Office 2013 Excel software.

### III. RESULTS

#### 3.1. Nitrogen (N) content in cultural substrates

The nitrogen contents as percent in the cultural substrates are given in Table 1. *Tithonia diversifolia* powder showed higher nitrogen proportions than the cow dung powder, with 3.32%, and 2.13%, respectively. The soil-sand mixture in a ratio of 2:1, contained 0.21% nitrogen and NPK, 23% of nitrogen.

#### 3.2. Physicochemical parameters of soil substrates in pots with tomato plants

##### 3.2.1. Kinetics of mineralization of fertilisers in experimental blocks

The results obtained from the determination of the EC (dS/m) in the  $\delta$  of the three blocks (HEE of *C. citratus*; Ridomil and Control) allowed to establish the curves expressing the kinetics of mineralization by measuring the EC (dS/m) as a function of time (Fig. 1).

The comparison of the different mean ECs as function of time in days, showed very significant differences ( $p \leq 0.05$ ).  $\delta Ti$  and  $\delta Cd$  showed mineralization kinetics that followed progressive dynamics over distributed time for the 48-day experiment; the EC  $\delta Ti$  varied from  $EC_{12} = 351.46 \times 10^{-3} dS/m$  to  $EC_{48} = 521.87 \times 10^{-3} dS/m$  with a slight decrease on the 24<sup>th</sup> day, whereas in the  $\delta Cd$  the EC ranged from  $EC_{12} = 212.55 \times 10^{-3} dS/m$  at  $EC_{48} = 521.87 \times 10^{-3} dS/m$ ;  $\delta Ti$  mineralized more rapidly than  $\delta Cd$ . In contrary, the  $\delta NPK$  and  $\delta C$  showed mineralization kinetics that followed regressive dynamics on the distributed time for the 48-day experiment. EC in  $\delta NPK$  ranged from  $EC_{12} = 1315.03 \times 10^{-3} dS/m$  to  $EC_{48} = 680.78 \times 10^{-3} dS/m$ ,  $\delta C$  had ECs varying from  $EC_{12} = 134.01 \times 10^{-3} dS/m$  to  $EC_{48} = 46.60 \times 10^{-3} dS/m$ .

##### 3.2.2. Turn Over of total minerals (TDS (ppm)) in each substrate in the experimental blocks

The total mineral concentrations in substrate in block treated with HEE of the three blocks (HEE of *C. citratus*; Ridomil and Control) were recorded, values from which the total mineral Turn Over (TDS (ppm)) were generated are presented in Table 2.

The determination of the mean values of total mineral concentrations (TDS (ppm)) of the substrates recorded in table 2, resulted from the averages of the different values of (TDS (ppm)) of each substrate, taken from each block, with the corresponding interpretations. In general the mineralization process was highlighted by the content in total minerals in different blocs.  $\delta Cd$  and  $\delta Ti$  showed progressive mineral congestion over time and the values ranged from  $TDS_{12} = 302.23$  ppm to  $TDS_{48} = 743.26$  ppm for  $\delta Cd$ , and from  $TDS_{12} = 502.22$  ppm to  $TDS_{48} = 741.22$  ppm for  $\delta Ti$ . In contrary the  $\delta NPK$  and  $\delta C$  blocks showed regressive mineral congestion, with TDS ranging from  $TDS_{12} = 2049.93$  ppm to  $TDS_{48} = 973.88$  ppm for  $\delta NPK$  and from  $TDS_{12} = 191.57$  ppm to  $TDS_{48} = 65.00$  ppm for  $\delta C$ .

*Summary of interpretation of the reactions ( $pH_{water}$ ) of each substrate of the three blocks over time*



The determination of the mean values of the reactions ( $\text{pH}_{\text{water}}$ ) as shown in Table 3, resulted from the averages of the different values of the  $\text{pH}_{\text{water}}$  of each substrate, taken from each block, with the corresponding interpretations.

In general, the reactions of the different substrates showed a highly significant difference ( $p \leq 0.05$ ) over the days. The  $\delta\text{Ti}$  and  $\delta\text{Cd}$  showed an improvement of the reactions in the direction of the optimal reaction of absorption of the minerals which had respective values between  $\text{pH}_{\text{water}12} = 7.00$  to  $\text{pH}_{\text{water}48} = 6.33$  and  $\text{pH}_{\text{water}12} = 6.12$  to  $\text{pH}_{\text{water}48} = 6.18$ . The  $\delta\text{C}$  had similar reaction with values varying from  $\text{pH}_{\text{water}12} = 6.31$  to  $\text{pH}_{\text{water}48} = 6.08$ . The  $\delta\text{NPK}$  reactions showed an improvement in the direction of the immobilization reaction in comparison with the reactions of  $\delta\text{Bv}$ ,  $\delta\text{Ti}$ , and  $\delta\text{C}$ , with values ranging from  $\text{pH}_{\text{water}12} = 5.65$  to  $\text{pH}_{\text{water}48} = 5.37$ .

### 3.2.3. Turn Over of Nitrogenous Minerals From Fertilisers of Each substrate In Experimental Blocks

#### -Concentration in Nitrate ( $\text{MNO}_3^-$ (ppm)) of pot substrates in experimental blocks

The determination of the concentration in  $\text{MNO}_3^-$ (ppm) in substrates of the three blocks (HEE of *C. citratus*; Ridomil and Control) allowed to obtain of Turn Over of  $\text{MNO}_3^-$ (ppm) as illustrated in Fig.2.

From Fig.2, it can be seen that the concentrations of nitrogenous nitrate minerals in the substrates showed a significant difference ( $p \leq 0.05$ ) as function of time in days. The  $\delta\text{Ti}$  and  $\delta\text{Cd}$  over the 48-day period showed mineral availability  $\text{NO}_3^-$ (ppm) which followed progressive dynamics; while in the  $\delta\text{NPK}$  and  $\delta\text{C}$ , mineral availability  $\text{NO}_3^-$ (ppm) followed regressive congestion dynamics after 48 days. Thus, with a mineral regression  $\text{NO}_3^-$ (ppm) ranging from  $\text{NO}_3^-_{12} = 168.28\text{ppm}$  to  $\text{NO}_3^-_{48} = 134.82\text{ppm}$  and  $\text{NO}_3^-_{12} = 20.83\text{ppm}$  to  $\text{NO}_3^-_{48} = 8.52\text{ppm}$ . The  $\delta\text{NPK}$ ,  $\delta\text{C}$  were denitrified with respect to  $\delta\text{Ti}$ ,  $\delta\text{Cd}$  which were nitrified. The  $\delta\text{Ti}$  showed the highest nitrification, with concentrations  $\text{NO}_3^-$ (ppm) ranging from  $\text{NO}_3^-_{12} = 47.79\text{ppm}$  to  $\text{NO}_3^-_{48} = 77.23\text{ppm}$ , and  $\delta\text{C}$  with  $\text{NO}_3^-_{12} = 25.68\text{ppm}$  to  $\text{NO}_3^-_{48} = 68.75\text{ppm}$ . The biofertiliser, *T. diversifolia* was better at nitrification than cow dung.

*\*Summary of interpretation the Turn Over minerals nitrates in substrates of the three experimental blocks for the trial period*

The determination of mean mineral concentrations  $\text{NO}_3^-$ (ppm) reported in Table 6, resulted from the averages of the different values of the mineral concentrations  $\text{NO}_3^-$ (ppm) of each substrate, taken from each block, with the corresponding interpretations.

The Turn Over of nitrate ( $\text{NO}_3^-$ ) in blocks showed a significant difference ( $p \leq 0.05$ ) as function of time in days. After 48 days  $\delta\text{Ti}$  and  $\delta\text{Cd}$  showed nitrate Turnovers (ppm) that followed progressive congestion dynamics, with values ranging from  $\text{NO}_3^-_{12} = 49.13\text{ ppm}$  to  $\text{NO}_3^-_{48} = 73.2\text{ ppm}$  for  $\delta\text{Ti}$  and  $\text{NO}_3^-_{12} = 29.90\text{ ppm}$  to  $\text{NO}_3^-_{48} = 63.59\text{ ppm}$  for  $\delta\text{Cd}$ . For the  $\delta\text{NPK}$  and  $\delta\text{C}$ , the Turn over of  $\text{NO}_3^-$ (ppm) followed regressive congestion dynamics after 48 days. Thus, the Turn over values of  $\text{NO}_3^-$ (ppm) decreased  $\text{NO}_3^-_{12} = 181.2\text{ ppm}$   $\text{NO}_3^-_{48} = 95.27\text{ ppm}$  for  $\delta\text{NPK}$  and  $\text{NO}_3^-_{12} = 18.79\text{ ppm}$  to  $\text{NO}_3^-_{48} = 6.55\text{ ppm}$  for  $\delta\text{C}$ .

#### - Concentration in Ammonium ( $\text{MNH}_4^+$ (ppm)) of pot substrates in experimental blocks

The determination of the concentration in  $\text{MNH}_4^+$ (ppm) in substrates of the three blocks (HEE of *C. citratus*; Ridomil and Control) allowed to obtain of Turn Over of  $\text{MNH}_4^+$  as illustrated by Fig.3.

The statistical analysis of  $\text{MNH}_4^+$ (ppm) data showed a significant difference ( $p \leq 0.05$ ) over days. The  $\delta\text{Ti}$ ,  $\delta\text{Cd}$ ,  $\delta\text{NPK}$  and  $\delta\text{C}$ , over the 48-day period, showed the Turn Over availability of  $\text{MNH}_4^+$ (ppm) that followed regressive congestion dynamics; with a regression in  $\text{NH}_4^+$ (ppm) ranging from  $\text{NH}_4^+_{12} = 75.07\text{ppm}$  to  $\text{NH}_4^+_{48} = 43.06\text{ppm}$  for  $\delta\text{NPK}$ ,  $\text{NH}_4^+_{12} = 5.725\text{ppm}$  at  $\text{NH}_4^+_{48} = 3.43\text{ppm}$ , for  $\delta\text{Ti}$   $\text{NH}_4^+_{12} = 5\text{ppm}$  at  $\text{NH}_4^+_{48} = 2.82\text{ppm}$  for  $\delta\text{Cd}$  and  $\text{NH}_4^+_{12} = 3.09\text{ppm}$  at  $\text{NH}_4^+_{48} = 1.44\text{ppm}$  for  $\delta\text{C}$ .

### 3.3. Nitrogen Use Efficiency (NUE) of tomato seedlings 35 days after transplanting in experimental blocks

The data reported in Figure4, represent the Nitrogen Use Efficiencies (NUE) of tomato seedlings 35days after transplanting, values by which the NUE graph ( $\text{Kg}^{-1}\text{DM}$ ) according to the treatments was constructed. The evolution of NUE for each treatment during the incubation period was higher in the amended and treated substrates than in the non-treated and non-amended control substrates. Seedlings obtained from combined amendment and treatments, NPK-HEE and NPK-R, showed an improvement in NUE of 38.49% and 37.45% respectively, as compared to seedlings from only amended plot NPK-C. The NUE values of 35-day-old seedlings from following treatment combinations Ti-HEE, Ti-R, Cd-HEE, Cd-R increased respectively by 27.74%, 52.07% and 93.93%, 70.52% as compared to the controls Ti-C and Cd-C (Fig. 4).

## IV. DISCUSSION

### 4.1. Nitrogen (N) content in cultural substrates

With the increase in the use of synthetic nitrogen fertilisers and the repercussions caused on the environment by their

mineralization, researchers have turned to new sources of nitrogenous sources to improve the use of available nitrogen minerals in the soil for crops. The results from the nitrogen content determination of the fertilisers gave the respective values of: 3.32% N, 2.13% N, 0.23% N, 23% N, in *Tithonia diversifolia*, cow dung, Soil/Sand and NPK. These results are in accordance with the findings of Roy and Kashem (2014). These authors reported that cow dung contained nitrogen and can be used as biofertilisers. Jama et al., 2000, reported on *T. diversifolia*'s ability to improve soil fertility. Roy and Kashem (2014), have shown the ability of cow dung to change the properties of a soil at different times and obtained similar results with the nitrogen content in cow dung of 2.13% and in the experimental soil of 0.52%. Jama et al., 2000 and Moke et al., 2013, obtained slightly different results for nitrogen content in *T. diversifolia* with respective values of 3.5% and 4.2%. Nitrogen contents in biofertilisers are elucidated by the state of the organic matter (OM), the effectiveness of the mineralization during the experiment, the parts of the plant used (leaves only, or whole plant) and the origin of the soil used.

#### 4.2. Physicochemical parameters of soil substrates in pots with tomato plants

The mineralization kinetics in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  over 48 days followed progressive dynamics, confirmed by total mineral congestion dynamics. The kinetics of mineralization of  $\delta\text{NPK}$  and  $\delta\text{C}$  followed regressive dynamics, confirmed by the Turn over of TMs that followed regressive congestion dynamics. These results are similar to those of Roy and Kashem (2014), which demonstrated the dynamics of mineralization of cow dung and poultry manure by determining the EC of the cultural substrates. In fact, Ti and Cd undergo primary mineralization, which in its execution process uses the young organic matter, in which the nutrients are incorporated in molecular form. The action of biotic and abiotic factors allows the partial release of minerals such as  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{CO}_2$ ,  $\text{PO}_4^{2-}$ ,  $\text{SO}_4^{2-}$  (Smith and Doran 1996). Some is used in the biochemical cycle to form humus, which combines with clay to form the Clay-Humic Complexes with a negative overall charge. This complex captures the positively charged minerals to form the adsorbent complex hence, for a slow mineralization of the OM; the minerals will have a longer hold time in the soil (Bationo et al., 2007, Duhan et al., 2005). The NPK and the non-treated control have undergone secondary mineralization, which in its execution process uses stable minerals or pre-existing adsorbent complex. This mineralization depends on the quantity, nature and composition of elements of the different fertilisers (De neves et al., 2004). This mineralization accounts for

leaching, volatilization, and uptake and plant utilization (USDA 2014). Chibane (1999) showed that the favorable EC for tomato cultivation is 0.625dS / m. The EC values obtained at 48<sup>th</sup> day in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  were 0.5229dS / m and 0.5218dS / m, respectively, very close to the optimal value for tomato cultivation. The  $\delta\text{NPK}$ , at the same date, revealed an EC of 0.68078dS / m instead of 1.315dS / m at the 12<sup>th</sup> day. During this period, this high concentration of minerals was phytotoxic for the tomato plants and the losses in biomass were observed, revealing the need to split in time fertilization with NPK. The soil reaction depend on the optimal pH of mineral absorption pH (6.5) and a water pH of immobilization of minerals water pH (4.5). Water pH kinetics in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  compared to  $\delta\text{C}$  revealed an increase towards 6.5; unlike water pH in the  $\delta\text{NPK}$ , the kinetics of water pH compared to  $\delta\text{C}$  revealed an improvement in the direction of 4.5. These data were in accordance with the findings of Azeez and Van Azerbeke, 2012 and Roy and Kashem 2014, who reported that the study of soil responses may vary over time depending on the type of amendment.

The results and illustrations obtained in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  find their elucidations in the degree of mineralization; the primary mineralization that undergoes Cd and Ti contributes to the formation of Clay-Humic Complexes (CHC) molecules as described by Duhan et al., 2005, which revealed that any organic matter incorporated into the soil disseminated the CHC molecules. These CHC molecules have a global negative charge, which gives it the capacity to potentiate the free protons in the medium (UNIFA, 1999), which will improve the water pH towards neutrality. The results and illustrations obtained in the  $\delta\text{NPK}$  find their elucidations in the degree of mineralization, the NPK being an already mineralized fertiliser makes less available the CHC molecules in the soil.

The residual CHC is rapidly saturated with the protons, leaving the free proton concentrations in the medium capable of immobilizing all life in the soil. The water pH values in the  $\delta\text{Ti}$  and  $\delta\text{Cd}$  corroborate with the interval recommended by Hendrickson, 2005, who revealed that for tomato, the pH of the soil should vary between 6 and 7. The pH values obtained in the  $\delta\text{C}$  are characteristic of the soil pH in the region.

The results from our experience revealed that the  $\text{MNO}_3^-$  Turn Overs (ppm) in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  followed progressive congestion dynamics. Turnovers of  $\text{MNO}_3^-$  (ppm) in  $\delta\text{NPK}$  and  $\delta\text{C}$  followed regressive congestion dynamics. These results are accordance with the observations of Khalil et al., 2005, who reported from a

90-day experiment on the rate of mineralization of nitrogen in different soil types under aerobic conditions of an organic material, a quality index on the transformation of organic nitrogen. The results obtained in  $\delta\text{Ti}$  and  $\delta\text{Cd}$  find their elucidations in nitrification; this nitrifying activity is favored by the pH of the soil, which reaches its optimum for water pH values greater than 6 with good availability of nitrate minerals. In addition, is the improvement of the soil texture that by dissemination of the CHC leads to the sequestration of nitrate minerals (Zaman et al., 2008).

The results obtained in the  $\delta\text{NPK}$  and  $\delta\text{C}$  found their elucidations in denitrification. This denitrification is favored by the water pH, which, when close to 5, amplifies volatilization (Zaman et al., 2007), the nature of the starting fertiliser, which can be in the form of a stable or residual product coupled with a well oxygenated soil, the daily watering frequency and with a less potential  $\text{MNO}_3^-$  texture only contribute to denitrification, which results in leaching, volatilization, immobilization by organic matter (OM) and a part used by the plant (Barton et al., 1999, Bowman et al., 2002, Bationo et al., 2007 and Baoqing et al., 2014).

The results from our experience with Turn OverM  $\text{NH}_4^+$  (ppm) in  $\delta$  revealed decongestion dynamics. These results are in agreement with those of Eigenberg et al., 2002, who evaluated the availability of  $\text{MNH}_4^+$  (ppm) in soils amended to animal waste. The results obtained in the  $\delta\text{Ti}$ ,  $\delta\text{Cd}$  and  $\delta\text{C}$  are not in accordance with those obtained by Roy and Kashem (2014), who revealed that soils amended with organic fertilisers were influenced by dynamics of progressive congestion of  $\text{MNH}_4^+$  (ppm) for the duration of experiment. By referring to the nature of the fertilisers, the same processes would have occurred in the organic  $\delta$ . These differences could be explained by the extraction efficiency. In fact, Roy and Kashem (2014), in their experiments suggested the use of 1N KCl solution to extract  $\text{MNH}_4^+$  (ppm). In this work  $\text{MNH}_4^+$  was extracted with water. These results confirm the importance of primary mineralization on the potentiation of  $\text{MNH}_4^+$  (ppm). The decongestion observed in the  $\delta\text{C}$  and  $\delta\text{NPK}$  reflected either an extension of the  $\text{MNH}_4^+$  (ppm) mineralization, which under the action of nitrifying microorganisms progressively oxidizes ammonium to nitrate, or losses by volatilization or immobilization by microorganisms. This mineralization of molecular nitrogen is influenced by the nature of the fertiliser, the watering regime, the volume of fertiliser applied, the type of soil and the duration of the experiment (Rahman et al., 2013).

#### 4.3. Nitrogen Use Efficiency (NUE) of tomato seedlings 35 days after transplanting in experimental blocks

The results from the NUE optimization trial of the (2%) hydro ethanol extract (HEE) of *C. citratus* and 2% Ridomil in 35-day-old tomato plants and at the beginning of flowering period, revealed 38.49% improvement in the  $\text{NPK}_{\text{HEE}}$ , and 37.45% in the  $\text{NPK}_{\text{R}}$  combinations compared to the  $\text{NPK}_{\text{C}}$  combination. An improvement of 27.74%, 52.07% in the  $\text{Ti}_{\text{HEE}}$  and  $\text{Ti}_{\text{R}}$  combinations compared to the  $\text{Ti}_{\text{C}}$  combination and of 93.933% in the  $\text{Cd}_{\text{HEE}}$  combinations, and 70.52% in the  $\text{Cd}_{\text{R}}$  combinations compared to the  $\text{Cd}_{\text{C}}$  combination. These results corroborated those of M rigout (2006), who demonstrated that green algae extracts are able to optimize the use of nitrogen in wheat plants. Smil (2001), revealed that nitrogen is the most important nutrient for agricultural production, because it is the yield' determinant. The high values of NUE obtained here, could be explained by the fact that NUE has been determined at the biomass level and according to Masclaux-Daubresse et al., (2010) who showed that the use of nitrogen in plants takes place in several stages: absorption, assimilation, translocation and remobilization. During assimilation, nitrogen integrates carbon chains to form proteins, cofactors, nitrogen bases and secondary metabolites. This assimilation leads to the formation of biomass and the high proportions in NUE at this stage positively influences the yields. This assimilation leads to the formation of biomass and the high values of NUE at this stage positively influence the yields since the assimilated nitrogen will be remobilized to allow the filling of the fruits or the seeds.

The NUE can be determined at fruiting stage and is influence by, the reasoning of nitrogen fertilization, the nature of the fertiliser and the degree of mineralization, the species of the plant, and the initial nitrogen status of the tomato plants from the nursery. The results obtained also find explanations for the climatic conditions, the soil type and the optimization approach used.

When compared the values of the NUE from the natural approach (biofertilisers and biopesticide) to the work of Lewandowsky and Schmidt (2006), Zub and Brancourt (2010), who used the genetic approach to optimize NUE in *Miscanthus* plants, the NUE obtained were lower than those reported in this work.

## V. FIGURES AND TABLES

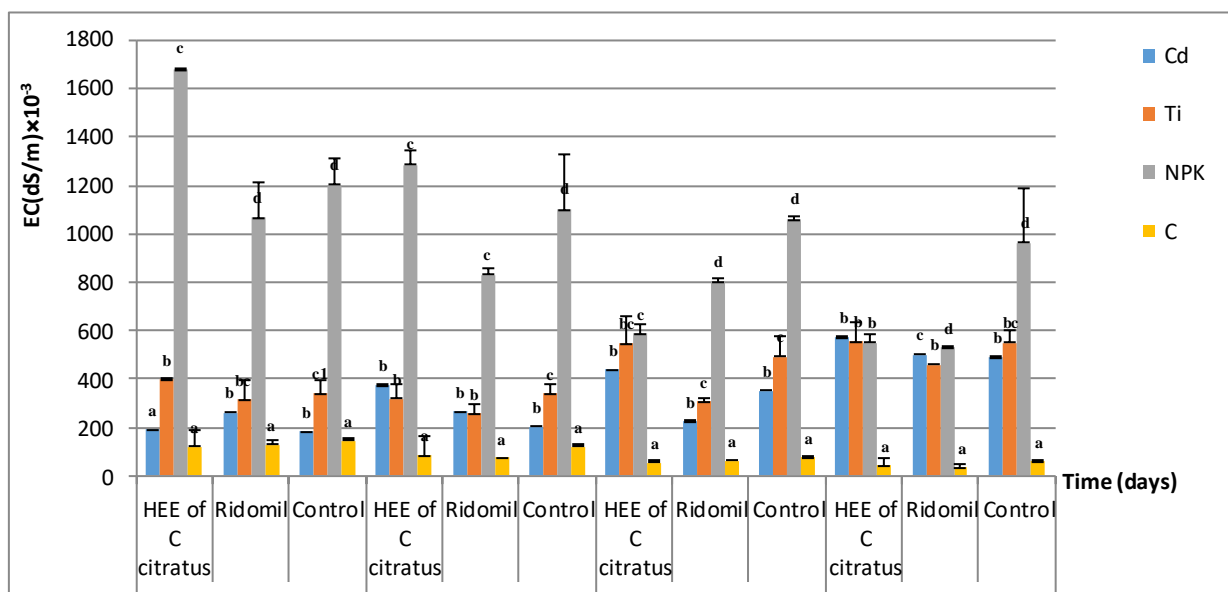


Fig. 1 Electro Conductivity EC  $((dS/m) \times 10^{-3})$  as function of time in bloc treated with 2% hydro ethanol extract (HEE) of *C. citratus*, Ridomil 2% and non-treated control.

a...d : Different letters indicate significant difference at  $p \leq 0,05$  (n =3). Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control

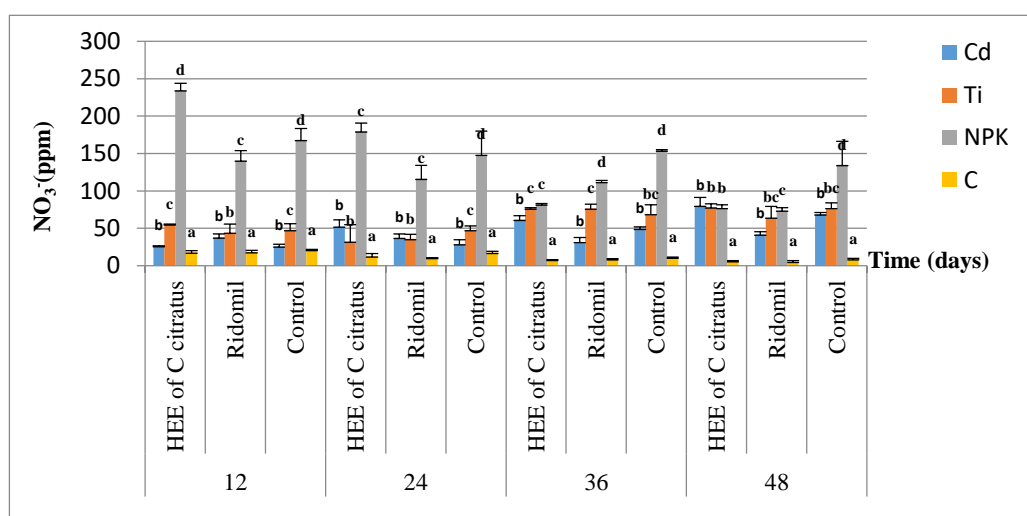


Fig. 2 Turn Over of  $NO_3^-$  (ppm) as function of time in bloc treated with 2% hydro ethanol extract (HEE) of *C. citratus*, Ridomil 2% and non-treated control.

a...d : Different letters indicate significant difference at  $p \leq 0.05$ ; Bars denote mean +SD (n =3).Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control



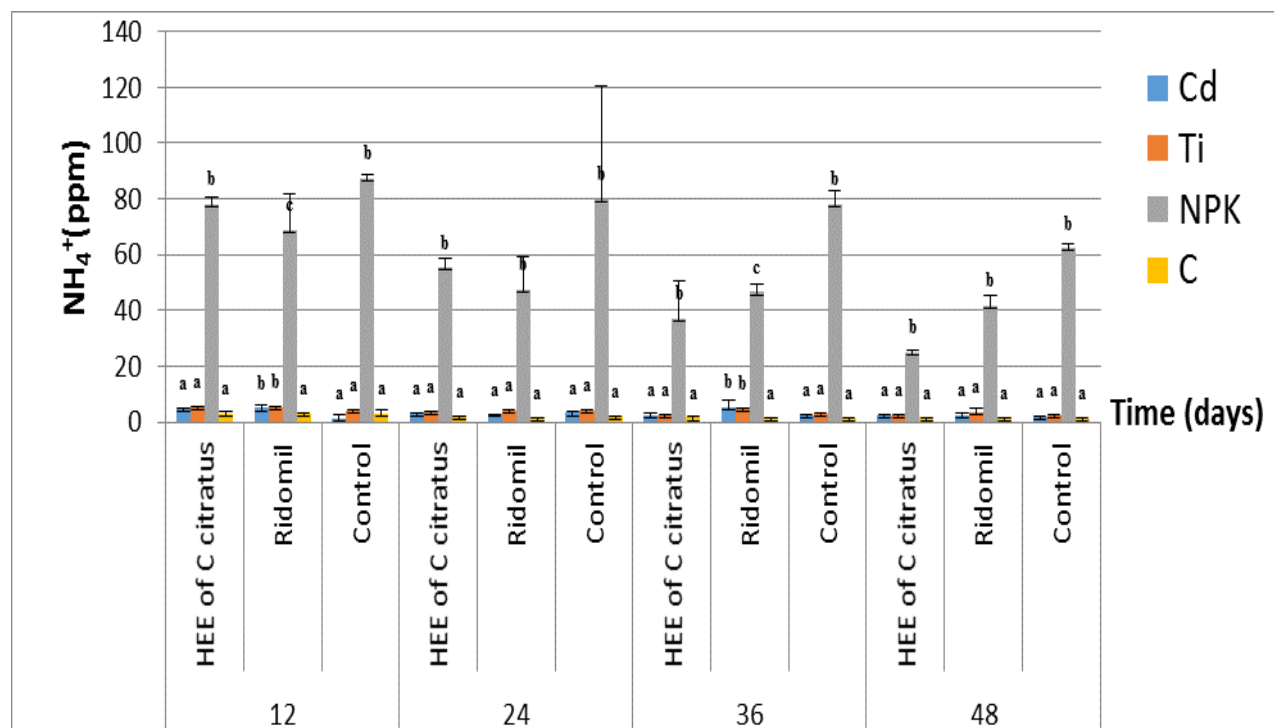


Fig. 3 Turn Over of  $\text{NH}_4^+$  (ppm) as function of time in block treated with 2% hydro ethanol extract (HEE) of *C. citratus*, Ridomil 2% and non-treated control.

<sup>a...c</sup> : Different letters indicate significant difference at  $p \leq 0.05$ ; Bars denote mean +SD (n=3). Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control

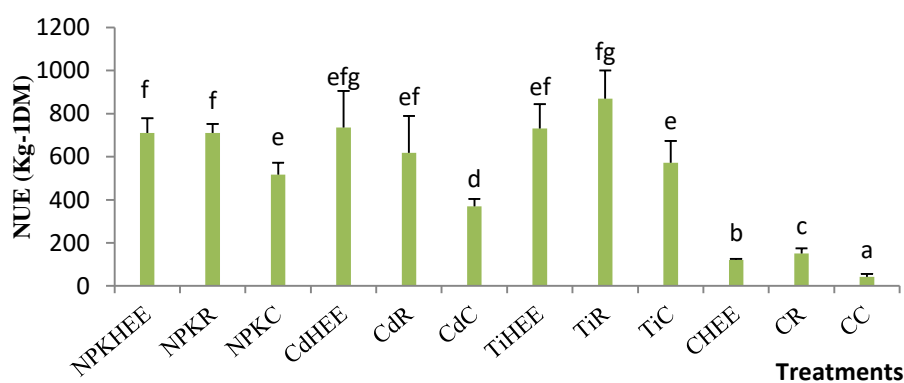


Fig. 4 NUE in 35-day-old tomato plants grown in non-treated and treated with 2%, HEE *C. citratus* and 2%, Ridomil 2% blocks.

<sup>ag\*</sup> : Different letters indicate significant difference at  $p \leq 0.05$  ; Bars denote mean +SD (n=3). Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control; HEE: Hydro Ethanol extract of *C. citratus*; NUE (Nitrogen Use Efficiency)

Table 1 Nitrogen (N) content as percent in substrates

Substrates	soil:sand (2:1)	cow dung	<i>T. diversifolia</i>	NPK
N (%)	0.21	2.13	3.31	23

Table 2 Mean Turn Over of total minerals (TDS (ppm)) in blocks non-treated and treated with 2%HEE *C. citratus* and 2% Ridomil

Mean TDS (ppm) in all blocks					
Time (days)	12	24	36	48	Interpretations
Fertiliser					
Cd	302.23 <sup>a</sup> ±136.14	387.27 <sup>a</sup> ±162.19	482.11 <sup>a</sup> ±81.38	743.26 <sup>b</sup> ±53.11	Minerals' congestion
Ti	502.22 <sup>a</sup> ±69.55	425.4 <sup>a</sup> ±63.75	634.91 <sup>b</sup> ±66.47	741.22 <sup>b</sup> ±88.96	Minerals' congestion
NPK	2049.93 <sup>c</sup> ±79.69	1494.17 <sup>c</sup> ±201.5	1185.53 <sup>c</sup> ±16.18	973.88 <sup>b</sup> ±133.37	Minerals' decongestion
C	191.57 <sup>a</sup> ±19.08	135.5 <sup>a</sup> ±19.95	92.24 <sup>a</sup> ±4.51	65.00 <sup>a</sup> ±14.77	Minerals' decongestion
Test	(0.05) **	(0.05) **	(0.05) ***	(0.05) **	

ac \*: the values in the same columns followed by the different letters are significantly different at a probability  $p \leq 0.05$ . Each value is a mean of three repetitions±SD. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control.

Table 3 Mean reactions ( $pH_{water}$ ) in different cultural substrates over time in days

EHE <i>C. citatus</i> (2%). Ridomil(2%). Témoin					
	$pH_{water12}$	$pH_{water24}$	$pH_{water36}$	$pH_{water48}$	Interprétations
Cd	6.12 <sup>a</sup> ±0.23	6.54 <sup>b</sup> ±0.08	6.30 <sup>b</sup> ±0.06	6.18 <sup>a</sup> ±0.14	Improved of $pH_{water}$ to 6.5
Ti	7.00 <sup>d</sup> ±0.19	6.72 <sup>d</sup> ±0.15	6.44 <sup>c</sup> ±0.11	6.33 <sup>b</sup> ±0.13	Improved of $pH_{water}$ to 6.5
NPK	5.65 <sup>a</sup> ±0.315	5.67 <sup>a</sup> ±0.10	5.32 <sup>a</sup> ±0.11	5.37 <sup>a</sup> ±0.09	Improved of $pH_{water}$ to 4.5
C	6.31 <sup>b</sup> ±0.22	6.27 <sup>b</sup> ±0.08	5.92 <sup>a</sup> ±0.14	6.08 <sup>b</sup> ±0.02	Improved of $pH_{water}$ to 6.5
Test	(0.05) ***	(0.05) ***	(0.05) ***	(0.05) **	

ac \*: the values in the same columns followed by the different letters are significantly different at a probability  $p \leq 0.05$ . Each value is a mean of three repetitions±SD in each block. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control; HEE: hydro ethanol extract.

Table 4 Mean Turn over of  $NO_3^-$  (ppm) in non-treated and treated with 2%, HEE *C. citratus* and Ridomil 2%

Time (days)	12	24	36	48	Interpretations
Fertiliser					
Cd	29.90 <sup>a</sup> ±2.57	39.41 <sup>a</sup> ±6.58	47.45 <sup>a</sup> ±8.23	63.59 <sup>a</sup> ±5.7	progressive nitrification
Ti	49.13 <sup>a</sup> ±6.56	38.49 <sup>a</sup> ±11.51	74.01 <sup>b</sup> ±7.78	73.2 <sup>b</sup> ±8.83	progressive nitrification
NPK	181.2 <sup>c</sup> ±12.36	150.07 <sup>c</sup> ±10.1	114.07 <sup>b</sup> ±11.7	95.27 <sup>b</sup> ±13.06	regressive denitrification
C	18.79 <sup>a</sup> ±1.88	13.39 <sup>a</sup> ±1.91	9.11 <sup>a</sup> ±0.34	6.55 <sup>a</sup> ±1.20	regressive denitrification
Test	(0.05) **	(0.05) **	(0.05) **	(0.05) **	

ac \*: the values in the same columns followed by the different letters are significantly different at a probability  $p \leq 0.05$ . Each value is a mean of three repetitions±SD in each block. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control.

## VI. CONCLUSION

The aim of this study was to evaluate the influence of hydro ethanol extract of *C. citratus* on nitrogen metabolism in relation to the increase of the NUE in tomato plants. The nitrogen content in the soil/ sand mixture, cow dung, *T.*

*diversifolia*, and NPK were, respectively, 0.21% N, 3.32% N, 2.13% N, and 23.00% N. These levels guided to reason the fertilization at 15g N for the first test according to the complete needs of the tomato and at 5g N for the optimization test. The results of the mineralization of the

various amendments led to the selection of two levels of mineralization. *Tithonia diversifolia* and cow dung showed primary mineralization by improving the pH in the direction of the optimal pH of mineral use of pH6.5. The kinetics of the EC, the Turn Over of the TM followed a progressive dynamic with progressive congestion dynamics in the  $\delta\text{Ti}$ ,  $\delta\text{Cd}$ . NPK and Control showed secondary mineralization by improving water pH in the direction of the pH of immobilization of minerals. The kinetics of the EC, the Turn Over of the TM followed a regressive dynamic with a regressive dynamic of congestion. Results from the  $\text{MNO}_3^-$  (ppm) revealed two changes in Turn Over over 48 days.  $\delta\text{Ti}$  and  $\delta\text{Cd}$  showed  $\text{MNO}_3^-$  (ppm) Turn Over that followed a progressive congestion dynamic.  $\delta\text{NPK}$  and  $\delta\text{C}$  showed  $\text{MNO}_3^-$  (ppm) Turn Over that followed a regressive congestion dynamic. The results obtained from the determination of  $\text{M NH}_4^+$  (ppm) revealed a significant evolution and disproportionality between the  $\delta\text{NPK}$  and the other treatments. The determination of NUE at the biomass level in tomato plants treated with 2% HEE of *C. citratus* and ridomil, showed an increase of 38.49% in  $\text{NPK}_{\text{HEE}}$  combinations, and 37.45% in  $\text{NPK}_{\text{R}}$  combinations. An increase of 27.74%, 52.07% in the  $\text{Ti}_{\text{HEE}}$  and  $\text{Ti}_{\text{R}}$  combinations and an increase of 93.93% in the  $\text{Cd}_{\text{HEE}}$  combinations, and 70.52% in the  $\text{Cd}_{\text{R}}$  combinations.

We conclude that the hydro ethanol extract of *C. citratus* justified the importance of the use of the natural approach to optimize the use of nitrogen by plants. The biofertilisers, *T. diversifolia*, cow dung showed a slow release nitrogen property, which is a challenge to the synthetic fertilisers like NPK. In fact, to formulate slow release synthetic chemicals, agro companies tend to incorporate the minerals into biodegradable films. The combined use of plant sprays with hydro ethanol extract of *C. citratus* and soil amendment with *T. diversifolia* or cow dung, improved significantly the nitrogen use efficiency (NUE) of tomato plants and could be used as alternative to conventional inputs.

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