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INFLUENCE OF ORGANIC AND MINERAL FERTILIZERS ON THE ANTIOXIDANTS AND TOTAL PHENOLIC COMPOUNDS LEVEL IN TOMATO (*Solanum lycopersicum*) VAR. MONGAL F1

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ABSTRACT

The availability of secondary metabolites in various plant parts can be influenced qualitatively and quantitatively by managing ecological factors or farming practices. Purpose of this study was to determine the impact of organic and mineral fertilization on the content of phenolic compounds and antioxidant activity in fruit of the tomato Mongal F1 variety. Experimentation was carried out in a randomized block factorial design. Results of study revealed that plant treated by organic fertilizers have 16.36 ± 0.07 mg Trolox Equivalent (TE)/100g fresh tomato (ft) antioxidant activity and 6.96 ± 0.63 mg Gallic Acid Equivalent (GAE)/100g ft total phenols content while the plant treated by mineral fertilizer have 15.57 ± 0.05 mg TE/100g ft for antioxidant activity and 6.09 ± 0.13 mg GAE/100g ft for total phenols content. Furthermore, it was reported that, as compared to mineral fertilization, organic fertilization significantly increases the antioxidant activity and phenolic compounds production in Mongal F1 tomato variety. This increase is probably due to the availability of various major and minor elements in organic fertilizer contrary to mineral fertilizer which has only three major elements, nitrogen, phosphorus, and potassium. 79 days after transplanting, fruit rippling has no obvious effect on the accumulation of these compounds.

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1 Introduction

In Burkina Faso, agriculture is the main source of income for the population and the mainstay of the country's food security. It employs more than 80% of the population and contributes for about 33% to the Gross Domestic Product (GDP) (MAHRH, 2011). Tomato production comes second after onion bulb and generates significant foreign currency influx for the national economy. The national tomato production is 157,086 tons a year and remains the most profitable vegetable farming in the country with total sales valued at €117 million (MAHRH, 2011). It is the main source of income for vulnerable populations in the fight against malnutrition and poverty. The main destinations of export tomatoes are Ghana, Togo, Benin, Equatorial Guinea and Ivory Coast.

Research on natural antioxidants is interesting in more than one way because it could lead to the production of natural substitutes to potentially toxic synthetic antioxidants such as butylatedhydroxyanisole and butylatedhydroxytoluen, which are commonly used for the conservation of certain foods (Fukumoto & Mazza, 2000; Lako et al., 2007; Koala et al., 2013a, Koala et al., 2013b). Natural antioxidants have preventive action against chronic diseases such as cancer, cardiovascular disease including risk factors such as hypertension, diabetes, and obesity (Diaz et al., 1997; Renaud et al., 1998; Willcox et al., 2004). Food intake of natural antioxidants known for their role in strengthening the immune system plays a major role in the prevention of this category of diseases but also infectious diseases and even the aging process and neurodegenerative diseases which are often linked to the deficit in antioxidants (Arts et al., 2005). Polyphenols and carotenoids are natural antioxidants with beneficial effects on human health (Osganian et al., 2003; Melendez-Martinez et al., 2004; Tapiero et al., 2004; Stahl & Sies, 2005; Li et al., 2011). Antioxidants are therefore a dual weapon which can be used to protect individuals at risk of over-nutrition but especially those most vulnerable to under-nutrition, infectious and parasitic diseases such as young children, the elderly, pregnant women and lactating women (Rice-Evans & Packer, 1998; Milner, 1999).

There is a growing interest to develop simple methods to increase the concentration of polyphenols in foods in order to strengthen their overall nutritional value (Parr & Bolwell, 2000; Schreiner, 2005; Schreiner & Huyskens-Keil, 2006). The literature suggested that many environmental factors and cropping practices could influence the biosynthesis of polyphenols and antioxidants (Daniel et al., 1999; Premuzic et al., 2001; Premuzic et al., 2002; Jeffery et al., 2003; Schreiner, 2005; Dragovic-Uzelac et al., 2007). Polyphenols and carotenoids are also produced by plants throughout their development for various reasons such as defense against microorganisms, insects, and herbivores (Dixon & Paiva, 1995). Exposure to ultraviolet radiation (Rozema et al., 1997), environmental stresses (Ehrhart, 2014), allelopathic interactions (Mann et al., 1987), and availability of nutrients in

the soil could also stimulate the biosynthesis of these natural products. It has also been shown that the levels of fertilization in nitrogen, phosphorus, potassium and calcium can affect the production of secondary metabolites in plants (Oloyede et al., 2012; Sabrina et al., 2012; Omar et al., 2012; Vignesh et al., 2012), and therefore that the presence of macronutrients is essential for plant growth and has a significant effect on the accumulation of polyphenols and antioxidants (Parr et al., 2000).

A reaction of plants to these stimuli varies with the plants and used fertilizers but mechanism is not well understood. Further, the use of these techniques to induce plants to produce secondary metabolites (and therefore potentially increase their nutritional value) is not common. The overall objective of this study was to explore the effect of organic and mineral fertilizer on the production of total antioxidants and polyphenols in the fruit of Mongal f1 tomato variety.

2 Materials and Methods

The study was conducted at the headquarters of the Research Institute for Applied Sciences and Technologies, located at the industrial area of Ouagadougou – Burkina Faso. The study area was located at the geographical coordinate 12° 25' North latitude, 1° 29' West longitude and altitude of 435m (IGB, 2014).

2.1 Materials

The tomato variety Mongal F1 used in this study was obtained from the National Institute for Agricultural Research (INRA, France). This variety was chosen because of its easy availability and nature of growing efficiently under dry hot weather or in wet tropical areas corresponding to Burkina Faso. It is also resistant to nematodes as well as bacterial and fungal diseases (Aïssa et al., 2014). The organic fertilizer used in this study was well-decomposed livestock manure while the mineral fertilizer consisted of N-P-K (23-14-23).

2.2 Methods

Farming operations such as land preparation, nursery watering and mulching, transplanting, weeding/ hoeing, fertilizing, harvesting and pest treatment were performed as per the instructions manual of the variety. The experiment was conducted in a Factorial Block with 4 replications. The two studied factors were fertilization (OF- Organic Fertilization; MF- Mineral Fertilization; C- Control) and harvest periods (R1, R2, R3) which correspond to fruit physiological maturity, to evaluate the effect of maturation on total phenolic contents and antioxidant level in the fruits.

Nine factorial combinations which studied were C/R1, C/R2, C/R3, OF/R1, OF/R2, OF/R3, MF/R1, MF/R2, MF/R3. Spacing between two blocks was 2.5m and elementary plots were 1.5m apart. Each plot consisted of 4 rows of 3m in length.

Spacing between adjacent rows was 0.8 m and plants within each row were 0.5 m apart. Two border lines were planted on both sides in each plot to reduce border effects. The first harvesting operation (R1) was carried out at 79 days after transplanting which corresponds to fruit physiological maturity. The second and third harvests (R2 and R3) were performed 85 and 89 days after transplanting, respectively.

2.2.1 Determination of antioxidant activity

The antioxidant activity of tomato fruit was determined by the ABTS method (Roginsky & Lissi, 2005). It is the ability of an antioxidant relative to trap the cation radical ABTS •⁺. The antioxidant power was measured according to the Trolox Equivalent Antioxidant Capacity (TEAC) method (Re et al., 1999). The principle of this method is that the total antioxidant activity of a molecule is derived from its ability to inhibit the radical 2,2'-azinobis- (3-ethylenzothiazoline-6-sulfonic acid) (ABTS•⁺). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is used as a reference antioxidant. Its cyclic molecular structure is similar to that of vitamin E (α-tocopherol), (Cuvelier et al., 2003).

For the formation of the radical ABTS •⁺, 10 mg ABTS are dissolved in 2.6 ml of distilled water simultaneously, 1.7 mg of persulfate potassium were dissolved in the same volume of distilled water. These two solutions were mixed together and placed in a dark at room temperature for 12 to 16 hours. And then, the ABTS •⁺ solution was diluted 1/200 by adding methanol. An aliquot of 500 µl diluted tomato extract was mixed with the 2 ml of the ABTS•⁺ - ethanol solution. After 15 min of incubation in the dark, absorbance is read at 734 nm, using a microplate (spectrophotometer SAFAS, MP96). The test was replicated three times and the results expressed as µmol Trolox Equivalent per 100 g of fresh material referring to the standard range.

2.2.2 Determination of total phenols

Total phenols content was determined by the Folin-Ciocalteu method modified by Singleton et al. (1999). This Folin-Ciocalteu reagent was prepared with phosphomolybdate of tungsten. It oxidized in a basic environment, the hydroxyl groups of the phenolic compounds present in the sample. The reduced molecules (quinones, oxides, etc.) have blue color which intensity is proportional to the polyphenols content of the sample. Total phenols content was determined by a calibration curve established from different concentrations of gallic acid (3,4,5-trihydroxybenzoic acid). Absorbance was measured with a microplate spectrophotometer (Microplate Autoreader MP96; SAFAS Instruments) at 760 nm wavelength by using a 96-well plate (glass vials 250 µL) after incubating the plate for 30 min at 37°C. Distilled water was used as a blank. Total phenolic contents of the samples determined from the calibration curve equation ($y = 46.41x + 0.063$, $R^2 = 0.998$) were expressed in mg of gallic acid equivalents (GAE) per gram of fresh weight. All measurements were performed in three replications. Results are expressed in gram gallic acid which was equivalent (GAE) to 100g of plant material.

2.3 Statistical analysis

All analysis was conducted in three replications, and data were processed in Microsoft Excel 2013 while analysis of variance (ANOVA) was performed using the software "XLSTAT version 7.5.2".

3 Results and Discussion

Results of the total phenolic content and antioxidant activity of tomato variety Mongol F1 during maturation are presented in the table 1.

Table 1 Effect of organic and mineral fertilizers application on the content of total phenols and antioxidant activity during tomato maturation.

Treatments	Content of total phenols (mg GAE /100g ft)	Antioxidant Activity(AOA) (mg Trolox Equivalent/100g ft)
C/R1	7.33 ± 0.12 ^b	16.20 ± 0.03 ^b
C/R2	6.65 ± 0.18 ^d	16.35 ± 0.01 ^{ab}
C/R3	6.00 ± 0.10 ^f	16.26 ± 0.05 ^c
OF/R1	7.70 ± 0.20 ^a	16.26 ± 0.04 ^c
OF/R2	7.00 ± 0.05 ^c	16.41 ± 0.04 ^a
OF/R3	6.17 ± 0.04 ^e	16.37 ± 0.04 ^{ab}
MF/R1	6.16 ± 0.12 ^e	15.54 ± 0.03 ^e
MF/R2	6.20 ± 0.33 ^e	15.53 ± 0.02 ^e
MF/R3	5.90 ± 0.12 ^f	15.65 ± 0.01 ^d

In the same column, values with different superscript letters are significantly different ($P < 0.001$); E- Trolox Equivalent; ft - fresh tomato; fm - fresh matter; GAE- Gallic Acid Equivalent; OF-Organic Fertilization; MF-Mineral Fertilization; C- Control; R1 - harvesting 79 days after planting, R2 - harvesting 85 days after planting, R3- harvesting 89 days after planting.

3.1. Antioxidant activity

Results of the present study revealed that the antioxidant activity of samples varies between 15.53 and 16.41 mg TE /100g fresh weight. These values correspond to treatment MF/R2 and OF/R2, respectively. Among various combinations, organically fertilized tomatoes have a higher antioxidant activity than tomatoes under mineral fertilization and the control (without fertilization) for all harvests. Overall, maturation has a positive effect for the accumulation of antioxidants and total phenols.

Results in the table are in agreement with the findings of Wu et al. (2013) who reported higher antioxidant activity of 5.5 mmol TE/100g fresh jujubes under organic fertilization while this value was only 4 mmol TE/100g fresh jujubes under mineral fertilization. The increases in secondary metabolites and antioxidant potential may be caused by the presence of various major and minor elements in organic fertilizers while in case of mineral fertilizer only three basic mineral elements (i.e. nitrogen, potassium and phosphorus) are available (Ibrahim et al., 2013). Furthermore, the use of organic manure increases soil fertility by improving its physical, chemical, and biological properties (Khalil et al., 2007). Lowest levels of antioxidants were observed in the presence or absence of mineral fertilizers. These results are in agreement with several authors those who reported that mineral nutrition has little or no effect on the production of polyphenols and antioxidants in some plants (Pavla & Pokluda, 2008; Gupta- Elera et al., 2012; Marilou et al., 2012). Premuzic et al. (2001) found in cherry tomato that vitamin C (a powerful antioxidant) content was higher under organic fertilization or fertilization combining mineral and organic inputs than under exclusive mineral fertilization.

3.2. Total phenols content

The total phenols content of the tomato samples was between 5.90 mg to 7.70 mg GAE/100g fresh tomato for all treatments. Fruits sample collected from plants under organic fertilization showed overall higher total phenols contents than those under mineral fertilization during maturation. The biosynthesis of polyphenols seemed to be positively influenced by the application of organic fertilizers. These results are in agreement with the observations of Toor & Savage (2005) who obtained an average of 12.7 mg GAE / 100g from fresh tomato. Zoran et al., (2014) reported very high amount of phenolic compounds in their study on tomato although they are in agreement with our results for the fertilization. They found 50.83 and 29.98 mg GAE/100g fresh tomato from organic and mineral fertilizations, respectively. Like tomatoes, other fruits such as peach and pear (Carbonaro et al., 2002), strawberry (Reganold et al., 2010) and jujubes (Wu et al., 2013) also showed similar type of phenolic content values. Cieslik et al. (2006) conducted study on the total phenol content of certain fruits and reported 28.1 mg GAE/100 mg for kiwi, 11.6 mg GAE/100 mg for watermelon and 7.8 mg GAE/100 mg

phenolic content for melon. These species are considered as interesting sources of natural antioxidants.

Levels of total phenols in present the study are lower than those from the literature (Toor & Savage 2005; Zoran et al., 2014). This would undoubtedly be related to plant varieties, cultural practices or methods of analysis used by these authors. Indeed, the literature showed that the micronutrient content of fresh fruits of different tomato varieties can vary significantly and it depending on the crop variety itself (Lenucci et al., 2006). Cropping practices, environmental factors, fruit maturity period and post-harvest storage can also cause changes in the composition of fruits of the same variety (Raffo et al., 2002; Slimstad & Verheul, 2005; Luthria et al., 2006). Part of the effective antioxidant activity of tomato could be also attributed to their phenolic compounds, mainly chlorogenic acid, rutin and gallic acid. The present study of the AOA in relation with GAE showed a slight correlation ($r^2 = 0.478$) between AOA and GAE.

Correlation revealed that the highest GAE had not the highest AOA which is contrary with some previous studies on potatoes which showed that polyphenolic compounds are mainly responsible for the antioxidative activity of plant extracts (Huang et al., 2005). The present study on tomato showed that polyphenols are slightly responsible for antioxidant activity of plant extracts since they contributed only 48% polyphenols content. Furthermore, it was also reported that carotenoids are powerful natural antioxidants and tomato fruit contained almost 449µg/100 fmof β-Carotene and 2573 µg/100 fm of lycopene (USDA, 2007). Total carotenoid contents assessed in tomato certainly contributed to increase their AOA. Phenolic compound content decrease during fruit ripening regardless of the of fertilization type (organic or inorganic)

Conclusion

Results of the present work highlight the importance of organic fertilization in the accumulation of total antioxidants and polyphenols in tomato variety Mongal F1. The antioxidant activity of tomatoes is reported higher than that of certain fruits consumed in Burkina Faso as a source of natural antioxidants. Results showed that tomato variety Mongal F1 is therefore a good source of natural antioxidants, and could potentially be used as a nutraceutical or functional food. In addition, tomato, a vegetable widely consumed fresh or processed, is recognized through the literature for its nutritional qualities and richness in micro-components such as carotenoids (especially lycopene), phenolic compounds and vitamin C.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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