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A FERTILIZER WITH NITRIFICATION INHIBITOR DMPP (3, 4-DIMETHYLPYRAZOL PHOSPHATE) AND UREA AS ALTERNATIVES TO AMMONIUM NITRATE FOR TOMATO CROP IN TUNISIA

KARIMA KOUKI KHALFALLAH[1], NEJIB TURKI[2], MALEK BEN KHELIL[3], AHMED ARBI[4] & MUSTAPHA SANAA[5]

[1,2,5]INAT: National Agronomic Institute of Tunis, 43 Avenue Charles Nicolle, Tunis, Tunisia

[3]ESA Mateur: Ecole Supérieure d'Agriculture Mateur. Mateur Tunisia

[4]GICA: Groupement Interprofessionnel des Conserves Alimentaires, 77 Avenue Taieb Mehiri, Tunis. Tunisia

ABSTRACT

The risk of pollution caused by nitrate leaching, the recent undesired (dangerous) uses and the safety issues, all associated with the storage of ammonium nitrate has led to seek an environmentally sound alternatives for this fertilizer. Three nitrogen fertilizers treatments have been tested on season crop tomato: ammonium nitrate (33.5%), urea (46%) and « Entec Solub 16-10-17 » fertilizer which contains 1% of 3,4-Dimethyl Pyrazole Phosphate (DMPP). All fertilisers were applied at a rate of 205 kg N ha^{-1}. Ammonium nitrate improved plant growth more than the two other treatments especially during the first crop month. and so, the trend was reversed in favor of Entec Solub from the 6^{th} cluster. Despite the similar yields in the three treatments, fruit size was smaller in plants fertilized with Entec Solub. And also the advantage provided by Entec Solub at fruit quality multiplied the cost by 1.8 times more than fertilization by urea or ammonium nitrate. The environmental effect of using Entec Solub seems to be reduced by high temperatures. At the same rate of nitrogen, Urea or Entec Solub can be an environmentally sound alternatives to substitute ammonium nitrate and generate similar yields for crop season tomato.

KEYWORDS: Fertilizer, Nitrification Inhibitor, 3, 4-Dimethylpyrazol Phosphate (DMPP), Urea, Ammonium Nitrate, Tomato

INTRODUCTION

Tunisia has chosen since the 60s to encourage the use of ammonium nitrate as a nitrogen source for higher yields in agriculture. The used amount of ammonium nitrate reached 155.150 tons in 2010 (FAO, 2010). The risk of pollution caused by nitrate leaching, the recent undesired (dangerous) uses and the safety issues, all associated with the storage of ammonium nitrate has led policy makers to seek alternatives for this fertilizer. The horticulture crops sector is the most affected because crop management is usually intensive. One of the alternatives considered is urea ($CO(NH_2)_2$), which is undoubtedly the most commonly used nitrogen fertilizer form around the world (IFA, 2010). However, urea is rapidly hydrolyzed to ammonium by the action of soil urease (Calvet, 2003). Although the ammonium is relatively better retained by the soil than nitrate, it may be subject to losses by volatilization (Kallenbach and Massie, 2005) and is usually oxidized rapidly to nitrate by nitrifying microorganisms in soils. Another alternative available to decision makers in Tunisia is to use nitrogen fertilizers incorporating 3,4-DimethylPyrazol Phosphate (DMPP), a compound which is very efficient in inhibiting the activity of Nitrosomonas bacteria, responsible for the first step in nitrification process in soil (Zerulla et al., 2001; Xu et al., 2005; Roco and Blu, 2006; Yu et al., 2007a; Menéndez et al. 2012). At present, most of the work on DMPP was focused on the effect of nitrification inhibitor in soil and improving the yields of food crops like maize (Jing et al., 2012) , barley (Ottow et al., 2012), rice (Hua et al., 2008), or industrial crops like sugar beet (Trenkel, 2010). Few research work focused on vegetables crops like melon (Egea and AlarcÓn, 2006), spinach (Renard et al., 2007), carrot (Smolen and Sady, 2009a; 2009b), lettuce and cauliflower (Pfab et al., 2012) . Tomato, which occupies the first place among the vegetable crops in the world with more than 4 million hectares in 2007 (FAO, 2008), has not yet been mentioned in this issue. Furthermore, the tomato is in fact a species of particular interest for Tunisian farmers, since it is a summer crop, inevitably irrigated, which occupies 32.200 ha, representing 18% of all vegetable crops in Tunisia (FAO, 2011). The objective of this research was to examine the effects of urea, poorly accepted among Tunisian farmers and "Entec Solub 16-10-17" a NPK complex fertilizer with nitrification inhibitor DMPP, recently introduced in Tunisia, all compared to ammonium nitrate used as control.

MATERIALS AND METHODS

Experimental Site and Soil

Data reported on this work were obtained from an experiment carried out in Bizerte North Tunisia (37°16'31.00"N; 9 ° 48'45 .92" E), from 26 May to 30 September 2013. The region benefits from a Mediterranean climate. The summer is warm and dry and the winter somewhat cold and rainy. The mean annual precipitation is 610mm. In summer, Sirocco wind can blow for 18 days per year with an average temperature of $26^{°C}$ and sometimes more during July and August. The soil of the field where the experiment was carried out is silty clay texture. The soil organic matter was 21 gkg^{-1}; pH (soil/water, 1:2.5) 7.9 and active limestone 90 gkg^{-1}. It has been cultivated over two-year rotation that included winter vegetables (spinach, parsley, radish ...) and forage crops (oats, berseem, maize ...) in the second year. A basal dressing was made during the preparation of the soil: 60 tha^{-1} of cattle manure and 100 $kgha^{-1}$ of potassium sulphate.

Experimental Device

The experiment consisted of the cultivation of tomato, cv. Sun 6200, a hybrid widely used in summer crops in Tunisia both for fresh market and for industry. The tomato seedlings were hand-hoe transplanted at stage 4-5 leaves on raised beds on 26 May in 2013. Tomato was subjected to three different treatments arranged in a completely randomized design with three replicates. Each experimental unit consisted of 39 m^2 plots with a density of ≈3.2 plant m^{-2}. Each treatment consisted of applying a fertilization program where a nitrogen fertilizer was introduced in order to induce a rapid release of mineral nitrogen: T1 ammonium nitrate (33.5%), T2 «Entec Solub 16-10-17» a fertilizer that contains a nitrification inhibitor: 3,4-Dimethyl Pyrazole Phosphate (DMPP) at a concentration of 1% and T3 urea (46%),. The three treatments received the same amount of nutrients (205 kg ha^{-1} N; 95 $kgha^{-1}$ P_2O_5; 291 $kgha^{-1}$ K_2O; 55 kg ha^{-1} MgO). In both programs which are using the ammonium nitrate and Entec Solub, half of nitrogen was made in nitrate form and half in the ammonium form. In dealing with Entec Solub, which contains 11% $N\text{-}NH^{4+}$ and 5% $N\text{-}NO^{3-}$, supplements of potassium nitrate and magnesium nitrate were used to balance the two forms of nitrogen. To balance phosphorus, potassium and magnesium rates among programs, phosphoric acid (cc = 85% ; density = 1.7); solupotasse (50% K_2O) and magnesium sulfate (16% MgO) were then used. The distribution of nutrients for the three treatments was made identically in strict respect of the phases of crop development (Table 1). Four applications of Calcium Sulfate (280 $kgha^{-1}$) were given every two weeks between July 7 and August 20. The foliar sprays of trace elements were made each 10 days between June 20 and July 20 using alternatively: Master (6.5% Fe EDDHA) and Nutrel (65 gl^{-1} Fe, 27 gl^{-1} Mn, 5.2 gl^{-1} Bo, 4.5 gl^{-1} Zn, 2 gl^{-1} Cu, 1.6 gl^{-1} Mo). Irrigation water was from a shallow well (EC 3.4 dSm^{-1}).

Table 1: Distribution Rate of Nutrients through the Development Phases of Seasonal Tomato Crop in Tunisia

Development Phases	Duration (Days)	Pourcentage of the Nutrient Absorbed by Phase			
		N	P_2O_5	K_2O	MgO
Plantation – First bloom	25	16%	42%	9%	23%
First bloom – First fruit set	20	20%	26%	18%	27%
First fruit set – First fruit ripening	25	27%	16%	32%	35%
First fruit ripening – 80% harvest	35	37%	16%	41%	15%

Plant Analysis

The yield evaluation and quality of fruits was realized during each harvest between August 15 and September 30.

Soil Analysis

Soil samples were collected five times every 15 days between June 25 and August 24. Two soil samples per block and per treatment were collected at each time and for each of the soil horizons 0-20 cm and 20-40 cm.18 samples have been collected every two weeks. They were stored at -20$^{\circ C}$ until analysis. Mineral nitrogen levels were determined by Devarda's alloy method (Pauwels et al., 1992).

STATISTICAL ANALYSIS

Data were analyzed using the one-way ANOVA of Statistical software for Window (Statistica, Version 5.5). The Duncan's new multiple range difference tests were used to compare at 0.05 level.

RESULTS

Rate of Plant Growth

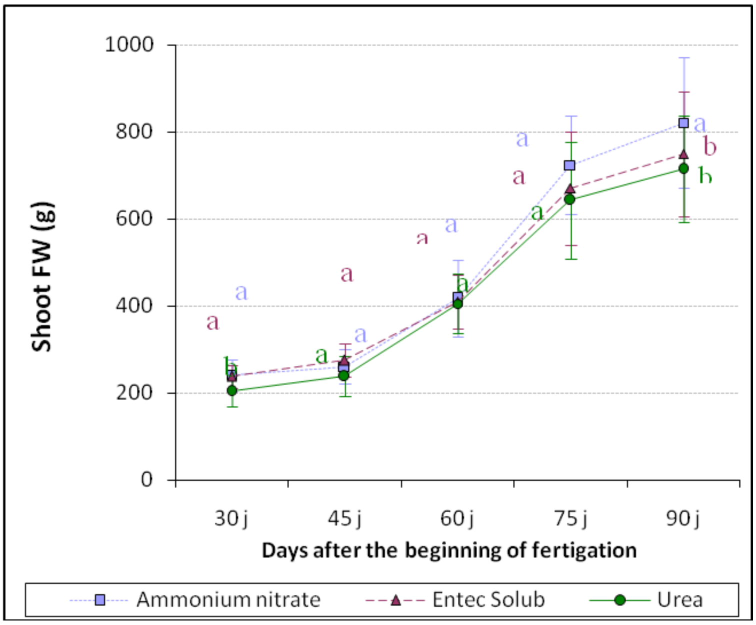
The change in the form of nitrogen fertilizer used in the fertilization of tomato late season showed significant effects on the growth of the culture, especially during the first month of the crop cycle. Indeed, by calculating the elongation (growth) rate of plants between two successive dates, from one week to another, significant differences were observed during the first week only. A slower rate was observed in plants receiving fertigation with urea (Table 2). However growth speed was no longer significantly different over the next few weeks, but a slight superiority of plants fertilized with ammonium nitrate base seems to emerge.

Plant Leaf Area

The measurement of the leaf area per plant developed, performed 60 days after fertigation, reveals significantly higher values in plants that received urea as a nitrogen source, compared to those fertilized with ammonium nitrate (Table 3). The differences are not significant between the urea treatment and that of Entec Solub for this parameter, but they are clearly significant for the average area of a leaf. This last parameter was highest in plants treated with urea.

Fresh Weight

The fresh weight of tomato at the aerial organs (Figure 1a) or in the roots (Figure 1b) was generally higher in plants fertilized with ammonium nitrate compared to those which received Entec Solub or urea. This superiority was most pronounced in the roots and especially between ammonium nitrate on the one hand and urea on the other, since the differences between the two treatments were significant at each pulling performed between the 30th and 90th day after the start of fertigation.



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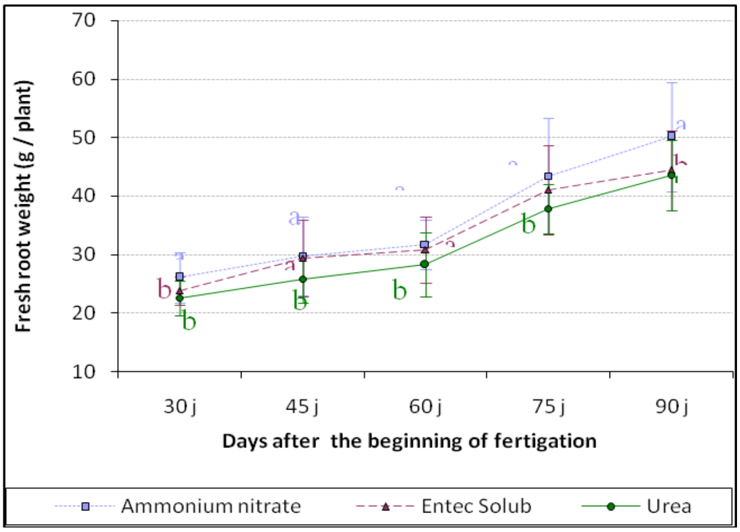

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Note: The values for the same date and assigned the same index are not significantly different at 5% risk of error.

Figure 1: Effect of the Nitrogen Fertilizer Used on Tomato Shoot (A) and Root (B) Fresh Weights

Table 2: Effect of the Nitrogen Fertilizer Used on Tomato Growth Rate

Growth Rate (mm d^{-1}) / Week after Starting the Fertigation	Ammonium Nitrate	Entec Solub	Urea
From the 3rd to 4th week	7,5 ± 1,5 ab [1]	7,8 ± 1,9 [a]	6,9 ± 1,3 [b]
From the 4th to 5th week	10,9 ± 2,2 [a]	10,3 ± 2,4 [a]	10,2 ± 2,2 [a]
From the 5th to 6th week	12,9 ± 2,1 [a]	12,3 ± 2,3 [a]	12,4 ± 2,2 [a]
From the 6th to 7th week	14,4 ± 2,2 [a]	13,9 ± 2,5 [a]	13,8± 2,3 [a]
From the7th to 8th week	15,0 ± 2,2 [a]	14,4 ± 2,5 [a]	14,3 ± 2,3 [a]
From the 8th to 9th week	16,2 ± 2,1 [a]	15,6 ± 2,5 [a]	16,6 ± 3,4 [a]

1Means followed by the same letter in lines are not different by Duncan HSD test, P < 0.05

Table 3: Effect of the Nitrogen Fertilizer Used on Developed Leaf Area per Plant and Leaf Size after 60 Days of Starting Fertigation

Parameters	Ammonium nitrate	Entec Solub	Urea
Total leaf area (cm^2)	2940,14 ± 442,89 [b]	2972,38 ± 156,44 [ab]	3193,43 ± 452,66 [a]
Average leaf area (cm^2)	41,75 ± 3,06 [c]	47,41 ± 1,62 [b]	49,82 ± 5,01 [a]

Note: The values for the same parameter measured and assigned the same index are not significantly different at 5% risk of error

Monitoring of Mineral Nitrogen in the Soil

The total nitrogen content in the topsoil (0-20 cm) has decreased remarkably for all the treatments between the initial state before planting and 30 days after starting fertigation (Figures 2a). This phenomenon seems greater with urea than with ammonium nitrate and Entec Solub although the differences did not prove to be significant. This could indicate that the amounts of nitrogen given during this phase are insufficient for the needs of plants. Such an imbalance has not been detected after this date, as the levels of soil nitrogen had an increasing trend over the sampling every 15 days up to 3 months after planting. However, leaching was relatively more important in the case of ammonitrate than in urea than in Entec Solub after one month of treatment because the rate of increase of N mineral in the soil horizon 20 -40 cm (Figure 2b) were respectively 62%, 22% and less than 1%. On the other hand, despite the general downward trend observed during the first month, the levels of nitrate in soil (Figure 3a, b) were much higher in the 0-20 cm soil layer than in 20-40 cm for treatment with ammonium nitrate.

Number of Flowers per Bouquet Formed

The monitoring of average number of flowers per bouquet formed showed that the effects of a nitrogen fertilizer was variable according to the number of the bouquet. Indeed, at the first and second, the number of flowers per cluster was significantly higher in plants that received ammonium nitrate as nitrogen source, compared with fertilized with urea or Entec Solub (Figure 4). At the 3rd, 4th and 5th bouquet, the differences between the types of fertilizer used were no longer significant. They have returned at bouquets 6, 7 and 8 with a significant difference in favor of treatment using Entec Solub compared to the other two treatment.

Fruit Calibration

The fruit yield, was similar among the three treatments. In contrast, the calibration of fruit yield by class size showed that fruit size was very different depending on the form of nitrogen fertilizers used in fertigation. Thus, the higher proportion of large caliber was recorded using urea, the medium size, using ammonium nitrate and the small caliber, using Entec Solub (Figure 5).

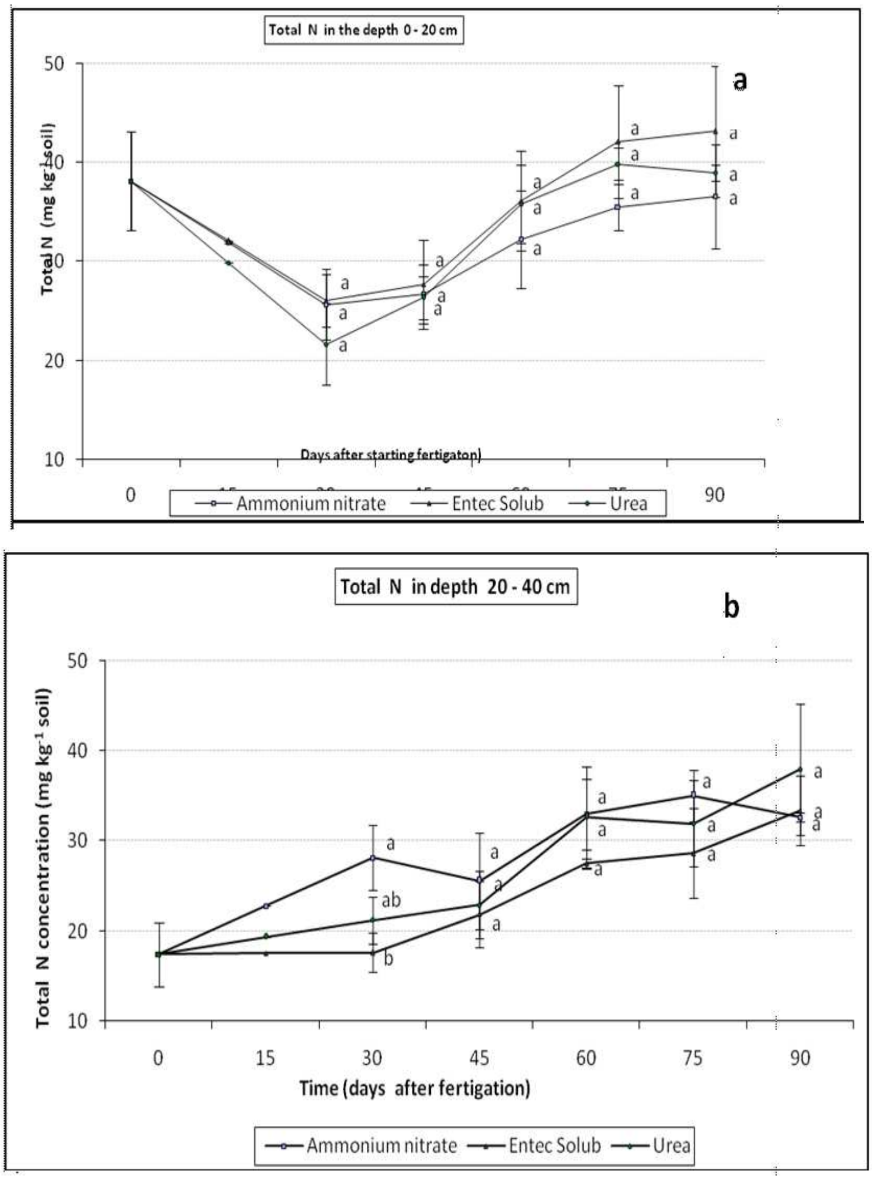
Fertilization Cost and Environmental Effect

The estimated cost of fertilizer in each of the treatments T1, T2 and T3 respectively using ammonium nitrate, Entec Solub and urea as nitrogen fertilizers, shows that the use of Entec Solub (T2) significantly increased the cost fertilization compared with other fertilizers.

Indeed, the overall cost of fertilization using Entec Solub (Figure 6) is 1.8 times that of ammonium nitrate-based fertilizer and the same for the urea.

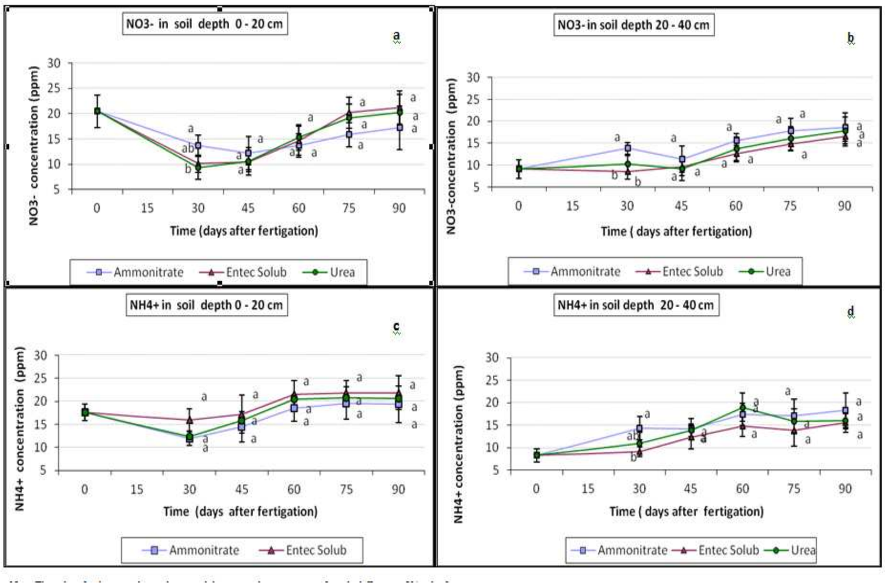
Fruit Quality

To assess the quality of the fruit, we evaluated the rate of apical necrosis in the total harvest, the sugar content and titratable acidity (Table 4). The results showed that the rate of apical necrosis was similar for all treatments and ranged from 2.5% in plants fertilized with urea, 3% of those fertilized with ammonium nitrate. The sugar was, for its part, significantly higher in fruit harvested from plants fertilized with Entec Solub and urea compared to those who received ammonium nitrate. In terms of total acidity, fruit related to treatment using Entec Solub were significantly more acidic compared to the other two treatments.



Note: The values for the same date and assigned the same index are not significantly different at 5% level.

Figure 2: Evolution of the Total Nitrogen Concentration in the 0-20 cm Layer (a) and 20-40 cm (b) for the three Nitrogen Fertilizers Used



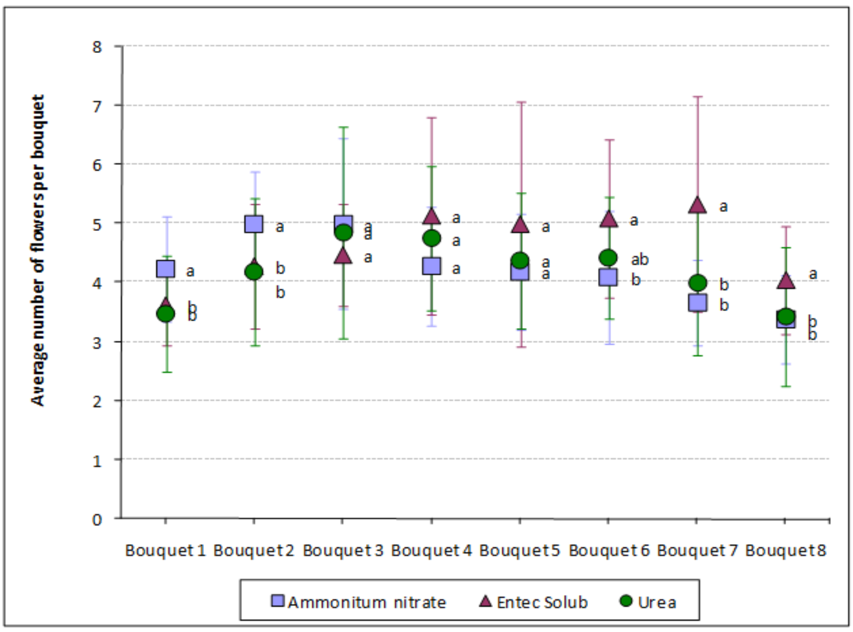
Note: The values for the same date and assigned the same index are not significantly different at 5% risk of error.

Figure 3: Evolution of Nitrate (NO3-) and Ammonium (NH4 +) Respectively in the 0-20 cm Layer (a, c) and 20-40cm Layer (b, d) for the three forms of Nitrogen Fertilizer Used

Table 4: Quality of Tomato Fruits According to Nitrogen Fertilizer Used in Fertigation

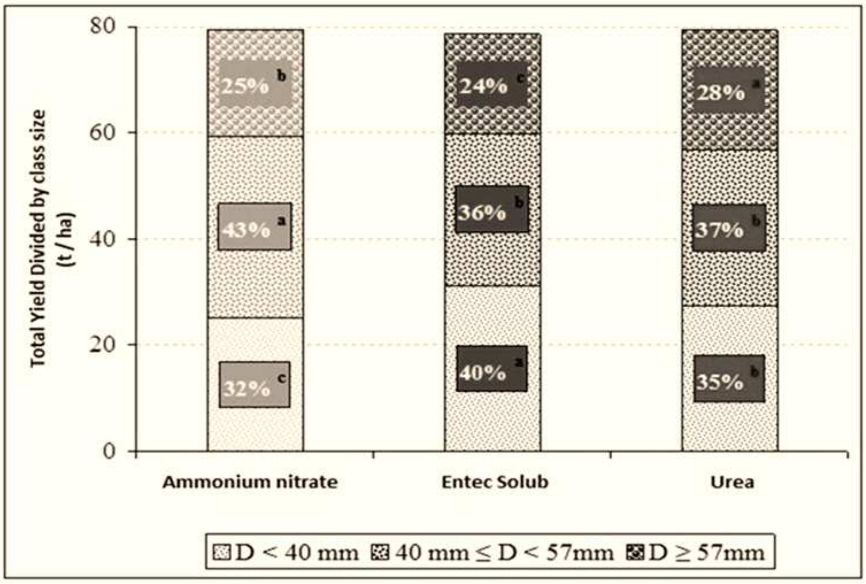
Traitements / Parameters	Ammonium Nitrate	Entec Solub	Urea
Rate of apical necrosis (%)	3,0 ± 0,4 ^{a}	2,8 ± 0,7 ^{a}	2,5 ± 0,5 ^{a}
% Brix	5,8 ± 0,3 ^{b}	6,5 ± 0,2 ^{a}	6,6 ± 0,3 ^{a}
Titratable acidity (% citric acid)	5,1 ± 0,3 ^{b}	5,5 ± 0,2 ^{a}	5,2 ± 0,3 ^{b}

NB: The values for the same parameter measured and assigned the same index are not significantly different at 5% risk of error



NB: The values for the same cluster assigned the same index are not significantly different at 5% risk of error

Figure 4: Average Number of Flowers per Bouquet Formed Depending on the Nitrogen Fertilizer Used in Fertigation



NB: The values for the same size class, and assigned the same index are not significantly different at 5% risk of error

Figure 5: Calibration of the Total Yield in terms of Nitrogen Fertilizer Used in Fertigation

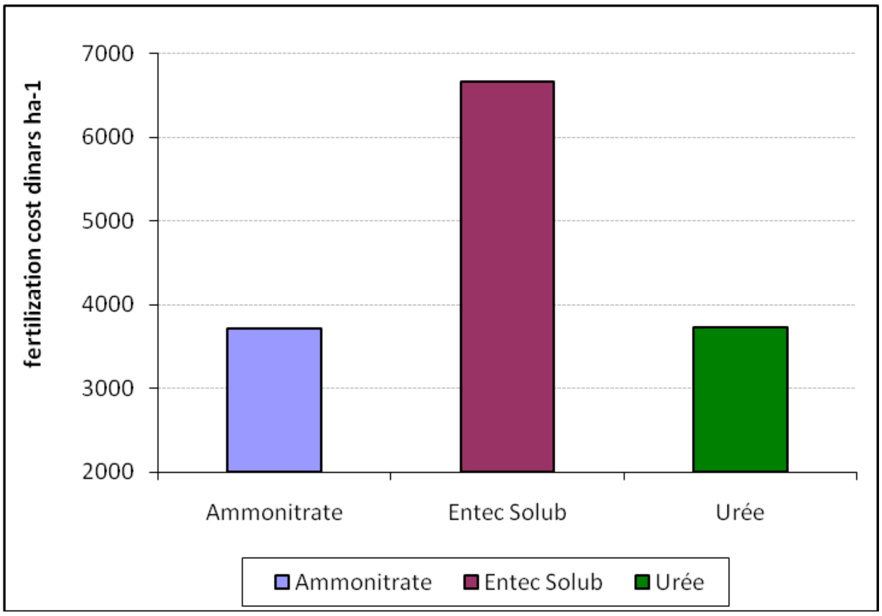


Figure 6: Fertilization Cost of Tomato late Season as Nitrogen Fertilizer Used in Fertilization

DISCUSSIONS

The use of ammonium nitrate as a nitrogen source is very favorable for the vegetative development of the tomato crop, which was monitored on the fresh weight of shoot and root parts. These results are in agreement with those obtained by Chaturvedi (2005), who compared the effect of different nitrogen fertilizers on rice growth and found a vegetative growth and biomass significantly higher using a nitrate fertilizer incorporating ammonium (DSC) by using urea. This improvement in biomass was associated with better rooting. The addition of ammonium nitrate seems therefore, provide the plant with nitrogen directly assimilable form, unlike Entec Solub ammonia fraction which is stabilized by the nitrification inhibitor DMPP, and urea which need to undergo hydrolysis to nitrification supplemented by that the nitrogen is available to plant roots. Treatment with Entec Solub caused effects on the fresh weight of the aerial part, very close to those of ammonium nitrate in a first time and those of urea in a second time, but differences remained non-significant with both treatments between the 45th and the 75th day after the beginning of the fertigation. The effects of a nitrogen fertilizer was variable according to the number of the bouquet. Indeed, it has been demonstrated by Nieves-Cordones et al. (2007) that a good diet improves nitric nitrogen flowering in tomato. This improvement of flowering was generally associated with better absorption of potassium (Heller et al., 1992; Marschner, 1995). Despite the fruit yield was similar among the three treatments, the calibration of fruit yield by class size showed that fruit size was very different depending on the form of nitrogen fertilizers used in fertigation, These results confirm those obtained on melon crop (Egea and Alarcon, 2006), yields were similar, but the fruit size significantly reduced by using nitrogen fertilizer containing DMPP. Competition for nutrients had to be stronger between fruit, which would have resulted in a reduction of the size. Indeed, it has been demonstrated by Yu et al. (2007a, 2007b) that by integrating nitrification inhibitor with urea has been a reduction in losses of about 34 %, 24% and 27% respectively for K^+, Mg^{2+} and Ca^{2+} in relation to the use of urea without DMPP. Regarding urea, its effect on improving the quality of the fruit could be explained by the release of ammonium, which is relatively slow compared to the ammonium nitrate. Indeed, it has been proven that hydrolysis of urea takes on average two days at 20$^{°C}$ in laboratory conditions (Amberger, 1989). The slow release of ammonia would have less competition creates against the increased potassium and therefore the accumulation of soluble sugars and increased as the acidity of the fruit has been proven by Epstein and Bloom (2005), Dobricevic et al (2008) and Al-Ajmi et al. (2009). This also seems likely to explain the rate of apical necrosis obtained with relatively smaller and Entec Solub urea versus ammonium nitrate

Leaching was relatively more important in the case of ammonitrate than in urea than in Entec Solub after one month of treatment, and the levels of nitrate in soil were much higher in the 0-20 cm soil layer than in 20-40 cm for treatment with ammonium nitrate despite the general downward trend observed during the first month. This could be explained by the immediate release of $N\text{-}NH^{4+}$ from ammonium nitrate. Also for the ammonium form, it seems easier when leached best preserved in the surface layer of soil with Entec Solub with ammonium nitrate. It seems to be more easily leached with ammonium nitrate, urea than with Entec Solub. The ammonium nitrate which provides, in effect, half of its nitrogen in nitrate form is directly accessible to the plant (0- 20 cm horizon) but has a greater risk of leaching (20-40 cm horizon), its concentration in use form is greater, unlike Entec Solub ammonia fraction which is stabilized by the nitrification inhibitor DMPP, and urea which needs to undergo hydrolysis supplemented by nitrification for nitrogen to be accessible to the roots. Entec Solub is considered very beneficial to the preservation of the natural environment since it integrates DMPP molecule appears to substantially reduce leaching losses (Yu et al., 2007a; 2007b) and volatilization (Belastegui et al., 2003; Akiyama et al, 2010) . Indeed, in the case of our experience and by soil testing, it has been shown that on the one hand the lowering of the ammonia nitrogen content in the 0-20 cm layer (Figure 3c), was less marked with Entec Solub with ammonium nitrate or urea, and secondly, the ammonia nitrogen content in the 20-40 cm layer (Figure 3d) had a more subdued increase with Entec Solub with the urea and ammonium nitrate. This proves that the DMPP molecule embedded in a more protected Entec Solub ammonia nitrogen nitrification in keeping with the highest concentrations in the surface layer of soil, and leaching by limiting its concentration in the deeper soil layer. Studies in the laboratory have shown that the increase in temperature accelerates the degradation of DMPP remarkably. Integrating this nitrification inhibitor at 10$^{°C}$ in ammonium can stabilize the soil for a period up to 100 days after application. While it is of the order of 18 and 8 days, respectively, at temperatures of 20$^{°C}$ and 30$^{°C}$ (Barth et al.,2008) . In fact, in cooler conditions (Southern Germany), it was shown that the effects of DMPP are much more intense and prolonged and contribute to a 40%

reduction of nitrogen leaching losses (Trenkel, 1997). The opportunity to use fertilizer incorporating nitrification inhibitors under conditions of Tunisia remains to verify that the above trials in Spain ranked first DMPP retardant nitrification compared to others such as dicyandiamide DCD (Belastegui et al., 2003), which proved much less effective in the stabilization of ammonia nitrogen.

CONCLUSIONS

Comparison of three fertilization programs, each involving a nitrogen fertilizer characterized by more or less rapid release of mineral nitrogen on tomato late season showed that ammonium nitrate improved growth plant more than the two other treatments especially during the first crop month. In the first two clusters, the treatment with ammonium nitrate advantaged over the other two treatments, and so from the 6^{th} bouquet, the trend was reversed in favor of Entec Solub . Despite the similar yields in the three treatments, fruit size was smaller in plants fertilized with Entec Solub. And also the advantage provided by Entec Solub at fruit quality multiplied the cost by 1.8 times more than fertilization by urea or ammonium nitrate. The environmental effect of using Entec Solub seems to be reduced because of the high temperatures that prevailed during crop season. At the same rate of nitrogen, Urea or Entec Solub can be an environmentally sound alternatives to substitute ammonium nitrate and generate similar yields for crop season tomato. Further studies are needed to confirm these results for other crops and under different soil and climate conditions.

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