Organic Agriculture: Geostatistical methods to evaluate the response of cherry tomato to soil nitrate

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ABSTRACT

The organic farming is characterized by high variance in abundance of soil nutrients and subsequent crop output. This high heterogeneity challenges the ability to locate lacks and surpluses of soil nutrients in the agricultural plots using the conventional analytical tools. In order to define these areas, we have used spatial tools based on kriging interpolation that allow constructing value maps of soil minerals and crop factors based on limited samples. We applied this advanced methodology to an organic greenhouse of cherry tomatoes *Solanum lycopersicum var. cerasiforme* and to a conventional greenhouse nearby that served as a control. Using these tools we defined the spatial patterns of the organic greenhouse area and succeeded in locating areas of lacks and excesses of nitrates. Overlapping the soil's nitrate values all over the tested area with the cherry tomatoes' growth, yield and physical parameters of the tested plot led to identification of plant-soil interactions that were defined, till implementation of this methodology, only by the conventional analysis methods (based on 'Random Blocks'). The high confidence of the results together with the compatibility to those documented in former studies indicated the validity of the applied spatial evaluation methods for studying the organic practice patterns.

Keywords: Spatial analysis, cherry tomatoes, organic vs. conventional, Nitrate effects

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INTRODUCTION

The use of organic practices in agriculture has risen rapidly in the recent years (Allen and Kovach, 2000) not only because of health benefits, but also due to their positive impact on ecosystem (Bengtsson *et al*., 2005; Goodman, 2000; Scialabba, 2000). In the case of conventional methods, the farmer has a good control over the supply and concentration of nutrients at the plant's root zone, whereas in the case of the organic methods, there is a high heterogeneity in the number and amounts of nutrients due to the excremental nature of the organic fertilizers (from flora and animal sources)[1]. The organic dissimilation rates are slow and inconsistent, which also causes irregularities in soil concentrations of nutrients (Worthington, 2001; Herencia *et al*., 2007). Subsequently, these soil heterogeneities lead to yearly differences in yield amount and quality (Herencia *et al*., 2007; Norton *et al*., 2009). Note, lately, there were attempts to legalize the production of organic fertilizers (Bowen and Niggli, 2009), in order to reduce the differences in the nutrients' concentration.

The conventional experimental method, based on treatments and replicates in random blocks manner (Johnson, 2009), is not suitable for organic fields due to the fertilizers' characteristics. To address this issue, an analytical methodology was developed in Kansas, enabling representation of agriculture field (Zhang and Taylor, 2001) upon implementation of the following three steps:

a. Defining soil and yield parameters
b. Fitting the data to geographic coordinates, using field processing tools combined with GPS (Bakash *et al*., 1998) or using remote sensing (Kishore *et al*., 2001).
c. Defining the links, in some cases by creation of values maps, (Vieira and Gonzalez, 2003) between the abiotic and yield parameters and assessing them by means of ANOVA analysis.

These methods are best suited to large fields with low heterogeneity as opposed to the situation in the organic greenhouses which are small scale places.

Considering all these, we have applied new spatial tools for assessing irregularities in the supply of nutrients to greenhouses planted with the cherry tomatoes (*Solanum lycopersicum var. cerasiforme*). This is a very small variety of tomato that has been cultivated since at least the early 1800 and thought to have originated in Peru and Northern Chile (Smith, 1994). The Cherry tomato are characterized by high yields, high marketing value (http://www.bae.ncsu.edu/programs/extension/publicat/postharv/tomatoes/tomat.html),short growing season, resistance to salinity (mainly the types developed in Israel, Mizrahi et al., 1998) and widespread scientific knowledge with regard to its biology. The organic cultivation portion of this crop is rapidly growing (http://www.uky.edu/Ag/CDBREC/introsheets/organictomatoes.pdf) mainly due to the high impact yields (Caris-Veyart *et al*., 2004). Thus, the organic cherry tomato is a good candidate for agricultural and physical research (Xiaoying *et al*., 2012).

The main goal of this research was to test different experimental methods for their capacity to measure the response of organically grown cherry tomatoes to the nutrients from the surrounding area. They were based on taking samples from a commercial area and analyzing a large number of variables that on one hand represent the environment and on the other hand, the reaction of plants to them.

Specifically the objectives were:

a. Assessing the differences in the concentration of major soil elements between the organic and conventional greenhouses.
b. Comparing the differences in distributions patterns of the major soil elements between the organic and conventional greenhouses.
c. Linking the distribution of Nitrate with the cherry tomatoes growth including the physical and yield parameters.

MATERIALS AND METHODS

The research took place in Moshav (cooperative Israeli settlement) Netzer Hazzani located in the Northern Negev (Longitude- 15º34', Latitude- 20º31'). The soil is defined as Sandy with deep and uniform pattern (USDA 1999). For description of parameters, see Table1. Climate is coastal Mediterranean with high moisture rates (IMS, Israel Meteorological services, Table 1). Irrigation water came from local wells (dissolved element content, Table 1, Gilat Field Services).

The research took place within two plots of organic and conventional practice greenhouses. The organic greenhouse had total area 1,920m^2 of which 1740m^2 were planted (the analyzed plot was 940m^2). The conventional greenhouse had total and planted area of 2,336 and 2,130 m^2, respectively, and the analyzed plot was 1,220m^2. The greenhouses were East-West oriented (planting order had North-South orientation) and covered with polyethylene. The organic and conventional greenhouses were composed from five and eight pediments, respectively (pediment length- 32m, width- 9m and height 3.5-5m).

Table 1: Site of study soil, climate and irrigation water data

<table>
<tr><td rowspan="3">Soil</td><td colspan="2">Sand (0.02-3mm)
90- 99%</td><td colspan="2">Silt (0.02-0.0002mm)
0.5%</td><td>Clay (<0.0002)
0</td></tr>
<tr><td colspan="3">Organic Matter
2-6.5%</td><td colspan="2">CECP[a] [meq 100gr]
0.4-0.8</td></tr>
<tr><td colspan="2">Saturation Point
30-40%</td><td colspan="2">Field Capacity
35-10%</td><td>Withering point
3-8%</td></tr>
<tr><td rowspan="2">Climate</td><td colspan="2">Day temperature
17- 24ºc</td><td colspan="2">Maximal moisture
66.2- 81.7%</td><td>Class'A' pan evaporation[b]
5.4 mm day^{-1}</td></tr>
<tr><td colspan="2">Solar radiation
415-925 Watt m^{-2}</td><td colspan="2">Sunny days[c]
121 days year^{-1}</td><td>Precipitation amounts
400-500 mm seasson^{-1}</td></tr>
<tr><td rowspan="2">Water</td><td>Ca[meq L^{-1}]
1.2</td><td>Mg[meq L^{-1}]
0.2</td><td>Na[meq L^{-1}]
2.2</td><td>Cl[meq L^{-1}]
1.5</td><td>S[meq L^{-1}]</td></tr>
<tr><td colspan="2">HCO_3[meq L^{-1}]</td><td>EC[ds m^{-1}]
0.5</td><td colspan="2">pH
8.0</td></tr>
</table>

[a] CECP- Cations Exchangeable Capacity
[b] (Wilson et al., 2001).
[C] Days that the global radiation above 800Watt/m^2

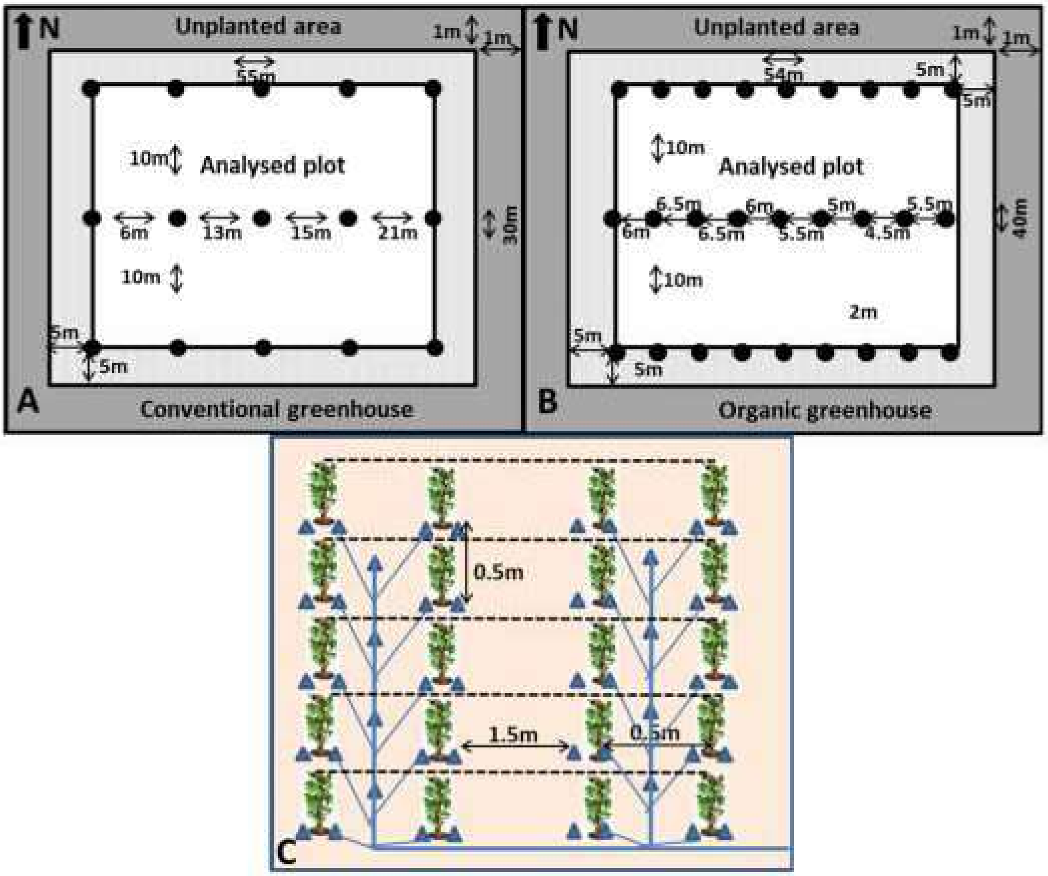

Figure 1: The greenhouses design.

A. Conventional greenhouse sampling plot

- Black point represents sampling point
- Solid gray represents unplanted area
- Dotted gray represents planted area
- White area represents the analyzed plot
- Arrows mean distances (between samples, between the sample and plot edges or between the plot and greenhouse edges)

B. Organic greenhouse sampling plot

C. Greenhouses planting unit

- Plant image represents Cherry tomato
- Triangle mark represents dripper location
- Solid blue lines are representing irrigation pipes
- Dashed black lines are representing trellising wires
- Arrows mean distances (between plants or between planting units)

Each 'Planting unit' was composed of two couples of four planting lines. Inside each couple, the distance between the planting lines was 0.5m, and between the couples, 1.5m. The distance between the plants in each line was 40cm, and the total density of the plants was 3 plants per m^2. Each plant was trellised over with iron wire (height of 2m) and irrigated directly with two drippers (1L $hour^{-1}$, three hours day^{-1}, and average of 2.25 L $plant^{-1}day^{-1}$), (Fig. 1A).

Agrotechnology

The plants were cherry tomatoes sub type '495' developed by Zeraaim Gadera Co. The seeds for the organic greenhouse were sprouted and grown on organic bed for three weeks separately and planted in the greenhouses at 15.02.2001, based on the organic farming protocols (Baker, 2009). Pollination was done by the Bombus bee (*Bombus terrestris*) from BioBee, Sde Eliyau. Fruit picking was carried out twice a week (10.5.2001-15.8.2001), once tomatoes reached minimal red cover of 50%. In the organic greenhouse, sulfur was sprayed against pests three times in the growing season (Jones and Howells, 2001), whereas in the conventional areas, chemical pesticides were applied.

Prior to planting, the soil of the organic greenhouse was mixed with 2.5 ton/ha of compost (Gaskell and Smith, 2007). The compost was from plant excrements and animal manures and was not uniform in its content. Average nutrient concentration from dry matter was: N- 5.53%, C-13.197%, H-6.24%, S-2.06%, $^{C}/_{N}$=1:3, Elemental Analyzer EA1108 (Eager, 2001). Along the growth season, 150 gr of Guano was added to each plant (Gaskell and Smith, 2007) and two litters of liquid urea (called also 'Cow urine', Silva *et al*., 1999) was added at planting and along the growth season.

Soil sampling were taken at 01.03.2001, 27 samples from the organic greenhouse, in three sampling lines shape (distance of 10m). The average distance was 5m between the samplings. In the conventional greenhouse, total of 18 samples were taken from three lines (distance of 10m). In that case the average distance between the sampling dots was 12m (Fig 1B and C). From both greenhouses, the extreme samples were taken 5m from the edges to illuminate side effects. Note, the accurate locations of the plots were determined by several factors such as distance from the plants, distance from the drippers, ease of digging and other factors that illuminate equal distances between the plots.

Tested parameters

Soil parameters:

a. As stressed by Heinze *et al* (2009), the organic practice has dramatic effects on the availability of the soil minerals, so firstly we have carried out comprehensive analyses of these elements. The soil samples were taken from depth of 0-20cm, dried and mixed with distilled water (70ml water per 100gr of soil) for 24 hours. Afterwards, the soil solutions were drained and analyzed using elemental analyzer (Eager, 2001). The tested molecules and parameters were: Na, K, Ca, $N\text{-}NH_4$, Cl, $N\text{-}NO_3$, P, EC, pH, C-Organic.

b. Soil CO_2 flux. This analysis represents the respiration of plant roots. It was carried out by means of LICOR 6400-09 Soil chamber (Licor, 1993), laid in 10cm depth adjacent to the main root of the cherry tomato (Ben Asher *et al*., 1994; Davidson *et al*., 2002). Note, the C-Organic was also measured by burning at 400ºC for ten hours (Sparks, 1996).

Several parameters related to cherry tomato growth and yields were examined. They were divided into yield, dynamic growth and physical parameters as described below.

Yield parameters:

a. Sweetness (the Brix value was tested using digital refractometer of Atago co. http://www.coleparmer.com/buy/category/atago-handheld-manual-refractometers).
b. Fruit pH was tested using pHmeter HI8733 of Hanna instrument
c. Number of fruit picking seasons per year and weight per year (Evans et al., 2010).
d. Texture and fruit color (Stommel, 2005).

Vegetative parameters:

a. Shoot elongation and thickening between two periods. Measurement was done from ground till the highest plant top (Schwarz and Klaring, 2002).
b. Changes of LAI (Leaf Area Index- represents the relationships between leaf area and soil unit, based on Beer law: Li and Stanghellini, 2001).

Physiological parameters:

This group of parameters is related to the inter-cellular, physiological activity in the photosynthetic cells (Yaptenco *et al.*, 2007) including the rate of gas exchange (CO_2 and O_2 emissions) together with the root respiration (Cramer *et al*., 2001). Four parameters were examined, all on the youngest fresh leave with area bigger than six cm^2 (measurements were done using LICOR 6400-09 Soil chamber, Licor, 1993).

a. Photosynthesis rates
b. Respiration rates
c. Inter-cellular CO_2 rates
d. Temperature differences between the outer and the inner parts of the leaves

Data analysis

Two types of analyses were implanted in this work, the conventional and the spatial one. The conventional one was based on comparisons between means and Standard Errors of various values together with confidence analysis, based on ANOVA using JMP ver. 5.0 (Sall and Lehman, 1996)

The spatial analysis was based on the assumption that there are reciprocal effects between plots in the tested area. By measuring these effects and crossing them over with the separation distances between the plots, one can estimate the parameter values all over the tested field. The analysis was based on the following five steps (GS+ ver 5.3, Gamma Design, 2013 and ESRI, 2001):

a. Entering the variable values together with their spatial location.
b. Calculating the semi-variances between the measured points, graphing them against the separation distances of the measured points and modeling them (the so called semivariogram: Meisel and Turner, 1998). Note, for the calculations we used uniform spherical model.
c. The obtained semi-variances were used to calculate the parameter values all over the tested area, based on the measured points using 'Kriging' method (Webster and Oliver, 1990).
   Note, for the calculation we used Uniform grid and Block kriging (Gamma design, 2013).
d. The calculated parameter values were used to illustrate values map (presented as groups of values). Note, the data from this step could be also used to identify the variable distribution patterns in the tested area (for example, locating irregularities in soil elements).
e. Crossing over of value maps belonging to different parameters for creating links between them. The results are usually presented as tables or graphs with groups of values belonging to the influenced parameter (as will be demonstrated on the nitrate) and the average of the affecting parameter such as the cherry tomato growth parameters)[3]. This step also defined 'Multi-layer analysis', ArcInfo ver. 9.0 (Fischer and Getis, 1997).

RESULTS AND DISCUSSION

Analysis of soil parameters in the organic and conventional greenhouses:

In order to evaluate the differences between the organic and conventional greenhouses, we firstly compared the amount of soil elements (the nutrients: N-NO_3, N-NH_4, P, K; the major elements: Na, Ca, Cl and EC& pH). These values were compared to the control (open area, unplanted and unprocessed soil nearby with area of 0.2ha, mix of eight samples). The results are presented in Fig. 2.

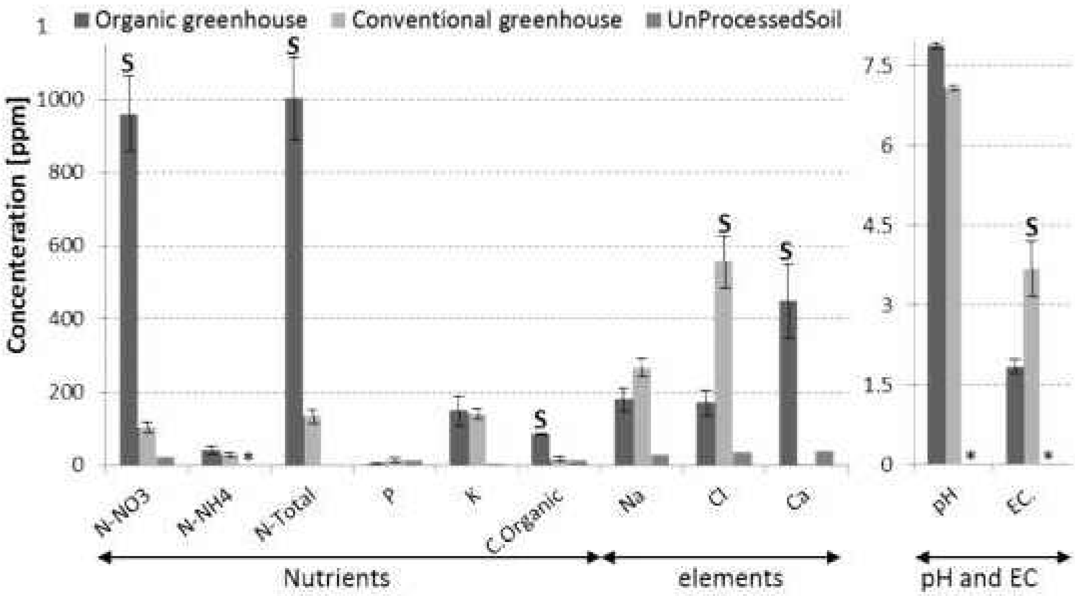

Figure 2: Nutrients' concentrations and physical characteristics of the different treatments

Thin lines above and beneath columns represent Standard Errors (SE).

'S' significance (except the EC which has significance of α=0.1 the other have α<0.01) pH measured in arbitrary units, EC measured in ds/m units

The conventional practice soil was characterized by high salinity as compared to the organic one (high EC, Na^+ and Cl^- values). The elements N-NO_3^-, N-NH_4^+, P-PO_3^- and Ca^{2+} had higher values in the organic greenhouse soil which could be attributed to the rich composition of the organic fertilizers (Burger and Jackson, 2003). From them, the values of the Nitrate and $C_{Organic}$ were significantly higher (α<0.001). The Potassium had similar values in both soils and sodium was low in the organic greenhouse as compared to the conventional one. In all cases, the unprocessed soil had lower values than both greenhouses (one exception is the Phosphorus). The differences in the nitrate content between the greenhouses, the high variability of it in the organic soil and its effects on cherry tomatoes growth and development as described in the literature (Ben-Oliel et al., 2005: Horchani *et al*., 2010) led us to explore its spatial distribution.

Analysis of soil nitrate in the organic and conventional greenhouses

In order to study the nitrate spatial distribution in the greenhouses, we analyzed the samples together with their location (the start point was determined at the North West corner). Even at the first analysis step (the semi-variogram analysis, section 3.4. of Materials and Methods, Data analysis, spatial analysis, step 'b') it was easily demonstrated that the sampled data of the organic greenhouse was well suited to the spatial analysis by the high fit to the spherical model. In contrast, in case of the conventional one no fit was found (Garrigues *et al*., 2006).

For studying the differences between the conventional and organic greenhouses we divided the concentrations values maps (based on the kriging analysis, section 3.4, spatial analysis, step 'c') into five equal levels.

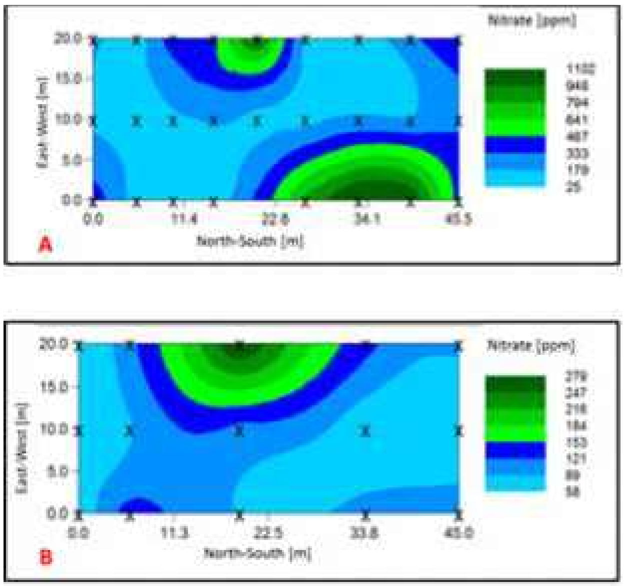

Figure 3: The calculated soil nitrate maps of the organic and conventional greenhouses plots

A- The organic greenhouse plot; B- The conventional greenhouse plot; The 'X' marks represent the sampling locations.

Firstly, using the values map of the organic greenhouses plot we located areas with extreme nitrate values, Excesses were found around 14, 19m (East-West and North-South locations, respectively) and around 0, 34.1m, respectively. Areas with shortage of nitrate were located in the middle of the tested plot.

Secondly, on first sight, both greenhouses have spatial distribution patterns, but the differences between the average sizes of their value groups indicate their spatial pattern. Whereas values group of the organic greenhouse was equal to 135ppm, values of the conventional one was equal only to 27-30ppm, which represents a 'weak' spatial pattern. Also, one has to remember that the map values, at the end calculation are based on the variogram values that in the case of the conventional greenhouse were highly different from the sampled ones, as noted before.

The effects of Organic fertilization on crop yield

For studying the interactions between the soil elements and the cherry tomato plants in the organic greenhouse plot, we chose the nitrate as representative and crossed over its map with growth and yield parameters of the cherry tomato. The most important parameter for the farmer, mainly in the organic culture (where the quality takes only second place: Evans *et al*., 2010) is the accumulated fruit biomass (a representative of all yield parameters), which leads to explore its relation to the soil nitrate. The relation of accumulated fruit biomass to soil nitrate is demonstrated in Fig. 4

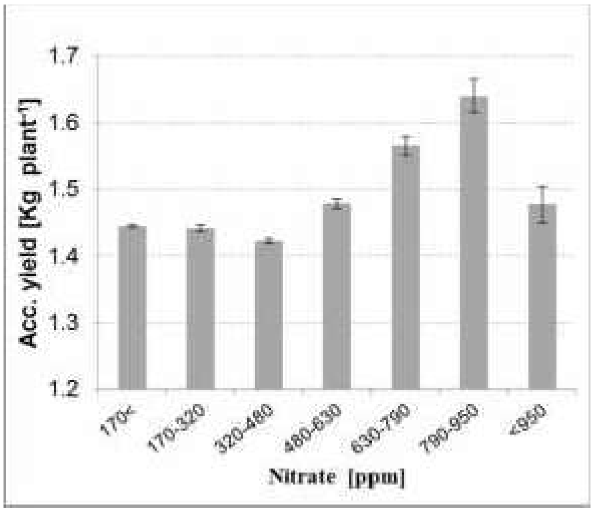

Figure 4: The effects of soil nitrate on cherry tomato accumulated yield per plant in the organic greenhouse (calculated by using spatial tools).

In case of nitrate soil concentration of less than 370ppm (the minimal needed amount), almost no fruit was produced. Higher nitrate values of 370- 650ppm were associated with polynomial increase in the accumulated cherry tomato biomass, while even higher soil nitrate values of 650-750ppm were linked to the maximal crop values. The maximal nitrate values of beyond 750ppm led to decrease (polynomial shape) in crop values which could be attributed to nitrate poisoning. The high fitness of the results to the graph (as expressed by the r^2 value) and the suitability of the data to the scientific literature (Si-Shuai *et al*., 2012) confirm the findings of this research.

In order to study more on the behavior of the cherry tomato under different amount of soil nitrates, we decided to also examine the effects of nitrates on vegetative parameters such as the LAI and shoot elongation. The results are presented in Fig. 5.

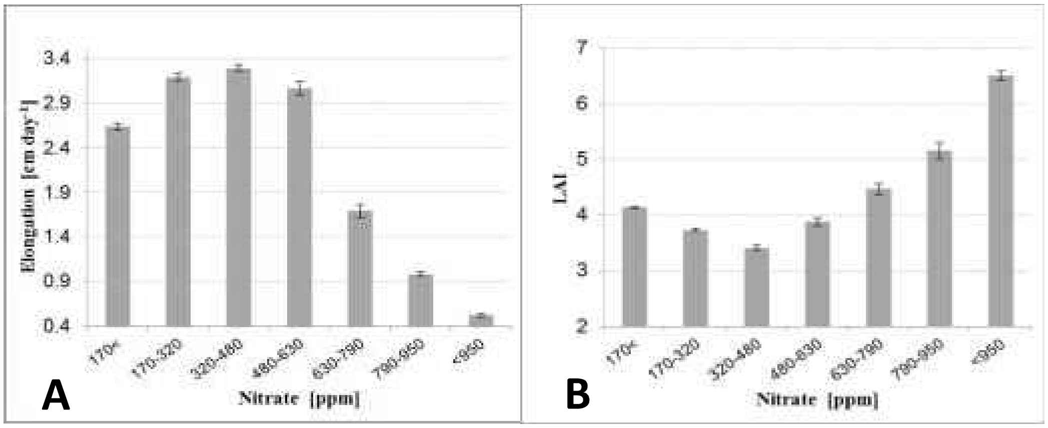

Figure 5: The effects of nitrate on different physical parameters of the cherry tomato in the organic greenhouse (was calculated by using spatial tools)

A- Main shoot elongation between 3/6/2001- 6/6/2001
B- LAI of the upper leave in the 12/6/2001 (defined with arbitrary units)

Till 0.5 kg per m^2 of nitrate there was an increase in the vegetative parameters: shoot elongation (Fig. 5A) and LAI (Fig. 5B). These results together with those presented in Fig. 4 demonstrate the 'investment' of the plant in vegetative growth on behalf of the reproductive phase. This means that the trends are turned over and plant invest in fruits (Evans *et al*., 2010; Schwarz and Klaring, 2002). After exploring the effects of nitrate on the vegetative and reproductive parameters, we examined several representative physiological ones such as root respiration and leaves transpiration (Fig. 6).

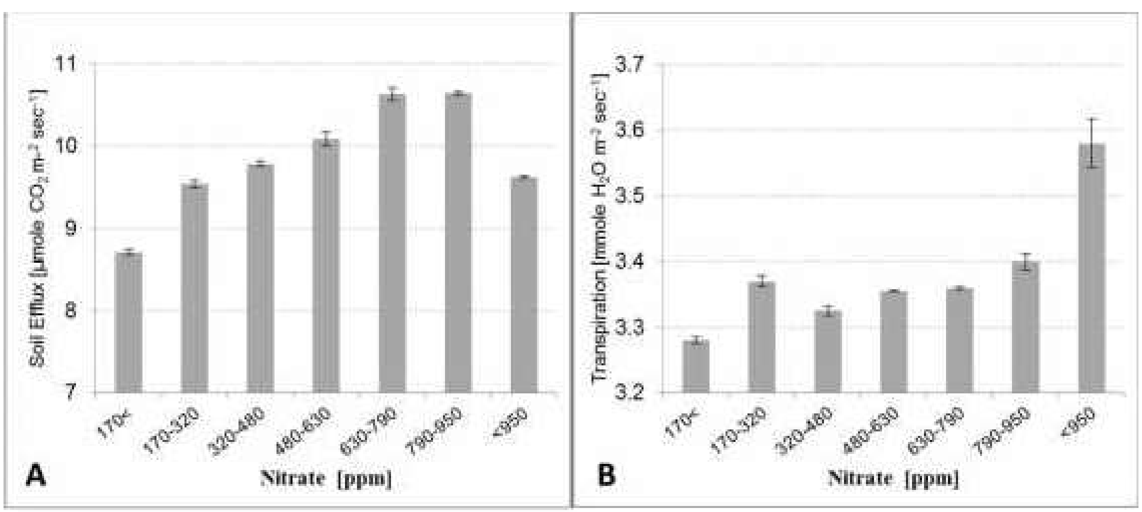

Figure 6: The effects of nitrate on different dynamic physiological parameters of the cherry tomato in the organic greenhouse (calculated by using spatial tools)

A- Soil CO_2 flux (represents roots activity of the plant)
B- Leaves respiration- transpiration value of the plant

In case of both parameters, there was a parallel increase in nitrate content. One has to take into concern two points:

a. Root respiration analysis represents the whole soil respiration, and not only that of the cherry tomato root system, so conclusions have to be taken with caution.
b. At nitrate concentrations higher than 0.95kg per m^2 the respiration decreased.

Similar trend, but less noticeable, was observed in the rate of photosynthesis (emission of CO_2), that together with the increase in LAI (Fig.5) stress the positive effect of the nitrate on the cherry tomato (Horchani *et al*., 2010).

The novelty of this manuscript lies in the application of spatial analyses tools for representing the variability in non-uniform fields, such as the organic plantings which have a limited number of samplings plots (Vieira and Gonzalez, 2003). In the scope of this research we analyzed several nutrients in order to identify those having considerable effects on the cherry tomato plants. Afterwards, we tested the nitrate (as a representative factor) using spatial tools for locating irregularities in its soil distribution. In order to assess the usability of the spatial tools and to study the effects of different nitrate levels on the cherry tomato plants parameters, we crossed over their spatial maps. The spatial analysis results were similar to those obtained by the conventional analysis as presented in the scientific literature ('Random Blocks': Jonhson, 2009). In Table 2 there is a brief summary of the differences between the two methods.

In general, the spatial tools allow getting practical results for the farmer in a fast manner with minimal extra field work using user friendly software. There are several available programs in the market. Of note, the software presented here does not imply authors' preferences. Still those who use this methodology has to take into concern, that in order to ascertain successful use several topics associated with the sampling scheme should be considered.

Table 2: The differences between the conventional research analysis (Random blocks) and the geostatistics.

Definitions	**Random Samplings**	**Calculation**			**Suitability**
Random blocks	Necessary	Average	Treatments variability	---	Limited number of parameters at the same time
Geostatistics	Unnecessary	Average	Treatments variability	Spatial Variability (additional)	Almost no limitation of parameters at the same time

Research scheme	**Sampling point**	**Treatments**	**Variability**	**Research areas**
Random blocks	Not important (only the average)	Has to be defined (based on former knowledge).	Suited to low variability	In areas aimed for researches (with control growth parameters)
Geostatistics	Important	Identified in the area	Suited also to high variability	Can be implemented in commercial field

Summary	**Last word…**	**Advantages**	**Disadvantage**
Random blocks	Assumption: variability, necessary negative impact	*Results are more accurate. *Results can be easily assessed by additional research set.	*High costs *Linking between parameters has to be done from the same locations
Geostatistics	Important part of the research and data analysis	*Low costs, no need for controlled research *Implemented in farmer area, no need to transfer data from research station * Parameters can be assesses parallel *Linking between parameters can be done with samples from different locations	* Wide parts of the results are virtually (based on computerized calculations). *Additional measuring (samples locating). * The results did not fit in some cases to tested semivariogram

Note, for the ease of the understanding, the table is separated into three parts: Definitions, Research scheme and Summary.

Firstly, as demonstrated in Table 2, although the spatial tools could be applied to a limited number of sample plots, the accuracy and fitness of the predicted values maps to the real field states will become higher with the rise in the number of the analyzed samples (Clark, 2010).

Secondly, our sampling scheme in both organic and conventional practice greenhouses was based on equal distances all over the greenhouses. This scheme is recommended in cases when the farmer does not have any former knowledge of the state of the nutrients' concentrations in his fields. Once he suspects irregularities in some parts, he should make more samplings in these areas[4].

Thirdly, one of the biggest advantages of the spatial tools is the ability to sample the tested factors in different plots and amounts for finding links and relationships between various factors[5]. Such a mode of action is superior (in terms of cost and allocated resources) to that of the conventional statistics which enforces sampling of the same plots for all the tested factors.

The tools presented in this paper could be adjusted for resolving other challenging aspects of the organic agriculture such as identification of crop subspecies unaffected by high variance in nutrients' concentrations by comparing of the relevant variogram patterns. Additionally and mainly for scientific use, following up of the nutrients' concentrations all over the growing seasons could be carried out by overlapping of the calculated values maps on time scale (termed 'Time series analysis'; Kyriakidis and Journel, 1999). Overall, the spatial analysis demands minimal investment in the realm of field sampling and relies on the user friendly software available in the market, in order to obtain meaningful data on the optimal fertilization regimes.

Notes:

[1] Note, in the market there are other software based on the same principles which could be also used for implementing the techniques described in this paper. Of note, the described software does not imply authors' preferences.

[2] The most frequently used factors for this analysis are the isotropic model, the uniform interval (also appear as the software defaults) and spherical model; Gamma design, 2009.

[3] The use of the Multi-Layer Analysis together with the spatial tools allows comparing factors sampled from different locations in the studied area, which is essential when there are difficulties in taking measurements from the same plot. This cannot be done with the usual statistics analyses due to their dependency on factors collected from the same plots. These results are in agreement with former publications (Si-shuai et al., 2012).

[4] The software currently available in the market also enables performing separate spatial analysis on the plot parts. One should use it in areas with high regularities in factors values; Gamma design, 2013.

[5] This state could be due to some reasons such as costs (for example, nitrate testing is cheaper and therefore could be carried in much higher frequency than examination of sulfur levels) and when the sampling process of one factor can affect the value of the other one.

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