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Effects of Biostimulant Application on Quali-Quantitative Characteristics of Cauliflower, Pepper and Fennel Crops under Organic and Conventional Fertilization

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***Abstract*—**Nowadays, the main goal for modern horticultural production is an increase the quality. In recent years, the use of organic fertilizers or biostimulants that can be applied in agriculture to improve quali-quantitative crop yields has encountered increasing interest. Biostimulants are gaining importance also for their possible use in organic and sustainable agriculture, to avoid excessive fertilizer applications. Consecutive experimental trials were carried out in the Apulia region (southern Italy) on three herbaceous crops (cauliflower, pepper, fennel) grown in pots under conventional and organic fertilization systems without and with biostimulants. The aim was to determine the effects of three biostimulants (Siapton®10L, Micotech L, Lysodin Alga-Fert) on quali-quantitative yield characteristics. At harvest, the quali-quantitative yield characteristics of each crop were determined. All of the experimental data were subjected to analysis of variance (ANOVA), and when significant effects were detected, the means were compared using Tukey's tests. These data show large differences in these yield characteristics between conventional and organic crops, particularly highlighting higher yields for the conventional crops, while variable results were generally observed when the biostimulants were applied. In this context, there were no effects of the biostimulants on the quantitative yield, whereas there were low positive effects on the qualitative characteristics, as related to higher dry matter content of cauliflower, and higher soluble solids content of pepper. Moreover, there were evident positive effects of the biostimulants with fennel, due to the lower nitrate content. These latter data are in line with most of the published literature obtained for other herbaceous crops.

***Keywords*—**Biostimulants, cauliflower, pepper, fennel.

I. Introduction

IN Italy, due to the characteristics of the geographical position and the climatic conditions, the cultivation of a large number of vegetable species throughout the entire year is widely diffuse. In some regions, such as Apulia (southern Italy), the horticultural sector has a very large income and supports a considerable number of employees. The most important of the vegetable crops grown in this region include cauliflower, pepper and fennel.

Nowadays, the main goal for modern horticultural production is an increase in quality. Furthermore, in consideration of the new demands of consumers, who are always more attracted by a diet based on greater consumption of fruit and vegetables without the risk of pesticide residues and with increased nutritional value, new important features in addition to the traditional quality attributes are now requested. For the agronomic practices, new opportunities are offered by sustainable management of the production factors that can be used to improve the plant–environment interactions, as well as to address the reduction in input needed for production, and finally, to induce specific stress conditions that promote higher quality at reduced input.

In recent years, many efforts have been put into developing new fertilizers and fertilization systems, and also for organic farming with lower fertilizer inputs. This aims to increase the nutrient uptake, growth and development of the plants, and to improve the quality, productivity and environmental impact. For such purposes, biostimulant products have appeared on the market. These are products derived from vegetable extracts, or seaweed, fungi, bacteria or animal hydrolysates, which consist of oligosaccharides, vitamins, humic substances (e.g., mixtures of humic and fulvic acids), microelements, and protein hydrolysates [1]. The use of biostimulants to promote plant growth has been widely studied. The soluble organic molecules contained in the biostimulants appear to have direct effects on some metabolic processes of plants, due to their particular molecular structures [2]-[8]. Isolation of the substances that are found in biostimulants is not easy, and the result of their application might be due to synergistic effects of these different components.

In most cases, the mechanisms behind the physiological and biochemical effects of biostimulants are still unknown. Indeed, the heterogeneous nature of the raw materials and substances that are used for biostimulant production does not allow an understanding of their mechanisms of action, or to individuate with certainty the components, or the main component, that are responsible for the biostimulant activity [9].

Biostimulants are usually applied to plants by sprinkling a solution on the leaves or by fertilization in addition to standard fertilization treatments. These are not used to provide nutrition, but rather to encourage and stimulate the plant metabolism processes, to improve nutrient use efficiency, or product yield and quality, and to reduce the impact on the environment [10]-[13].

It has been reported that biostimulant molecules can induce yield increases through direct actions on the plant physiology

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and nutrition processes, even when applied in a purified form [14]-[17]. For example, humic acids derived from various composts can stimulate vegetative growth of chicory [18], increase root growth of pepper [19], and increase biomass of cucumber and tomato [20].

The application of biostimulants to leaf vegetables can increase the chlorophyll content, and consequently the color and visual appearance of edible leaves, thus enhancing the attractiveness for consumers [21]. Apart from the positive effects of biostimulants on plants, there have also been cases in which their application had no effects on the yields of tomato, wheat and lettuce, but produced higher mean weights and dry matter content of tomato fruit, as well as lower nitrate content and higher dry matter content in lettuce, than seen for the control [22].

The purpose of the present study was to investigate the effects of some biostimulant products that are available on the market, in terms of the qualitative and quantitative yield characteristics of cauliflower, pepper and fennel crops.

II. Materials and Methods

Three experiments were carried out over 3 years, from 2011 to 2013, at the Department of Agriculture, University of Foggia (southern Italy; 41°27'27"N; 15°31'56"E; 75 m a.s.l.). Cauliflower (cv. 'Casper'), pepper (cv. 'Akron') and fennel (cv. 'Tarquinia') were investigated, grown consecutively in cylinder pots (height, 29 cm; Ø, 35 cm), with the testing of three biostimulant products: Siapton® 10L, Micotech L, and Lysodon Alga-Fert. These biostimulants are available on the open market, and the use of all of them is also allowed in organic farming. The soil in the experimental pots was collected from a conventional farming field, and its principal characteristics are given in Table I. The characteristics of the biostimulants used are given in Table II.

For each crop, four treatments were compared: conventional fertilization, conventional fertilization plus biostimulant, organic fertilization, and organic fertilization plus biostimulant.

Different biostimulant formulae were applied to the crops considered. They were all applied to the plants by foliar application (foliare distribution or 'fertigation'), at different times, and at the label doses. The fertilizers and the biostimulants used for each treatment are reported in Table III.

The cauliflower, pepper and fennel crops were transplanted (one plant per pot) on September 28, 2011, May 31, 2012, and October 17, 2012, respectively. Each crop was harvested on March 19, 2012, August 7, 2012, and February 15, 2013, respectively. For each crop, the plots were arranged in a randomized block design, with five replicates for each of the four treatments.

The water irrigation times and volumes per pot were according to the soil water balance approach. Therefore, the gravimetric soil moisture was measured weekly by weighing each pot, to evaluate the soil water depletion. The amount of water supplied by the irrigation re-established the soil water content to field capacity.

The inorganic fertilizers used in the course of the trials were ammonium nitrate (26%-27% nitrogen), ammonium phosphate (20%-21% P_2O_5) and ammonium sulfate (47% K_2O). The organic fertilizers used were Prodigy 4 (7% organic nitrogen, 6% P_2O_5, 1% K_2O, 25% organic carbon, 45%-55% organic matter) and chicken manure (4% nitrogen, 4% P_2O_5, 4% K_2O, 41% organic carbon, 71% organic matter).

At harvest, the quali-quantitative yield parameters determined for each crop were predefined. For the cauliflower, these were total leaf surface; weight, diameter, compactness index and dry matter of corymbs, and nitrate content of corymbs, and stems. For the pepper, these were plant height and weight, and fruit yield and soluble solids content. For the fennel, these were plant height and weight, leaf number, total weight, and dry matter, and nitrate content of the marketable yield.

The data collected in each experimental trial were analysed statistically using analysis of variance (ANOVA), and when significant differences were detected, the mean values were compared using Tukey's tests.

TABLE I
CHARACTERISTICS OF SOIL

Sand [2.0> Ø < 0.02 mm]	36.1%
Loam [0.02 > Ø < 0.002 mm]	39.2%
Clay [Ø < 0.002 mm]	24.7%
Typology (USDA)	Medium texured
Organic matter (Walkley-Black)	1.30%
pH (in H_2O)	8.14
Electrical conductivity (water-satured extract; ECe)	1.19 d S cm^{-1}
Sodio adsorption rate (SAR)	3.51
Total nitrogen (Kjeldhal)	0.81‰
Available P_2O_5 (Olsen)	85.6 mg kg^{-1}
Exchangeable K_2O (Shollemberger)	1430 mg kg^{-1}

TABLE II
CHARACTERISTICS OF USED BIOSTIMULANTS

Product	Particulars	Chemical composition
Siapton® 10L	Amino acids and peptides obtained by chemical hydrolysis from animal epithelium. Raw materials: connective tissue, fleshings, shavings	Water-soluble organic nitrogen (N) 8.7% Organic carbon (C) 25% C/N ratio 2.9
Micotech L	Nitrogen organic fertilizer inoculated with micorrhizal fungi	Micorrhizal fungi 10% Bacteria of rizosphere 2×10^4 CFU/g Trichomycetes 1×10 CFU/g
Lysodin Alga-Fert	Alginate liquid fertilizer made of extracts of seaweed with ammonium and nitrogen	Organic nitrogen (N) 7% Organic carbon (C) 21% Extract of *Ascophyllus nodosum* 5%

TABLE III
FERTILIZERS AND BIOSTIMULANTS USED IN CONVENTIONAL AND ORGANIC TREATMENTS

Treatment	At transplant					During the crop cycle				
	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Chicken manure (kg ha^{-1})	Prodigy 4 (kg ha^{-1})	N (kg ha^{-1})	Siapon (ml hl^{-1})	Micotech L (kg ha^{-1})	Lysodin (kg ha^{-1})	Alga-Fert (g hl^{-1})
Cauliflower (cv. 'Casper')										
Conventional	60	110	110	-	-	70	-	-	-	-
Conventional + Biostimulant	60	110	110	-	-	70	6× 200a	6× 6b	-	-
Organic	-	-	-	1500	800	-	-	-	-	-
Organic + Biostimulant	-	-	-	1500	-	-	6× 250a	6× 6b	-	-
Pepper (cv. 'Akron')										
Conventional	50	150	200	-	-	150	-	-	-	-
Conventional + Biostimulant	50	100	200	-	-	150	-	-	6× 4b	-
Organic	-	-	-	1000	--	-	-	-	-	-
Organic + Biostimulant	-	-	-	1000	-	-	-	-	6× 4a	-
Fennel (cv. 'Tarquinia')										
Conventional	42	65	145	-	-	83	-	-	-	-
Conventional + Biostimulant	42	65	145	-	-	83	-	-	-	7× 250a
Organic	-	-	-	-	800	-	-	-	-	-
Organic + Biostimulant	-	-	-	-	800	-	-	-	-	7× 250a

a, by foliar distribution
b, by fertigation

III. RESULTS AND DISCUSSION

The yield characteristics of the cauliflower, pepper and fennel crops under the conventional and organic fertilization systems without and with biostimulants are reported in Tables IV, V and VI respectively.

For the cauliflower crops, there were several effects seen (Table IV). The total leaf area of the plants was significantly changed only according to the two fertilization systems, as significantly greater under the conventional system than under the organic system, both without and with biostimulants. Although not reaching significance, within each cultivation system, the total leaf area tended to be greater with the biostimulants than without. For the mean weights of the marketable corymbs, these were significantly greater under the conventional fertilization system, both without and with biostimulants, than under the organic system. There were significant increases with the biostimulants for the mean corymb diameters under the conventional treatment and for the mean compactness index under the organic system. The dry matter contents of the inflorescences and stems were generally significantly greater under the organic system than the conventional, regardless of biostimulants. In contrast, the nitrate content of corymb heads and stems were significantly greater under the conventional system than the organic, but again regardless of biostimulants.

With respect to the pepper crops (Table V), the heights of the plants showed no significant differences. The marketable yields and numbers of fruit were generally significantly greater under the conventional system than the organic, with no effects of the biostimulants. However, the fruit weights and soluble solid contents were generally significantly increased by the biostimulants under both fertilization systems. These biostimulants effects are considered positive for the qualitative characteristics of the pepper fruit.

For the fennel crops (Table VI), all of the plant and yield quali-quantitative–related parameters were significantly greater under the conventional fertilization system than the organic system, without and with biostimulants in both cases. Moreover, it is worth noting that although the biostimulant had no significant effects on the nitrate content of the fennel bulbs, its addition did show a tendency for lower nitrates under both fertilization systems. This is particularly positive, as nitrates and nitrites accumulation in plant tissues can constitute a danger for human health, and can lead to different health disturbances. Indeed, an epidemiological study has shown a positive correlation between the intake of nitrates and nitrites and gastric cancer in humans. This lower nitrate content in the fennel with the biostimulant is in agreement with a previous study that highlighted that the use of a biostimulant that contained an extract derived from a brown alga (seaweed) reduced the nitrate content in the leaves of lettuce and rocket [23]. The biostimulant Lysodin Alga-Fert appears to affect nitrogen metabolism by speeding up the incorporation of nitrate in the plant, through the activation of the related enzymes [24].

TABLE IV
PLANT AND MARKETABLE YIELD CHARACTERISTICS OF CAULIFLOWER (CV. 'CASPER')

Treatment	Total plant leaf area (cm^2)	Corymb						
		Weight (g)	Diameter (cm)	Compactness index[a]	Head dry matter (%)	Stem dry matter (%)	Head nitrate content (mg/kg dm)	Stem nitrate content (mg/kg fm)
Conventional	3,787 a	601.0 a	13.0 b	1.0 b	9.1 c	9.3 b	4587 a	5875 a
Conventional + Biostimulants	4,369 a	573.2 a	17.8 a	1.8 ab	9.7 bc	8.9 b	4681 a	6231 a
Organic	1,423 b	100.2 b	9.7 c	2.0 ab	12.3 a	10.9 c	3800 b	4862 b
Organic + Biostimulants	1,749 b	100.4 b	9.8 c	2.8 a	11.5 ab	10.8 c	4037 b	5025

[a], Rating scale: 1 (compact), 2 (average compact), 3 (non-compact).
Different letters within the columns indicate significant differences (P <0.05; Tukey's tests)

TABLE V
PLANT AND YIELD CHARACTERISTICS OF PEPPER (CV. 'AKRON')

Treatment	Plant height (cm)	Fruit			
		Yield (g)	**Number** (n)	**Weight** (g)	**Soluble solids** (°Brix)
Conventional	38	1432 a	11.7 a	122.4 b	7.0 b
Conventional + Biostimulants	36	1064 a	9.8 a	146.3 a	7.3 b
Organic	38	903 b	6.6 ab	136.8 b	6.9 b
Organic + Biosimulants	32	836 b	6.4 b	154.8 a	7.2 a

Different letters within the columns indicate significant differences (P <0.05; Tukey's tests)

TABLE VI
PLANT AND YIELD CHARACTERISTICS OF FENNEL (CV. 'TARQUINIA')

Treatment	Plant		Leaves		Marketable yield		
	Height (cm)	Weight (g)	Number (n)	Total weight (g)	Weight (g)	Dry matter (%)	Nitrate content (mg/kg f.m.)
Conventional	71.5 a	799.7 a	5.9 a	436.8 a	359.0 a	15.2 a	2676 a
Conventional + Biostimulants	73.8 a	830.4 a	5.6 ab	444.4 a	382.3 a	14.6 a	2227 ab
Organic	48.1 b	457.3 b	4.9 b	251.5 b	207.1 b	12.0 b	1259 b
Organic + Biosimulants	53.4 b	495.3 b	4.9 b	266.1 b	226.3 b	11.7 b	685 bc

Different letters within the columns indicate significant differences (P <0.05; Tukey's tests)

IV. CONCLUSIONS

The data from these three agronomic trials carried out for cauliflower (cv. 'Casper'), pepper (cv. 'Akron') and fennel (cv. 'Tarquinia') under conventional and organic fertilization systems and without and with these biostimulants were generally quite variable. However, the yields for all of these crops were greater under the conventional systems than the organic systems (both without and with biostimulants).

The yield increases in the conventional systems was due to the mineral fertilizer applications. Instead, organic systems often rely on organic matter and are based on nutrient (nitrogen, phosphorous) availability that derives from organic matter mineralization. The macronutrient availability patterns under organic farming therefore differ significantly during the growing period from those of conventional systems. Organic crops are often subjected to limited nitrogen and phosphorous availability, especially during periods when the soil temperatures and the water availability reduce the mineralization capacity of the soil biota [25]. In terms of the crop yields, only slight, although sometimes significant, benefits were seen for the biostimulants.

For the qualitative characteristics of the crop products, the plants under the organic fertilization system (without and with biostimulants) showed higher percentages of dry matter (cauliflower corymbs) and lower concentrations of nitrates (pepper fruit, fennel bulbs) than the plants under the conventional system.

In conclusion, as demonstrated by other studies carried out with different herbaceous crops, the present study indicates that while the use of these biostimulants show some benefits on some of the productive parameters, in general, these subtle differences are difficult to evaluate [22].

On the whole, the present study confirms that these biostimulants can be effectively applied in such primary production activities. Undoubtedly, such research on the supply of biostimulants to crops is relatively complex, because of the numerous variables involved. Therefore, further studies are needed to determine optimal doses and frequencies of application of such biostimulants on various crops and cultivars, and also how these might be affected in terms of climate and soil conditions.

REFERENCES

[1] L. Cavani, C. Ciavatta, 2007. Attività biostimolante negli idrolizzanti proteici. *L'Informatore Agrario*, 44, 46-52.

[2] M. Ayuso, T. Hernandez, C. Carcia, J.A. Pascual, 1996. Stimulation of barly growth and nutrient absorption by humic substances originating from various organic materials. *Bioresource Technology*, 57, 251-257.

[3] S. Diver, L. Greer, 2001. Sustainable small-scale nursery production. Horticulturae System Guide. ATTRA, http://www.attra.org/attra-pub/PDF/nursery.pdf

[4] P. Morard, B. Eyheraguibel, M. Morard, J. Silvestre, 2011. Direct effects of humic-like substance on growth, water and mineral nutrition of various species. J. *Plant Nutr.*, 34, 46-59.

[5] S. Nardi, D. Pizzeghello, A. Muscolo, A. Vianello, 2002. Physiological effects of humic substances on higher plants. *Soil Biology & Biochemistry* 34, 1527-1536.
[6] C. Ciavatta, 2006. Biostimolanti: fertilizzanti a tutti gli effetti. *L'Informatore Agrario*, 43, 37-39.
[7] B. Eyheraguibel, J. Silvestre, P. Morard, 2008. Effects of humic substances derived from organic waste enhancement on the growth and mineral of maize. *Bioresource Technology*, 99, 4206-4212.
[8] H. Khaled, H.A. Fawy, 2011. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil & Water Res*., 6, 21-29.
[9] G.P. Berlyn, R.O. Russo, 1990. The use of organic biostimulants to promote root growth. *Belowground Ecol.*, 2, 12-13.
[10] J.R. Heckman, 1994. Effect of an organic biostimulant on cabbage yield. J. Home and Consumer Hort., 1(1), 111-113.
[11] S.K Chen, C.A. Edwards, S. Subler, 2003. The influence of two agricultural biostimulants, microbial activity and plant growth in soil microcosms. *Soil Biology and Biochemistry*, 35, 9-19.
[12] S. Tagliavini, C. Kubiokin, 2006. Effetti della biostimolazione in Ortofrutticoltura: alcune esperienze a confronto. *Fertilitas Agrorum*, 1, 23-28.
[13] J. Parrado, J. Bautista, E.J. Romero, A.M. Garcia-Martinez, V. Friaza, M. Tejada, 2008. Production of a carob enzymatic extract: potential use as a biofertilizer. *Bioresour. Technol*., 99, 2312-2318.
[14] J.S. Virgine Tenshia, P. Singaram, 2005. Influence of humic acid application on yield, nutrient availability and uptake in tomato. The Madras Agr. J., 92, 670-676.
[15] T.K. Hartz, T.G. Bottoms, 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. Hort. Science 45, 906-910.
[16] E.M. Selim, A.A. Mosa, A.M. El Ghamry, 2009. Evaluation of humic substances fertigation through surface and subsurface drip irrigation systems on potato grown under Egyptian sandy soil conditions. Agr. *Water Management*, 96, 1218-1222.
[17] V. Mora, E. Bacaicoa, A.M. Zammareno, E. Aguirre, M. Garnica, M. Fuentes, J Garcia-Mina, 2010. Action of humic acid on promotion of cucumber shoot growth involves nitrate related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. *J. Plant Physiol.*, 167, 633-642.
[18] M.M. Valdrighi, A. Pòera, M. Agnolucci, S. Frassinetti, D. Lunardi, G. Vallini, 1996. Effects of composts derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*) soil system: a comparative study. *Agr. Ecosys. Env*., 58, 133-144.
[19] N.Q. Arancon, S. Lee, C.A. Edwards, R. Atiyeh, 2003. Effects of humic acids derived from cattle, food and paper-waste-vermicomposts on growth of greenhouse plants. P*edobiologia*, 47, 741-744.
[20] R.M Atiyeh, S. Lee, C.A. Edwards, N.Q Arancon, J.D. Metzger, 2002. The influence of humic acids derived from earhworm processed organic wastes on plant growth. *Bioresource Technology*, 84, 7-14.
[21] A. Ferrante, L. Incrocci, R. Maggini, G. Serra, F. Tognoni, 2004. Colour changes of fresh-cut leafy vegetables during storage. *J. Food, Agri. and Environ.*, 2(3&4).40-44.
[22] G. Disciglio, L. Frabboni, A. Tarantino, E. Tarantino, 2014. Applying natural fertilizers to herbaceous crops. *Journal of Life Science*, Vol. 8, no. 6, 504-510 ISSN 1934-7391.
[23] A. Alberici, M. Valagussa , A. Piaggesi, A. Ferrante, 2009. Effects of biostimulants on quality of baby leaf lettuce grown under plastic tunnel. *Acta Horticulturae*, 807, 407-412.
[24] P. Vernieri, E. Borghesi, A. Ferrante, G. Magnani, 2005. Application of biostimulants in floating system for improving rocket quality. *J. Food, Agri and Environ*., 3(3&4), 86-88.
[25] P.R. Warman, 1998. Results of the long-term vegetable crop production trials: conventional vs compost-amended soils, in: *International Symposium on Composting and Use of Composted Materials for Horticulture*, pp. 333-34.