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Current Pharmaceutical Biotechnology, 2015, *16*, 000-000

Improving of Nutraceutical Features of Many Important Mediterranean Vegetables by Inoculation with a New Commercial Product

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Abstract: Several epidemiological studies show that fruits, vegetables and cereals can play a nutraceutical role for their content of many antioxidant phytochemicals such as carotenoids, ascorbic acid and phenolics. A commercial inoculant (MICOSAT F®) containing arbuscular mycorrhizal fungi (AMF) could improve the nutritional value in crops. The goal of this work was to evaluate the effect of AMF on the production level of carotenoids, AsA, phenols including antocyanins and saponins, proteins, total antioxidant activity and nitrates in fruits, vegetables, legumes and durum wheat var. grecale, whose consumption is largely recommended according to Mediterranean diet. The treatment increased the antioxidant activity in strawberries (37.50%), in giant lentils (29.17%) and in durum wheat (63.63%) but decreased it in kiwi (31.81%) and in grape (19.81%). Nitrate levels decreased significantly in strawberries (39.78%) and in tomato intended for transformation (37.79%). The application of MICOSAT F® enhanced the levels of several secondary metabolites. However, the amount of phytochemicals and respective by-products were reduced in some cases. Environmental conditions and modality of AMF inoculation could module both primary and secondary metabolites.

Keywords: Arbuscular Mycorrhizal Fungi, nutraceutical vegetables, antioxidant activity, Micosat F®, nitrates.

INTRODUCTION

In the last years consumers have revealed an increasing interest in crops recognized as "functional food" or "nutraceutical food". Many epidemiological studies show health-promoting properties of fruits, vegetables and cereals whose consumption is highly recommended according to the principles of Mediterranean diet. These foodstuffs are largely investigated not only for their content in dietary fibres, vitamins and minerals, but also for the levels of secondary metabolites commonly called "phytochemicals" [1]. They play a beneficial role mainly as antioxidants, in stimulation of immune system and in prevention of oxidative stress [2] and chronic non-communicable diseases (CNCD) including cardiovascular diseases (CVD), such as hypertension, coronary heart disease, diabetes, and obesity [3]. Phenols include flavonoids that are known to exert a protective action against intestinal inflammation and rheumatoid arthritis [4,5]. Among these compounds, anthocyanins are believed to play a role in the antioxidant response in the tissues, especially in berries, affected by biotic or abiotic stress factors [6]. Saponin (2-phenyl-benzopyrane), present especially in legumes, defines a group of isoprenoidal-derived aglycone with a demonstrated long-term beneficial impact on serum glucose and lipids concentrations, as well as anti-carcenogenic [7], anti-inflammatory [8], and anti-bacterial [9] effects. Some studies reported that ascorbic acid (AsA) can prevent cancer by neutralizing free radicals before they can damage DNA and initiate tumor growth [10], while carotenoids, in particular lycopene, show antioxidant and DNA repair activities and protection against immune system [11]. Plant breeding and biotechnology approaches constitute successful ways to increase nutritional properties of fruit and vegetables [12]. Nevertheless, these techniques are associated with some difficulties represented by the public acceptance and risk assessment [13]. Therefore, alternative strategies in order to improve functional properties of crops are auspicable. In this context arbuscular mycorrhizal fungi (AMF) represent an efficient unconventional method. It provides to establish a strong symbiosis between mycorrhizal fungi and their host plants. AMF colonize the root cortex and develop an extraradical mycelium which spreads through the soil surrounding roots [14] determining metabolic transformations in plant roots, increased biomass, enhancement of plant tolerance to biotic and abiotic stresses [15]. The observed modifications in plant metabolism and physiology are the result of multiple transcriptional changes [16]. In particular arbuscular mycorrhizal (AM) symbiosis regulates genes involved in both primary (nitrogen, carbohydrate, protein metabolism) and secondary metabolism of mycorrhizal plants [17]. Zouari *et al.* (2014) [18] found that tomato mycorrhizal plants under low nutritional stress produce fruits with a nutrient content similar to those from non-mycorrhizal plants under high nutrient conditions. So AMF fungi can help replace exogenous fertilizer for fruit crops. Interestingly, physiological transformations are due to the activation of host defense reactions,

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1389-2010/15 $58.00+.00

involving the production of reactive oxygen species (ROS) in roots and stimulating of the antioxidant metabolism and the accumulation of total phenolics, AsA, carotenoids in several tissues and, as a consequence, in fruits [1-19]. Some evidences showed that AMF can influence the level of nitrates in plant due to the capability of AM hyphae to take up and transport them to the host plant [20]. Reg CE 1881/2006 [21] fixed limits for nitrates in spinach and lettuce, comprised between 2000 and 4500 mg/kg dependent on the month of harvest and condition of growing.

Many data regarding the evaluation of AMF effect on a single crop or a single metabolite are available. Moreover, the ability of AMF to improve nutritional quality need further and more global investigations. Therefore the aim of the present work was to evaluate the transversal and general impact of the commercial inoculant MICOSAT F®, containing a mixture of selected mycorrhizal fungi, bacteria and streptomices derived from rhizosphere, on different crops and several metabolites. In particular the effect of this product on carotenoids, AsA, phenols including antocyanins and saponins, proteins, nitrates and total antioxidant activity was evaluated in fruits, vegetables, legumes and durum wheat var. grecale.

MATERIALS AND METHODS

Experimental Design

Grown Conditions

The experiments were carried out on seeds from a field located in Angri (SA). Fruits and vegetables were grown under greenhouse conditions at an ambient temperature of 20-27°C and 75% relative humidity.

Inoculum Preparation

The product MICOSAT F®, containing a mixture of selected mycorrhizal fungi, bacteria and streptomices derived from rhizosphere, was applied as a layer of 200 ml mycorrhizal inoculum per 1 L pot at sowing. The inoculum consisted of rhizosphere soil from 6-months-old sorghum pot cultures containing spores, hyphae and heavily colonized root pieces. In order to obtain a homogenous colonization, a system characterized by a central inoculum compartment with two lateral test plant compartments was adopted. The central compartments included beans with an inoculum of the commercial product. One month later the symbiosis was well taken place and the system was ready for inoculation [22].

Plants Treatment and Effectiveness of Inoculation

Pre-germinated plants were transferred from pots into the lateral compartments, that were joined with the inoculum compartments. Two weeks after inoculation plants were harvested.

After clearing in 10% KOH, staining in 5% ink-vinegar solution and destaining in water, 20 root pieces of 1 cm were mounted on slides and observed with a light microscope (100 x magnification). Mycorrhizal colonization was determined as reported by Vierheilig *et al.* [23].

Plants from the control treatment received 200 ml of rhizosphere soil and root pieces from non-colonized sorghum plants, and were randomly selected and tested for mycorrhizal colonization in every experiment [23]. Three biological replicates were performed for each analyzed crop.

Sample Preparation

Edible parts of fruit and vegetables were chopped, ground by Ultra-turrax T25 Basic (Staufen, Germany) and kept at -80°C until the analyses. Each crop was analyzed for its main phytochemicals. In particular lycopene and β carotene were detected in Sicilian tomatoes, in tomatoes intended for transformation and in squash, total carotenoids in kiwi, giant lentils, both types of tomatoes, squash and wheat, total phenols were investigated in all analyzed categories, anthocyanins in strawberries, while AsA in kiwi, white grape, strawberry, giant lentil, squash and tomato, saponins in giant lentils, proteins in lentils and wheat. In addition the content of nitrates was evaluated in strawberries, giant lentils, Sicilian tomatoes and tomatoes for transformation. Results of determined parameters are expressed as mean ± standard deviation.

Materials

Butylated hydroxytoluene (BHT), acetone, ethanol, methanol, 2,6-diclorophenol-indophenol (DIF), $NaHCO_3$, CH_3COOH, Na_2CO_3, H_2SO_4, HCl, KOH, Folin-Ciocalteau's phenol reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), $FeCl_3 \cdot 6H_2O$, saponin, malvin, vanillin, Trolox were purchased from Sigma-Aldrich.

Determination of Bioactive Compounds

Proteins

Proteins were estimated according to the Kjieldahl method, by using a PBI International model Mineral SIX digester (PBI International, Milan, Italy) and a Buchi model B-324 distillation unit (Buchi, Flawil, Switzerland) according the method reported by Prosky *et al.* [24].

Carotenoids

Total carotenoids were extracted according to the method of Talcott and Howard [25] with slight modifications. The absorbance at 470 nm was measured at a spectrofotometer (Jasco V-530 UV–vis spectrophotometer, Tokyo, Japan). Total carotenoids were calculated according to the method of Gross [26] using the following equation:

$$\text{Total carotenoids (mg/g)} = (Ab \times V \times 10^6)/A^{1\%} \times 100\ g$$

where Ab is the absorbance at 470 nm, V is the total volume in mL of extract, $A^{1\%}$ is the extinction coefficient for a mixture of carotenoids solution (1 g/100 mL) at 2500, and g is sample weight (g).

The determination of lycopene was performed reading the absorbance at 503 nm and by using the Lambert Beer equation, with the coefficient of molar extinction ε (L/mol*cm) 152989 for lycopene dissolved in chloroform [27].

β carotene was determined by the spectrophotometric method reported by Lichtenthaler and Buschmann [28]. Each

sample was analyzed in triplicate. Results were expressed as mg/kg fresh weight (FW).

Ascorbic Acid

Ascorbic acid determination was carried out according to the AOAC official method [29] by titration with a solution prepared by weighting 50 mg of DIF and dissolving them in 50 ml H_2O added with 42 mg of $NaHCO_3$. AsA content was expressed as mg/kg (FW).

Total Phenolic Compounds

The method for determination of total phenolic compounds was described by Choi *et al.* [30] with some modifications. Two grams of analyzed sample were weighted, placed into a 50 ml Falcon tube and extracted with 25 ml CH_3OH/H_2O (60/40) by Ultra-turrax T25 Basic (Staufen, Germany) at 4000 rpm for 2 min and then into an ultrasonic bath (Branson 5200 Ultrasonic Corp., CT, USA) for 60 min at temperature of room. The sample was centrifuged at 4000 g for 10 min at 4°C.

Total polyphenol amount was evaluated by using the Folin-Ciocalteau's assay as reported by Singleton and Rossi [31]. In a falcon (15 ml), 625 µl methanolic extract, 625 µl of Folin-Ciocalteau's phenol reagent and 2.5 ml dd H_2O were added and shaken. After 6 min, 6.25 ml of 7% Na_2CO_3 solution were added to the mixture. The solution was diluted with 5 ml dd H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 760 nm by spectrophotometer. All biological replicates of samples were analyzed in triplicate. Total phenolic content of tomato fruits was expressed as mg gallic equivalents (GAE)/kg FW.

Saponins

The saponin quantification was carried out in accordance with Helaly method [32] with slight modification. An aliquot of 6 g of sample was extracted with MeOH 80% and the resulting solution was filtered through a 0.45 µm membrane and dried under vacuum. The residue was redissolved in 0.5 mL MeOH 80%. The following solutions were added to the last solution: 0.5 mL of 8% vanillin in ethanol and 5 ml of 72% H_2SO_4 in water. The mixing of the reagents was carried out in a thermostat ice bath at 0°C. The mixture was then set in a thermostat at 60°C for 20 min and at 0°C for 5 min and then measured at a wavelength of 544 nm. A calibration curve was constructed using a standard saponin (Soyasaponin I, Sigma-Aldrich) which was also treated in a similar manner. The standard saponin curve was linear over a concentration range of 0.012–0.36 mg/mL.

Anthocyanins

The total monomeric anthocyanin content of the samples was evaluated applying a pH-differential method [33]. Samples (5 g) were placed in a 100 ml methanol-HCl 0.75% (w/w) solution at room temperature. The extraction was monitored for 24 h. An aliquot of 1 mL of extract and of calibration solutions of malvin (Sigma-Aldrich) (0.1-10 mg/100 mL) have been added to two vials containing 10 mL of acetate buffer (pH 3.6) and HCl 1N, respectively. The difference between the absorbances read at 530 nm has been calculated. Total anthocyanin content was expressed as mg malvin equivalents (ME)/100 g.

Antioxidant Activity

The antioxidant activity was evaluated in both lipophilic and hydrophilic fractions obtained by extraction with chloroform 100% and methanol respectively. Both extracts were tested by the ferric reducing/antioxidant power (FRAP) method as described by Tenore *et al.*[34] and by ABTS test [35].

The percentages of the variations of quantitative parameters between mycorrhizal samples and controls were calculated by using the following formula: % increase and/or decrease = (value in mycorrhized sample-value in unmycorrhized sample)/ value in unmycorrhized sample * 100.

Total antioxidant activity (TAA) was calculated by adding lipophilic antioxidant activity (LAA) to hydrophilic antioxidant activity (HAA) in both adopted tests.

Nitrates

A portion of 100 g of edible part of vegetable was homogenized by a mixer (BUCHI B-400, BUCHI Italia s.r.l., Assago, Milan, Italy); homogenized sample was extracted with 200 mL ultrapure water and placed at 70°C for 5 min. So the mixture was filtered through Whatman No. 41, 150 mm filters (Whatman, Springfield Mill, UK) and then 3 mL of the filtrate was purified using ISOLUTE® Alumina Neutral Cartridges (Biotage AB, Uppsala, Sweden) previously activated by 3 mL ultrapure water. The purified extract was filtered through Anotop 10 LC, 0.2 µm, 10 mm filters (Whatman, Springfield Mill, UK) prior to chromatographic analysis. All the chromatographic determinations were performed on a Dionex system (Dionex Corporation, Sunnyvale, CA, USA) composed of a GP50 quaternary gradient pump, an electrochemical detector set to conductivity mode equipped with a temperature-compensated conductivity cell (model ED40) and a Rheodyne injection valve (model RH9125, Cotati, CA, USA) with a 25 µL injection loop. The mobile phase consisted of 9 mmol/L Na_2CO_3 and was set in isocratic mode at a flow rate of 1.0 mL/min. Total run time was 20 min.

Statistical Analysis

All data were analysed with respect to the variance using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between experimental and control groups was determined by the Student's *t* test. Differences were declared significant at $p<0.05$.

RESULTS AND DISCUSSION

Effectiveness of Inoculation

Fig. (**1**) shows the effectiveness of inoculation in analyzed crops.

At the end of the experiment root colonization reached values comprised between 50 %±2 (in squash) and 82 %±3 (in Sicilian tomatoes) and several arbuscules could be observed. Larose *et al.* [22] studied the accumulation of fla-

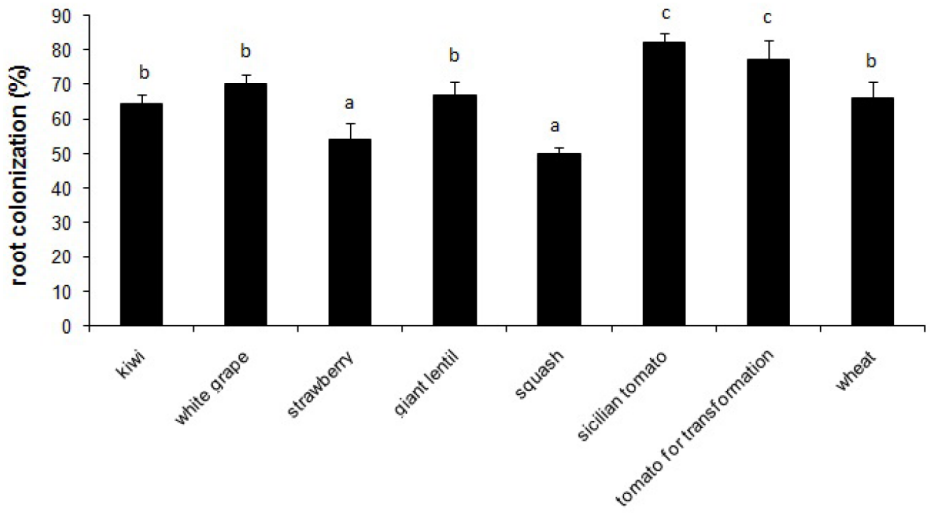

Fig. (1). Root colonization by MICOSAT F® in analyzed crops. Data are means ± standard deviations of three replicates from three individual plants. Values with different letters are significantly different ($p<0.05$).

vonoids in roots of *Medicago sativa* colonized by 3 species of *Glomus*. They found a maximum value of root colonization equal to 78%±3.

Total Carotenoids

Table **1** shows the levels of health-promoting phytochemicals in analyzed fresh fruits, vegetables and cereals.

Relatively to the fruits, mycorrhization induced a significant ($p<0.05$) mean increase of total carotenoids from 1.0±0.1 to 2.8±0.3 mg/kg in kiwi, while in non mycorrhizal squash the mean value of total carotenoids was 58.1±3.1 mg/kg FW with a mean increase of 55% after the treatment. In tomatoes from Sicily and in those intended from industrial transformation, the amount was 55.4±4.5 mg/kg FW and 60.7±5.9 mg/kg FW respectively with a relative mean increase of 24.90% and 35.09% after the treatment. In particular, mean lycopene amount after the inoculation exceeded of 19.88% and 25.17% the controls in tomatoes from Sicily and in tomato destined to transformation respectively. Giant lentils did not reveal detectable levels of carotenoids neither in untreated nor in treated legumes, while the mean level of carotenoids in non mycorrhizal durum wheat var. grecale was equal to 1.4±0.1 mg/kg with an increase of 42.86% after the treatment.

In general, it is known that AMF can stimulate the metabolism in plant roots, since AM symbiosis activates the plastidial methylerythtitole (MEP) pathway related to the increasing of carotenoids [36]. Our results are in concordance with those reported by other authors. Ordookhani and Zare [37] investigated the effects of inoculating tomato roots with plant growth-promoting rhizabacteria and AMF on lycopene and observed an increase of mycorrhizal tomatoes until 40% respect to the control. Analogously, inoculation with a microbial mix of AMF and different bacteria in tomato plants determined higher contents of lycopene, β-carotene, and lutein when the substrate contained microbial mix and green compost [38]. Giovannetti *et al.* [19] found an increase of lycopene content in tomato from a mean value of 53.2 mg/kg to 63.06 mg/kg after the inoculation. On contrary, Di Cesare *et al.* [39] reported a negative effect of mycorrhyzation on lycopene amount in tomatoes. This different pattern could depend on different composition of mycorrhyzae used in the considered studies. Also environmental parameters and cultural practices play a significant role in the effect of mycorrhization. For example an increase of total carotenoids in mycorrhizal lettuces was observed when plants were subjected to different degrees of water deficit [40].

Phenolic Compounds

Phenolic compounds showed a decrease of 65.4% in kiwi after the treatment from a mean value of 2812.1 mg/kg, while a reduction of 42.5% was observed in white grape from the mean level of 124.1±9.1 mg/kg FW in untreated plant. However, different AMF strains vary in their efficacy to increase the synthesis of different biochemicals. In fact, contrary to our results, Krishna *et al.* [41] found an increase of phenolic compounds in micropropagated grape (*Vitis vinifera L.)* plantlets.

Interestingly, phenolics increased significantly in strawberries by a value of 64.67% after the mycorrhization, from an initial content of 1123.7±93.1 mg/kg FW, but considering only anthocyanin content, they did not show a significant change after the treatment (105.1±9.2 mg/kg before treatment and 95.5±7.5 mg/kg in treated plant). Our values are in according with levels reported by Tulipani *et al.* [42]. They found levels of phenolic in strawberries comprised between 1730 and 3130 mg GAE/kg FW.

The effect of AMF colonization on the amount of phenols in strawberries was evaluated for the first time by Castellanos-Morales *et al.* [43], who showed that symbiosis induces an increase in some phenolics. Lingua *et al.* [44] demonstrated that the use of a consortium of AM fungi in combination with selected *Pseudomonas* strains in conditions of reduced fertilization induced an increasing of antho-

Table 1. Levels of analyzed compounds in non mycorrhizal (NM) and mycorrhizal (M) crops.

Category	Type	Compounds							
Fruit		**Lycopene (mg/kg)**	**β carotene (mg/kg)**	**Total carotenoids (mg/kg)**	**Phenols (mg/kg)**	**Antocyanin (mg/kg)**	**AsA (mg/kg)**	**Saponins (g/kg)**	**Proteins (%)**
Kiwi	NM			1.0±0.1[a]	2812.1±10.4[b]		788.0±7.3[a]		
	M			2.8±0.3[b]	971.2±8.2[a]		1120.2±10.7[b]		
White grape	NM				124.1±9.1[b]		35.2±4.3[a]		
	M				71.3±2.1[a]		55.3±3.9[b]		
Strawberry	NM				1123.7±93.1[a]	105.1±9.2[a]	590.2±10.7[a]		
	M				1850.4±97.5[b]	95.5±7.5[a]	620.1±11.4[b]		
Vegetables/ legumes									
Giant lentil	NM			n.d	1824.1±12.1[a]		48.8±2.3[a]	15.4±1.6[a]	24.9±3.2[a]
	M			n.d	2413.3±10.1[b]		77.5±2.8[b]	17.6±1.5[a]	25.7±2.9[a]
Squash	NM		45.2±2.4[a]	58.1±3.1[a]	42.0±3.4[a]		95.1±2.1[a]		
	M		71.4±2.7[b]	90.1±3.8[b]	66.0±5.3[b]		145.7±3.4[b]		
Sicilian tomato	NM	40.7±3.8[a]	14.7±1.6[a]	55.4±4.5[a]	393.0±3.2[a]	n.d	141.8±4.3[a]		
	M	50.8±4.5[b]	18.4±1.7[a]	69.2±5.3[b]	347.1±4.2[a]	n.d	218.7±4.0[b]		
Tomato for transformation	NM	49.0±4.2[a]	11.7±2.2[a]	60.7±5.9[a]	407.2±3.1[a]	n.d	132.1±3.9[a]		
	M	65.3±5.5[b]	16.7±1.8[a]	82.0±9.3[b]	425.3±2.9[a]	n.d	203.5±4.1[b]		
Cereal									
Wheat	NM			1.4±0.1[a]	2600±150.2[a]				18.9±2.2[a]
	M			2.0±0.4[a]	8600±367.2[b]				14.5±1.6[b]

Values are means ± SD (n=3). Within each product values with different letters are significantly different (p<0.05).

cyanins in strawberry fruits. In non mycorrhizal giant lentils, phenols showed the mean value of 1824.1 mg/kg with an increase of 32.30% after the treatment, whilst in squashes the levels increased significantly of 36.36% from a mean initial value of 42.0±3.4 mg/kg. In both Sicilian tomatoes and in tomato intending for transformation, the amount of total phenols did not change significantly after the treatment from the initial values of 393.0± mg/kg and 407.2 mg/kg respectively, while antocyanins were not detectable. In durum wheat var. grecale, phenols increased of 230.76% from a mean value of 2600±150.2 mg/kg in untreated cereal. The impact of inoculation on content of phenolics in vegetables was previously reported by Ceccarelli *et al.* [45] that studied the effect of AM fungal species *Glomus intraradices*, either alone or in mixture with *Glomus mosseae* in artichoke. They found significant differences of total phenolics in all edible parts of inoculated plants from control, with the highest value in plant inoculated with Glomus mix, with an increase of 50.0%. Kim *et al.* [46] reported values of phenols in some typologies of commercial wheat bran comprised between 336 and 396 mg GAE/100 g. Moore *et al.* [47] examined eight selected Maryland-grown soft wheat varieties for their presence of phenols and other phytochemicals. They found a level of total phenolics ranging between 400 and 800 mg gallic acid equivalents (GAE)/100 g. Therefore our results demonstrated that mycorrhization can significantly improve phenols content in wheat respect to the mean common values of commercial varieties. In general, the change in levels of phenolic compounds observed in plants colonized by AM fungi is induced since the primary contact between AMF and plant roots [48]. Volpin *et al.* [49] found a suppression of isoflavonoid phytoalexins induced in alfalfa roots, when the mycorrhization was established.

Ascorbic Acid

Mean AsA level in kiwi was equal to 788.0±7.3 mg/kg before mycorrhization with a significant increase of 42.13% after the treatment. Interestingly these levels are higher than those reported by other studies in kiwi. Szeto *et al.* [50] found a content of ascorbic acid of 520 mg/kg, while Frenich *et al* [51] reported a level of 307 mg/kg. In white grape the

average amount of AsA before the treatment was 35.2±4.3 mg/kg with a mean increase of 57.10%, while in strawberries we observed an increase of 5.0% from an initial value of 590.2±10.7 mg/kg. Among vegetables, we found a significant increase in giant lentils, in squashes, in Sicilian tomatoes and in transformed tomatoes of 58.81%, 53.20%, 54,23%, 35.08% respectively.

The effect of mycorrhizal symbiosis on AsA content in vegetables was studied by Baslam *et al.* [52] that found an enhanced amount of total ascorbate in greenhouse-cultivated lettuce. In giant lentils, total proteins increased, but not significantly, after mycorrhization, from 24.9% to 25.7% after the inoculation, while in wheat a decrease from a mean initial value of 18.9% to 15.5% was observed.

Proteins and Saponins

The proteins are investigated in mycorrhizal crops since in symbiotic interactions between host plants and microbes are involved lectins binding proteins that exert a role of defense against predators [53]. Latef [54] found an increased content of total proteins in mycorrhizal pepper leaves due to the alteration in gene expression known to occur in mycorrhizal plants. Martins *et al.* [55] reported that mycorrhization can enhance the level of proteins in leaves *of Castanea sativa plants from* 10.0 to 15.6 mg/g. An analogous behaviour was observed in the content of saponins in giant lentils, with an amount of 15.4±1.6 g/kg and 17.6±1.5 g/kg in non mycorrhizal and mycorrhizal plants respectively. Saponins occur in legumes as defense system. Their amount in plants is related to many environmental factors such as biotic stimuli like infection, or involved in mutualistic symbioses with mycorrhizal fungi and rhizobial bacteria [56]. In addition, saponins can exert signalling role in legume-rhizobia colonization [57]. *Medicago truncatula* is normally used as a model legume for processes of symbiosis, since it establishes symbioses with nitrogen fixing arbuscular mycorrhizal fungi Glomus spp and roots of this plant contain triterpene saponins. Schliemann *et al.* [58] found that mycorrhization of the roots of this legume resulted in reduction of saponin malonylation.

Antioxidant Activity

Table **2** shows the antioxidant power in analyzed products before and after the inoculation, evaluated by FRAP and ABTS tests. Regarding the kiwi, a significant mean decrease

Table 2. Antioxidant activity (µmol TE/kg FW) in non mycorrhizal and mycorrhizal products. evaluated by FRAP and ABTS tests. Data are the mean values ± SD (n=3). Within each product values with different letters are significantly different (p<0.05).

Sample		FRAP	ABTS
Fruit			
Kiwi	NM	2707300 ± 8000[a]	1123.6 ± 11.2[a]
	M	1846000± 4970[b]	2105.2 ± 12.3[b]
White grape	NM	1222300± 9780[a]	844.6 ± 8.2[a]
	M	980100± 6830[b]	1301.7 ± 9.5[b]
Strawberry	NM	880± 65.4[a]	1421.5 ± 10.4[a]
	M	1210± 79.5[b]	1987.0 ± 12.1[b]
Vegetables/legumes			
Giant lentils	NM	133.28 ± 8.20[a]	1893.0 ± 10.2[a]
	M	172.17 ± 18.20[a]	2489.6 ± 11.9[b]
Squash	NM	1120 ± 104.3[a]	1848.3 ± 9.2[a]
	M	80000 ± 4720[b]	3651.3 ± 13.5[b]
Sicilian tomato	NM	1140 ± 95.6[a]	2170.1 ± 9.9[a]
	M	1210 ± 103.4[a]	2824.8 ± 10.0[b]
Tomato for transformation	NM	1290 ± 78.8[a]	2271.4 ± 11.6[a]
	M	1260 ± 97.9[a]	2988.7 ± 12.4[b]
Cereal			
Wheat	NM	1100 ± 83.7[a]	2981.0 ± 12.0[a]
	M	1800 ± 74.5[b]	3687.4 ± 13.1[b]

of antioxidant activity evaluated by FRAP test was found, equal to 31.81%, from an initial level of 2707300±8000 µmol TE/kg FW in non treated sample, while from ABTS test we observed a mean increase of 46.62% from the value of 1123.6 µmol TE/kg FW.

A similar effect was evidenced in grape, with FRAP reduction of 19.81% from an initial value of 1222300±9780 µmol TE/kg, while ABTS value increased of 54.12% from the mean value of 844.6±8.2 µmol TE/kg. These opposite trend observed for FRAP and ABTS tests can be due to the significant decrease of phenolics together with a significant increase of AsA.

FRAP in strawberries showed a significant increase of 37.5% from a level of 880±65.4 µmol TE/Kg in untreated fruit, while ABTS test revealed an increase of 39.78% from the initial value of 1421.5±10.4 µmol TE/kg FW.

Surprisingly, the trend of total antioxidant activity after the mycorrhization in kiwi determined by ABTS test was opposite to that reported for phenols, so we can speculate that AsA and total carotenoids contribute significantly to its antioxidant power. On the other hand, in grape and strawberry, the changes of antioxidant power determined by ABTS test after the mycorrhization is congruent to that observed for total phenols.

Untreated giant lentils showed a level of FRAP of 133.3±8.2 µmol TE/kg FW and an increase of 29.17% after the treatment, while ABTS value showed an increase of 31.51% from the initial value of 1893.0±10.2 µmol TE/kg FW. These results are in concordance with the trend observed in saponins.

In durum wheat, the treated crops showed a mean antioxidant activity evaluated by FRAP equal to 1800.0±102.7 µmol TE/kg, exceeding significantly (63.63%) the calculated level in untreated samples, while the values evaluated by ABTS test after the treatment exceeded of 23.69% the level found in untreated product

Interestingly, squashes showed an increased of FRAP value from 1120.0±104.3 to 80000±4720 µmol TE/kg, while ABTS test revealed an increase of 97.53%.

Moore *et al.* [59] found in eight tested wheat grain samples a scavenging capacity of 14300-17600 µmol TE/kg. Ceccarelli *et al.* [45] reported an increase of antioxidant activity by 30% in artichoke leaves inoculated with *Glomus* species respect to control plants.

Sicilian tomatoes showed a FRAP level of 1140.0±95.6 µmol TE/kg FW and did not reveal a significant increase (6%) of antioxidant activity, while an increase of 30.17% was found by ABTS.

In tomato intended for transformation, a not significant decrease was detected by FRAP from 1290.0±78.8 µmol TE/kg FW to 1260±97.9 µmol TE/kg FW in inoculated plant, while an increase of 31.57% was found by ABTS from the value of 2271.4±11.6 µmol TE/kg FW.

These values significantly exceeded those reported by Cano *et al.* [60] that found a total level of 524.0 µmol TE/100 g FW in tomatoes cultivated in a greenhouse using standard horticultural practices in SE Spain. Latef and Chaoxing [61] reported that AMF colonization in tomato plants was accompanied by an enhancement of activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX). Analogously, an increased antioxidant enzyme activity was found in mycorrhizal pepper [54].

Nitrates

Table **3** shows the content of nitrates in some analyzed categories. In strawberries the treatment reduced significantly the amount of nitrates (39.78%) from an initial value of 606.8±70.4 mg/kg. In tomatoes intended for transformation this level was reduced of 37.79% from a mean value in not inoculated fruit equal to 488.7±51.1 mg/kg. Sicilian tomatoes showed a not significant increase (3%) in the content of NO_3^- from the mean initial value of 606.8±40.4 mg/kg. On the opposite, giant lentils, showing a mean value in non mycorrhizal legume equal to 13246.1±759.1 mg/kg, revealed a significant increase of nitrates equal to 19.24% compared with untreated crop. Bago *et al.* [20] reported that the extraradical hyphae of *G. intraradices* strongly increased the pH of nutrient-free medium and a depletion of nitrate in the media accompanied this PH variation. Baslam *et al.* [52] found that mycorrhization increased the levels of NO_3^- in the outer leaves of fertilized greenhouse-grown lettuce.

Table 3. Levels of nitrates in non mycorrhizal (NM) and mycorrhizal (M) crops. Data are the mean values ± SD (n=3). Within each product values with different letters are significantly different (p<0.05).

Sample	Type	mg NO_3^-/kg FW
Strawberry	NM	606.8 ± 70.4[a]
	M	365.4 ± 59.9[b]
Giant lentil	NM	13246.1 ± 759.1[a]
	M	15839.6 ± 451.0[b]
Sicilian tomato	NM	606.8 ± 40.4[a]
	M	629.4 ± 75.7[a]
Tomato for transformation	NM	488.7 ± 51.1[a]
	M	304.6 ± 28.3[b]

Nevertheless the levels of nitrates detected in our study were significantly lower than limits established by Reg CE 1881/2006 [21] with the exception of giant lentils. A more complete legislation including limits for most commonly consumed vegetables is auspicable.

CONCLUSION

AMF symbiosis is an efficient strategy to improve nutritional value of crops. In this study we evaluated the potential of MICOSAT F®. The application of this new commercial product enhanced the levels of several secondary metabolites

analyzed in foodstuffs. However, the amount of phytochemicals and respective by-products were reduced in some cases. So these different patterns are needed further investigations in order to optimize the treatment conditions in each examined plant.

LIST OF ABBREVIATIONS

AMF	=	Arbuscular Mycorrhizal Fungi
APX	=	Ascorbate peroxidase
AsA	=	Ascorbic acid
BHT	=	Butylated hydroxytoluene
CAT	=	Catalase
CNCD	=	Chronic non-communicable diseases
CVD	=	Cardiovascular diseases
DIF	=	2,6-diclorophenol-indophenol
FRAP	=	Ferric reducing/antioxidant power
FW	=	Fresh weight
GAE	=	Gallic acid equivalent
HAA	=	Hydrophilic antioxidant activity
LAA	=	Lipophilic antioxidant activity
LDL	=	Low-density lipoprotein
MEP	=	Plastidial methylerythtitole
POD	=	Peroxidase
PAL	=	Phenylalanine ammonia lyase
ROS	=	Reactive oxygen species
SOD	=	Superoxide dismutase
TAA	=	Total antioxidant activity
TE	=	Trolox equivalent
TPTZ	=	2,4,6-tripyridyl-s-triazine

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank doctor Daniela Giannetti and doctor Emanuela D'Urso for their valuable technical assistance.

SUPPLEMENTARY MATERIALS

Supplementary material is available on the publisher's web site along with the published article.

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Received: January 12, 2015 **Revised: April 10, 2015** **Accepted: April 13, 2015**