



EFFECT OF ORGANIC FERTILIZATION ON THE QUANTITY AND QUALITY OF MELON PRODUCTION

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ABSTRACT

Growing environmental impact of conventional farming practices, gradually impacts soil structure and biological balance. This has forced the development of alternative practices to reduce and mitigate these effects. Types of fertilizers influence the quantity and quality of production. Following this idea, in 2016, in a crop of melon in a polyethylene tunnel, Lignohumat organic fertilizer was used, a granular humic product, which was applied during vegetation, in three stages and three fertilization doses: 100; 150 and 200 g/ha. Melon plants (Charentais variety) fertilized with Lignohumat at different doses showed positive results compared to the unfertilized variant. Results showed that application of the 150 g/ha dose significantly improved ($P \leq 0.05$) the average number of fruits/plants, their average weight, fruit/plant production and the production/m² but also the quality of fruits: soluble dry matter – 8.55%, total dry matter – 12.12%, reducing sugars – 3.40%, vitamin C - 21.12 mg/100 g, carotene - 36.42 mg/100 g f.m and antioxidant activity of 175.92 μMTE/100 g (ABTS method) and 228.16 μMTE/100 g (DPPH method). Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS). Significant positive correlations were identified for very important parameters.

1. Introduction

High demand for agricultural products in order to obtain food and consumer goods in modern society has generated significant increases in agrarian activities over the past decades. Consequently, it has been necessary to

implement methods that will, among other things, improve crop efficiency, mitigate the negative impact on soil, reduce the use of chemical fertilizers and increase crops economic return.

Organic fertilization involves use of organic fertilizers, decomposition of organic matter debris, use of domestic waste water, manure and microorganisms (fungi and bacteria) (Chirinos *et al.*, 2006). In recent years, lignocellulosic materials have been used as fiber raw materials in the production of biodegradable nutrient pots for vegetable seedlings in containers. Considering the high degradation potential of these materials, they can also be used as sources of biofertilizers (Nechita *et al.*, 2010).

All these are used to improve nutrient fixation in the rhizosphere, to produce growth stimulators for plants, to improve soil stability by ensuring effective biological control, and promote micorization, symbiosis and bioremediation in soils contaminated with toxic substances (Rivera-Cruz *et al.*, 2008).

Bio-fertilizer application improves surface productivity in a relatively short time, reduces soil and water contamination, increases soil fertility, thus promoting antagonism and biological control of phytopathogenic organisms (Chirinos *et al.*, 2006). These issues provide real benefits for farmers as a result of lower fertilization costs and higher yields. Implementation of fertilization techniques requires feasibility studies, monitoring of environmental variables involved in metabolic processes, capital investment, time and trained personnel (Vanegas, 2003).

Biofertilizers have a significant effect on production due to nutrients that mobilize microorganisms that contribute to the availability of macro and micro elements and their forms (El-Sanafawi, 2006, Ahmed and Fraihat, 2011). Application of biofertilizers improves plant growth morphological elements, chlorophyll content and N, P, K elements, production elements (weight and number of fruit/plant) and quality (Dinu *et al.*, 2013). Therefore, the purpose of this study was to evaluate Lignohumat organic fertilizer based on humic acids on quantitative and qualitative elements of melon fruit and to establish an optimal dose for a polyethylene tunnel culture,

thus reducing the negative impact on soil and environment. *Cucumis melo* L. culture was chosen to diversify the diet and because melon fruits are also a significant source of polyphenol antioxidant phytochemicals which provide health benefits, especially to the cardiovascular system, as largely shown for other foods.

2. Materials and methods

2.1. Plant material

Experience was placed in a polyethylene tunnel of the Faculty of Horticulture and Agriculture (University of Craiova), Romania. Biological material was represented by Charentais variety. The experience had four variants and was placed in randomized blocks in three rehearsals. Variant specifics were as follows: V₁ (Control) - unfertilized; V₂-fertilized with Lignohumat 100 g/ha; V₃-fertilized with Lignohumat 150 g/ha; V₄-fertilized with Lignohumat 200 g/ha. Culture was set up by planting on 03.05.2016 according to the following technological scheme: 50 + 110 (3) + 50 x 30 cm (3.2 pl/m²).

Lignohumat organic fertilizer was applied foliarly at 10 days interval. Lignohumat is a highly effective humic product with chelating microelements with growth stimulating effect and plant protection against stress. Prior to the establishment of the crop, agrofond was given 30 t/ha of composted grape marc. Culture technology was that specific to yellow melons grown in polyethylene tunnels.

2.1.1. Samples preparation

A quantity of 100 g of each sample was broken up with a blender. 1 gram of homogenized sample (pulp) was mixed with 10 ml 80% methanol, intensely agitated for 10 minutes using a vortex. Extraction of phenolic compounds was carried out by keeping the mixture in an ultrasonic bath for 70 minutes. The mixture was filtered, and to the solid residue was added 5 ml of 80% methanol and the extraction procedure repeated. The two obtained extracts were combined and analyzed

using protocols specific work to determine the total polyphenol and antioxidant capacity.

2.2.Soluble dry matter (S.D.M) content was determined gravimetrically and the result was expressed in percentages.

2.3.The soluble solids content (SSC) % was determined using a digital refractometer (Kruss Optronic DR 301-95) from the juice pressed from the fruit the result was expressed in percentages.

2.4.The titratable acid content (acidity) was determined by titration with 0.1N sodium hydroxide (NaOH) and expressed as % citric acid.

2.5.Carbohydrates (reducing sugars) (%) were extracted in distilled water (1:50 w/V) and assayed colorimetric with 3,5 dinitrosalicylic acid using glucose as standard. Ascorbic acid was extracted in 3% metaphosphoric acid (1:50 w/V) and determined by using redox titration with 2,6-dichloroindophenol.

2.6.The determination of total carotenoids

The weighed samples, having been put separately in 95% in acetone (50 ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbances were read at 400-700 nm on Cary 50 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total caroten at 470 nm. The value of these pigments was calculated using the method described by Dinu *et al.*, (2013).

2.7.Total phenolic content

The amount of total phenolic compounds in the leaf and petiol of sweet potato extract was determined colorimetrically with Folin-Ciocalteu reagent by using the method

described by Singleton and Rossi (1965) with some modifications. To 1 ml extracts (diluted 1:10 with ultrapure water), 1 ml bidistilled water (blank), 1 ml of each standard solution were introduced in laboratory flasks of 25 ml and added every 5 ml reactive Folin-Ciocalteu (diluted 1:10 with ultrapure water). After 2 min, 4 ml of sodium carbonate solution 7.5% was added and they were kept in the incubator during 2 h at the room's temperature.

The absorbance was measured at 765 nm by using a model evolution 600, double beam scanning UV-visible spectro-photometer, PC control with VISION pro software. A standard curve was prepared by using 50, 100, 150, 200 and 250 mg/L solutions of gallic acid in methanol and water (60:40, v/v). Gallic acid was used as the reference standard and the results (total phenolic content) were expressed as gallic acid equivalents (GAE) and the results were expressed as mg/g F.W.

2.8.Antioxidant activity

Extracts for the determination of antioxidant activity were prepared into 80% aqueous methanol (1:10 w/v) at 24°C for 16 h. The resulting slurries were centrifuged at 4000g for 5 min and the supernatants were collected. The total phenolics content was determined colorimetric by using the Folin-Ciocalteu reagent (Dinu *et al.*, 2016) and expressed as mg of gallic acid equivalents (GAE)/100g f.w. The capacity of extracts to reduce the radical 2,2-diphenyl-1-picrylhydrazyl was assessed colorimetric. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as μM Trolox equivalents (TE)/100g.

The ABTS radical cation scavenging activity of the methanolic extract was assessed colorimetric. The final results were expressed as μM Trolox equivalents (TE)/100g fw.

2.9.Statistical analysis.

The significance of differences between variants was statistically determined using variance analysis using ANOVA and

calculating the limit differences, $LSD \leq 0.05\%$, ($LSD =$ least significant difference). The correlation coefficients between quality parameters were performed by multiple correlation coefficients.

3. Results and discussions

3.1. Production Determinations

Production determinations in the experimental variants concerned the average number of fruits/plants, average weight/fruit, production/plant and production/m².

Concerning the average fruit/plant number (Table 1), it varied from 5.4 at V₁ to 7.2 at V₃, the variant with significant results. Regarding the average weight/fruit this was higher in the fertilized variants compared to unfertilized variant, which shows that the fertilization dose directly influences production. Significant differences between variants are observed in production obtained per plant (4.7 kg per V₃ versus 2.7 kg at V₁) and production per m² - 15 kg/m² at V₃ versus 8.6 kg/m² at V₁ unfertilized. Results are similar to those obtained by Mohamed and Mohamed (2016) in an experiment with fertilizer variants in melon

culture, but also those reported by Adam *et al.*, (2009) and Naidu *et al.*, (2014).

3.2. Biochemical determinations

Main biochemical components of melon fruit are shown in Table 2. High content of soluble solids (SSC) corresponds to a good accumulation in sugars, which is very important from a sensory point of view. It was different at each variant. Highest content, of 8.55% was recorded at V₃, followed by V₂ and V₄ and the lowest in V₁, non-fertilized variant. Dry substance (%) values recorded in this study confirm results of Ahmad and Al-Fraihat (2011). Total soluble dry matter had the highest value (12.12%) in the fertilized variant with 150 g/ha Lignohumat and the lowest in the unfertilized version (6.80%). V₂ and V₄ had values close to each other but greater than V₁ and much lower than V₃ (Table 2). The total dry substance values (%) obtained in the study at the University of Craiova, Romania, are superior to those obtained by Mohamed and Mohamed (2016) in a study with several variants of fertilization of melons, one of which was compost + humic acids + microorganisms.

Table 1. Elements of production at studied variants

Variant	Average fruit/plant (number)	Average weight / fruit (g)	Production/plant (kg)	Production./m ² (kg)
V ₁ - control	5.4 ^b	471 ^b	2.7 ^c	8.6 ^c
V ₂	6.0 ^{ab}	552 ^{ab}	3.5 ^{bc}	11.2 ^b
V ₃	7.2 ^a	643 ^{ab}	4.7 ^a	15.0 ^a
V ₄	6.4 ^{ab}	680 ^a	4.5 ^{ab}	14.4 ^a
LSD \leq 0.05	1.2	180.4	1.1	1.8

The different letters in the same row indicate significant differences $P \leq 0.05$ between the variants

Table 2. Biochemical determinations of melon fruits

Variant	Soluble solids (%)	Soluble dry matter (%)	Acidity (citric acid %)	Carbohydrates (%)	Vitamin C (mg/100g f.m.)
V ₁ - control	5.80 ^c	6.80 ^d	0.085 ^d	2.75 ^b	16.88 ^c
V ₂	7.25 ^b	8.29 ^c	0.105 ^c	2.66 ^b	20.10 ^b
V ₃	8.55 ^a	12.12 ^a	0.147 ^a	3.40 ^a	21.12 ^a
V ₄	7.05 ^b	8.91 ^b	0.133 ^b	3.01 ^a	19.40 ^b
LSD \leq 0.05	0.944	0.514	0.008	0.451	0.887

The different letters in the same row indicate significant differences $P \leq 0.05$ between the variants

Acidity recorded low levels ranging from 0.085 to 0.147% citric acid in melon fruit on experimental variants. The highest content was recorded at variant fertilized with 150 g/ha Lignohumat (0.147%), still small quantities compared to other fruit or vegetable species. These values are also supported by Kolayli *et al.*, (2010) and Obando-Ulloa *et al.*, (2009). Carbohydrates, an important parameter for determining fruit quality, ranged from 2.66% to V₂ to 3.40% at V₃ with statistically significant differences for V₃ and V₄ variants that were fertilized with the highest doses of Lignohumat. Lingle and Dunlap (1987) stated that sugary melons are influenced by environmental factors. Thus, climatic factors, fertilization regime and nature of fertilizer influence the content in sugars, and implicitly the quality of the fruit. Vitamin C is a very important natural antioxidant. Its biological importance is based on the ability to participate in various enzymatic processes, hydroxylation, oxidation-reduction. Vegetable vitamin C content may vary depending on environmental factors and stress, such as: light intensity, temperature, humidity, pollution (Dinu *et al.*, 2016). The values in Table 2 for vitamin C content ranged between 16.88 mg/100g f.m. in unfertilized variant and 21.12 mg/100g f.m. in the fertilized variant with 150 g/ha of Lignohumat. The determined values are similar to those in literature. Thus, Laster and Hodges, (2008), in a study of three melon varieties, determined an ascorbic acid content of 34.7-44.7 mg/100 g. Obando-Ulloa *et al.*, (2009) reported an ascorbic acid content, determined at 29 melon isogenic lines from 6.0 to 19.7 mg/100g. Highest values of ascorbic acid were determined for melon varieties followed by grafted melons and the lowest values for hybrids. Laster and Hodges, (2008) stated that culture technology directly influences the accumulation of ascorbic acid in melon varieties.

Total polyphenols represent the largest class of secondary metabolites in plants. Phenolic content and composition of fruits and

vegetables depend on genetics, environmental conditions, and storage conditions after harvest. Table 3 presents the values of total melanin fruit polyphenols for the experimental variants. It is noted that the highest content was recorded in variant 3 of 94.10 mg GAE/100 g f.m followed by V₄ with 40.65 mg GAE/100 g f.m. Variance analysis highlighted variant 3. The results of our study are superior to those obtained by Preciado-Rangel *et al.*, (2015) in a melon culture treated with different types of organic fertilizers. They claim that low nitrogen, magnesium and phosphorus content in organic fertilizers could cause nutritional stress in melon plants during growth, thus favoring the increase in phenolic compounds.

Carotenoids are one of the main lipophilic constituents, which contribute to total antioxidant activity and to content of provitamin in potato but also in other vegetables (Lachman *et al.*, 2016). Variance analysis for total carotene content showed a significant difference ($P < 0.05$) between studied variants. Results show a variation from 24.96 mg/100 g f.m to V₁ to 36.32 mg/100 g f.m to V₃. Highest value was at V₃, fertilized with 150 g/ha of Lignohumat, followed by V₄ and V₂. High values and differences between variants may be the result of different fertilizer doses and orange pulp color (Charentais variety) and are also supported by Henan *et al.*, (2013).

Results of this study are similar to those obtained by Woblang *et al.*, (2010), which studied the influence of pre and post-harvest factors on β -carotene content and antioxidant capacity of melon. In the present study, values greater than 31 mg/100 g f.m are the differentiated result from fertilization dose. Menon and Ramana Rao, (2012) reported values of carotene content close to those of this study, their research being focused on melon plants nutrition at various stages of ripening.

Evaluation of antioxidant activity is a parameter for assessing nutritional quality. DPPH radicals are generally used as a screening method for evaluating a wide variety of compounds. Antioxidant activity of the

studied variants was determined by the ability of the extracts to reduce the ABTS radical cation. It had values between 103.30 $\mu\text{MTE}/100\text{g f.m}$ to V₂ and 175.92 $\mu\text{MTE}/100\text{g f.m}$ to V₃. DPPH method varied between 104.0 $\mu\text{MTE}/100\text{g f.m}$ at unfertilized variant and 228.16 $\mu\text{MTE}/100\text{g f.m}$ at variant fertilized with 150 g/ha of Lignohumat. From the results

it can be said that the fertilizer dose of 150 g/ha has significantly influenced antioxidant activity of melons. Other doses did not influence antioxidant activity compared to non-fertilized variant.

Table 3. Biochemical determinations of melon fruit

Varianta	Total phenolics content* (mg GAE/100g f.m.)	Total carotene (mg/100 g f.m.)	Antioxidant activity	
			ABTS ($\mu\text{MTE}^{**}/100\text{g f.m.}$)	DPPH ($\mu\text{MTE}^{**}/100\text{g f.m.}$)
V1-Control	34.76 ^c	24.96 ^c	123.00 ^b	104.3 ^b
V2	36.90 ^c	31.28 ^b	103.32 ^b	112.0 ^b
V3	94.10 ^a	36,42 ^a	175.92 ^a	228.1 ^a
V4	40.65 ^b	31,52 ^b	134.09 ^b	107.5 ^b
LSD \leq 0.05	2,44	1,62	36,7	39,42

*gallic acid equivalents; ** equivalents Trolox; The different letters in the same row indicate significant differences $P\leq 0.05$ between the variants

Table 4. Correlations between the biochemical compounds analyzed in the melon variants fertilized with Lignohumat

Specification	S.S.M (⁰ Bx)	S.D.M (%)	Carbo-hydrates (%)	Total phenolics content mg (GAE/100g f.m)	Total caroten (mg/100g f.m.)	Antioxidant activity ABTS ($\mu\text{MTE}/100\text{g f.m}$)	Antioxidant activity DPPH ($\mu\text{MTE}/100\text{g f.m}$)
Vitamin C (mg/100g f.m)	0.936***	0.856***	0.598*	0.682*	0.972***	0.471	0.679*
S.S.M (⁰ Bx)	-	0.960***	0.771**	0.853***	0.994***	0.680*	0.847***
S.D.M (%)	-	-	0.915***	0.946***	0.950***	0.857***	0.930***
Carbohydrates (%)	-	-	-	0.922***	0.767**	0.982***	0.885***
Total phenolics content (mg GAE/100g f.m)	-	-	-	-	0.812**	0.924***	0.996***
Total carotene (mg/100 g f.m.)	-	-	-	-	-	0,660*	0,799**

* $r - P 5\% = 0.58$; ** $r - P 1\% = 0.71$; *** $r - P 0,1\% = 0.82$

Variation of antioxidant potential depends on plant maturity stage or analyzed plant parts, genotype, culture method, environmental conditions (Soare *et al.*, 2016) but also of the fertilizers used, therefore, Preciado-Rangel *et al.*, (2015). recommend fertilization with organic nutrients applied foliar to melon culture

to improve the nutritional qualities of production.

Antioxidant activity begins to increase with the onset of fruit maturation, reaches a peak in the ripening stage, after which it decreases at overmaturation (data not shown). Although all four variants of melon were grown under same

environmental conditions, study results show that 150 g/ha Lignohumat dose significantly influenced the production elements as well as biochemical characteristics of melon fruit, thus there were significant differences between unfertilized variant and V₃. The correlations between certain quality parameters of melon fruit obtained in the fertilized experience with different doses of Lignohumat are presented in the table 4. For the majority of the parameters, significant positive correlations were identified. Literature confirms that when fruits and vegetables with high carotene content are consumed, there is a strong antioxidant activity in the body because there is a strong correlation between these two elements.

Corrigan *et al.*, 2000 support the view that pumpkin fruits with a high sugar content and high total carotenoids are considered to be good quality fruits and can be eaten raw.

4. Conclusions

Our results have shown that the use of the Lignohumat natural fertilizer in three different doses at melon culture in polyethylene tunnel has led to the production of qualitative and quantitative yields superior to the unfertilized control. The average number of fruit/plant (7.2), average fruit weight (643 g) and average yield/m² (15 kg/m²) were recorded at V₃ (150 g/ha). Also in this variant, the organoleptic properties of melon fruit recorded the highest values compared to the non fertilized control but also with the other two variants fertilized with different doses.

Significant positive correlations were registered between all parameters studied regardless of the fertilization dose. These results may recommend the use of Lignohumat 150 g/ha for the fertilization of some vegetable crops in the polyethylene tunnel, knowing that in the vegetable sector, due to the superintensive character, high doses of fertilizers can be used which can cause soil and groundwater pollution. Organic fertilization with Lignohumat is a viable alternative to a source of organic nutrients in order to obtain melon

productions grown in the polyethylene tunnel with superior nutraceutical qualities.

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