ASSESSMENT OF BIOLOGICAL SOIL FATIGUE IN HORTICULTURAL MONOCULTURES

EVALUAREA OBOSELII SOLULUI DIN PUNCT DE VEDERE BIOLOGIC ÎN MONOCULTURILE HORTICOLE

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Abstract. *Biological soil fatigue in horticultural monocultures awakes the highest interest and presents the highest discrepancy. Different studies associate it with soil pathogens as causing agents, while others determine it in the lack of these. This work deals with the assessment of fatigue existence, a phenomenon which can be appreciated in the field, but a numerical model that provides data about yield losses and lack of plant vigour has not been found. Trial planning consisted of adding fresh organic matter through biodisinfection techniques and providing knowledge about the phenomenon and its relation with the content of organic matter and soil microbiota. For this reason, soils of two greenhouses were compared, the differences between them were the type of crop (cucumber and tomato respectively) as well as the supplying or non supplying of organic amendments. The content of organic matter and the soil or telluric microbiota (fungi, bacteria and oomycetes) in the soils were studied, as well as its effect on cucumber and tomato seedlings under controlled conditions. The results showed that fatigue appeared in soil with low content of organic matter, which showed at the same time lower density and diversity of fungal population. The addition of fresh organic matter seems to reconstitute the productive capacity of the soils, and this mitigates the fatigue and monoculture effects.*
Key words: *Edaphic microbiota, fungi, bacteria, oomycetes, seedlings.*

Rezumat. *Oboseala solului din punct de vedere biologic în monoculturile horticole trezește cel mai mare interes și prezintă totodată și o mare diferență. Diferite studii asociază acest fenomen cu agenți patogeni din sol ca agenți care îl provoacă, în timp ce alții afirmă că fenomenul se produce şi în lipsa acestora. Acestă lucrare se referă la evaluarea existenței oboselii solului, un fenomen care poate fi apreciat în domeniu, însă un model numeric, care să ofere date despre pierderile de randament și lipsa de vigoare a plantelor nu a fost găsit. Experimentul a constat din adăugarea de materie organică în stare proaspătă prin tehnici biodezinfectie și furnizarea de cunoștințe despre fenomenul și relația acestuia cu conținutul de materie organică și microbiotei solului. Pentru aceasta, solurile de două sere au fost comparate, diferențele dintre ele au fost*

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de tipul de cultură (castraveți și tomate), precum și administrarea, sau nu, de amendamente organice. A fost studiat conținutul de materie organică din sol și microbiota solului (ciuperci, bacterii și oomicetelor), precum și efectul asupra răsadurilor de castravete și tomate în condiții controlate. Rezultatele au aratat ca oboseala solului a apărut în varianta cu conținut scăzut de materie organică, care a avut în același timp și densitate mai mică dar diversitate a populației fungice. Adăugarea de materie organică în stare proaspătă poate reconstitui capacitatea productivă a solurilor, iar acest lucru atenuează efectele oboselii solului în monocultură.

Cuvinte cheie: *Microbiota edafică, fungi, bacterii, oomicete, răsad*

INTRODUCTION

This work tries to assess the existence of fatigue in horticultural soils in monoculture, as well as quantifying its intensity, this phenomenon can be appreciated in the land, but a numerical model which allows us to obtain reliable data about yield losses and lack of plant vigour has not been found. The studies that consider the existence of the phenomenon date from the years 70-80, and base their empirical character on the observations made in the land, in many cases, by the farmers themselves, without giving a numerical quantification. For this reason, the first question that must be posed by any person interested in the study of soil fatigue is: how do authors define fatigue if there is not data that supports its existence? From here on, the phenomenon complexity of fatigue, considered as an agronomic concept and defined by different authors as "fertility disorder of soils due to multiple causes that can be accumulative, successive or simultaneous in the land" (Bouhot 1983c, Chen *et al*. 1991) that causes a partial reduction of productivity and it is attributed mainly to reiterated monoculture in the same plot (Cebolla & Maroto 2004, Scotto 1982). However, this ambiguous term has been used with different approaches throughout the years. So that, physical fatigue has been defined as the loss of soil fertility due to labours or amendments which cause losses in their structure, and with this, a soil impoverishment (Maroto 2000, Bouhot & Dumas 1982). Chemical fatigue is described as the modification of chemical parameters of soil due to different fertilizers (Casado 1925, Bodet 1982), as well as the negative effect of allelopathic substances in the root development (Maroto 2000). And, finally, biological fatigue which is due to microbiological imbalances in favour of some organisms and/or to the detriment of others (Casado 1925), which is the most studied and the most controversial. In this sense, the main difference is found in the "thin line" that separates the phenomenon soil fatigue, that affects the normal development of plants, from pathogenicity that implies the presence of pathogenic organisms that act as causal agents in the expression of disease. These two terms, soil fatigue and pathogenicity, seem to walk hand in hand in the first studies about fatigue. Different authors, after observing yield losses in land with symptoms attributed to soil fatigue, carried out studies which concluded that the presence of pathogens as responsible agents of

such losses (Bouhot 1975, Bouhot 1979, Bouhot 1982a, Bouhot 1982b, Meynard & Bouhot 1982, Hoestra 1982, Vigouroux 1982, Roudeillac 1982, Bouhot & Bonnel 1982), even in some of these cases, biological fatigue coincides with chemical fatigue (Bouhot 1982a, Bouhot 1982c, Gindrat *et al*. 1982). On the other hand, due to the intensification of the expression of fatigue phenomenon after the withdrawal of methyl bromide, disinfecting substance of horticultural soils which achieved "covering up" its adverse effects (Tello & Lacasa 2004, Lacasa *et al*. 1999, Guirao *et al*. 2004), new studies arose which showed the existence of the phenomenon in the absence of pathogens (Otto *et al*. 1994, Lacasa *et al*. 2002, Guerrero *et al*. 2004, Guerrero *et al*. 2014). In this way, these studies showed that monoculture can involve that some microorganisms increase their presence in soils, to the detriment of others, which can affect negatively production, and in the case of pepper, it can be reduced to 60% (Lacasa *et al*. 2002, Guerrero *et al*. 2004). So that, before the possible existence of fatigue, other studies showed how adding organic matter through biodisinfection in agricultural soils in monoculture, affected fungal soil microbiota which reached a new balance (Martínez *et al*. 2009). Furthermore, biodisinfection seems to improve physical-chemical characteristics of soil (Fernández *et al*. 2005). These results have not been corroborated until now with new studies that evaluate and quantify soil fatigue caused by monoculture. For this reason, this study allows us to explore a model to be characterised in the intensive crops under plastic in the Spanish southeast. The way the trials are set out consists of adding fresh organic matter through biodisinfection techniques to compost soil, and it tries to provide knowledge about fatigue phenomenon or soil exhaustion in monoculture and its relationship with the content of organic matter and the soil microbiota. To that end, soils of two greenhouses of the province of Granada were compared which carried out monoculture year after year, and that they differ one from the other by the type of crop (cucumber and tomato respectively), as well as the supply or non supplying of organic amendments to the soil. The content of organic matter and the soil or telluric microbiota (fungi, bacteria and oomycetes) in the soils was studied, as well as its effect on cucumber and tomato seedlings under controlled conditions. Furthermore, in the case of soil with low content of organic matter, the assessments were made after the first incorporation of organic amendments through biodisinfection, to compare the results obtained before and after the disinfecting amendment.

MATERIAL AND METHOD

Selected greenhouses. Location.

Greenhouse 1. Soil with low content of organic matter.

Greenhouse with "raspa y amagado" Almería type structure, located in the subtropical coast of Granada (Motril). This soil has been cultivated for 14 years with cucumber monoculture always with the same cultivation lines, and no organic matter has ever been supplied. In the first sampling carried out, the greenhouse showed high incidence of disease in plants caused by *Fusarium oxysporum* f.sp. *radicis cucumerinum.*

When the crop ended, a biodisinfection treatment was carried out through the incorporation of mustard+radish+manure+rest of the previous crop (including sick plants) and then later solarisation (biosolarisation) during approximately 3 months.

Greenhouse 2. Soil with high content of organic matter.

Greenhouse with "raspa y amagado" Almería type structure, located in the interior of Granada (Fornes). Tomato monoculture has been cultivated in this soil for 14 years. Tomato crop has always been planted in this soil with the exception of a cucumber crop during 3 seasons. Every year supplies of fresh organic matter are made to be composted in the soil. Specifically, mustard+radish+vetch which are added once the tomatoes have grown, in autumn-winter, and prior to this, they bury tomato plants with manure at the end of the harvest.

Sampling.

The samples were made following a determined spatial distribution, with the purpose of obtaining a sample as homogeneous as possible. Therefore, with the help of a spade, the samples of approximately 10 kg were taken at a depth of between 0 to 30 cm, at three different points within the same cultivation line, and then they were mixed and homogenized inside the same bag. The samples were taken in the centre of the cultivation line to avoid the possible "edge effect" of the corridor between cultivation lines.

The samples that were taken were the following:

Greenhouse 1: - Before the incorporation of organic matter (8/5/2014).

- After the incorporation of organic matter through biodisinfection (21/1/2015). When samples were taken, organic matter had not been fully decomposed (the typical smell of geosmin produced by actinomycetes was appreciated).

Greenhouse 2: - A single sample was made (29/5/2014). The sample was taken when double stem plants had the third cluster opening.

Determination of the percentage of organic matter in soils.

Determination of organic matter was made through oxidation method with potassium dichromate and chloridric acid and a later valuation with Mhor's salt.

Assesment of plant vigour in controlled environmental chamber.

Vegetable material

Evaluations were made for the two horticultural species that are grown usually in the soils that are being studied: tomato (*Solanum lycopersicum* L. cv. Río Grande; Ramiro Arnedo S.A.), and cucumber (*Cucumis sativus* cv. Marketmore 76; Ramiro Arnedo S.A.). Therefore, seedlings of the two horticultural species were grown in each of the studied soils, with the purpose of determining the fatigue, if any, was specific for a particular species or if it was shown for both horticultural species.

Description of trials

Each horticultural species was sown separately at a rate of one tomato seed and one cucumber seed, in 175 ML volume pots (experimental unit) which contained the soil subject to study mixed with vermiculite at a rate 2:1 v/v, to avoid soil compaction which was observed in previous trials with non mixed soil.

Seeds were previously disinfected through immersion in a commercial dissolution of sodium hypochlorite at 20% during 15 minutes, and then they were rinsed and soaked during 48 hours in a wet chamber before sowing. Trials were carried out during 30 days in a controlled environmental chamber at 12.000 lux, photoperiod of 14 h light/day and maximum and minimum temperatures of 24,9±0,6 and 21,2±0,8 ºC respectively. Plants were irrigated on demand, but they were not fertilised during the trials.

Five replications were made with each soil for each horticultural species, and the trials with the different soils were repeated twice over the trial period.

Evaluated parameters in seedlings

- *Number of leaves.* Only the true leaves were considered, cotyledons as well as non fully developed leaves were rejected.

- *Length of the aerial part*. The total length of the aerial part of the seedling was considered as the distance between the part of the stem at the substrate level area and the apical bud of the seedling.

- *Aerial dry weight and root dry weight*. The aerial part was separated from the root part, and they were dried in a heater at 72ºC during 48 hours. After this time, each part was weighed separately on a precision scale (Model PB 303-S, with 1mg sensibility).

- *Leaf area*. To calculate the leaf area, the free software ImageJ 1.48 (NIH Image, Maryland, USA) was used. Previously, the leaves and/or leaflets of each seedling were scanned (Scanner Epson Perfection 1240).

Study of the fungal and bacterial microbiota and oomycetes.

Sampling preparation

Following the instructions of Tello *et al*. (1991) and Rodríguez-Molina (1996) the samples were subject to a drying, grinding and sieving process. After placing the samples in plastic trays, drying was done at environmental temperature, during a variable time (7-10 days) depending mainly on the humidity of the sample when arriving at the laboratory. A porcelain mortar was used for grinding, and for sieving a 200 µ mesh size sieve. The mortar and the sieve were washed and disinfected between the different samples flaming them with alcohol.

Analytical methods

The analytical methods used to know the microbiological composition of soils were (Tello *et al*. 1991):

Successive suspension-dilutions method for the evaluation of the total microbiota (fungi and bacteria) . In this case, soil is added to a culture medium in the form of a suspension of sterile water. The culture medium was acidified malt extract agar. Ten repetitions of each sample were made at the dilutions 10^{-3} and 10^{-4}. Incubation was carried out in the laboratory at room temperature during 4-7 days. After the indicated time, the fungi Colony-Forming Units (CFU) were counted and identified at the levels of the genera, as well as the CFU counting of the total bacteria present in each repetition. In this case, the 10^{-4} dilution was chosen, the results are expressed as $x10^4 UFC \cdot g^{-1}$ of dry soil.

Komada's selective medium (1975) modified by Tello et al. (1991) to assess the Fusarium flora. It's a selective culture medium for *Fusarium* genus which has three solutions. In this case, soil dilution is carried out directly in a dish with the melted culture medium. For each soil fraction or replication (4 in total), 4 dishes were prepared, which supposes a total of 16 dishes per sample. Incubation was carried out in the laboratory at room temperature during 4-7 days. After the indicated time, the CFU present in each Petri dish were counted and identified at the levels of the species.

Vegetable trap method to evaluate the presence of oomycetes. Immature petals of carnation were used. Five repetitions of each sample were made for each of the sampling carried out. Repetitions had 5 carnation petals. Incubation was made in the laboratory at room temperature during 4-5 days. After the indicated time, data was taken, determining the presence or absence of oomycetes in each of the replications, for this reason, presence was considered when oomycetes were found in at least one of the petals. The

results are expressed, for each of the samples carried out as a % of replications with presence of oomycetes.

Statistical Analysis

Student's t-test was carried out to compare the parameters evaluated in cucumber and tomato plants between soils. As they were parametric analysis, the assumptions of normality and homoscedasticity were checked previously. With the purpose of evaluating significant differences in the microbial variables analysed (Fungi CFU, Bacteria CFU) a non-parametric test was applied (Kruskal-Wallis), given that a data transformation was not found that fulfilled the assumptions of normality and homoscedasticity required by the parametric tests (due to the high variability of the values of the variables studied as effect of the heterogeneity that these variables show naturally in the soil). The statistical package used was Statgraphic Plus 5.1 (Manugistic Incorporate, Rockville, MD, USA) for Windows.

RESULTS AND DISCUSSIONS

Percentage of organic matter on soils.

Soil of Greenhouse 1 showed a 0.54% content of organic matter before the incorporation of organic amendments through biodisinfection, and reached 1.56% after the application of the treatment. Soil of Greenhouse 2 showed a 4.46% content of organic matter.

Plant vigour in controlled environmental chamber

Greenhouse 1 vs Greenhouse 2

Table 1 showed that when plants were grown in tomato monoculture greenhouse soil, where during the last 14 years, organic amendments have been added (Greenhouse 2), in all the parameters evaluated, the values are significantly higher compared with the plants grown in cucumber monoculture soil without supplies of organic amendments (Greenhouse 1). These differences were shown in the case of cucumber plants as well as tomato plants.

Table 1

Values (average±typical deviation) of the parameters considered for the assessment of cucumber and tomato plant vigour in controlled environmental chamber, depending on the soil of two greenhouses.
Greenhouse 1: soil with low content of organic matter and cucumber monoculture;
Greenhouse 2: soil with high content of organic matter and tomato monoculture.

	No. of leaves	Height (cm)	Root dry weight (g)	Aerial dry weight (g)	Leaf area (cm^2)
Pepino cv. Marketmore					
Greenhouse 1	2,3±0,5	4,01±0,75 b	0,05±0,01 b	0,15±0,05 b	17,63±9,51 b
Greenhouse 2	4,0±0,0	6,08±0,74 a	0,14±0,04 a	0,34±0,06 a	89,97±14,16 a
Tomate cv. Rio Grande					
Greenhouse 1	2,4±0,5 b	6,05±1,44 b	0,05±0,02 b	0,11±0,04 b	19,54±9,66 b
Greenhouse 2	4,4±0,8 a	8,95±0,95 a	0,10±0,03 a	0,29±0,05 a	92,52±13,91 a

Different letters in the same parameter and horticultural species show significant differences (P≤0.05) through t-Student test.

Greenhouse 1 before and after the incorporation of organic amendments through biodisinfection

Likewise, with reference to the results shown in Table 2, vigour of plants which were grown in cucumber monoculture soil without organic amendments supplies during 14 years (Greenhouse 1) improved after the first incorporation of organic amendments through biodisinfection (biosolarisation). In this way, in tomato as well as cucumber plants, the values of all the evaluated parameters are significantly higher after the addition of organic matter.

Table 2

Values (average±typical deviation) of the parameters considered for the assessment of cucumber and tomato plant vigour in controlled environmental chamber, depending on the soil of Greenhouse 1 (Soil with low content of organic matter and cucumber monoculture) before and after the incorporation of organic matter through biodisinfection

Greenhouse 1	No. of leaves	Height (cm)	Root dry weight (g)	Aerial dry weight (g)	Leaf area (cm^2)
Pepino cv. Marketmore					
Before	2,3±0,5 b	4,01±0,75 b	0,05±0,01 b	0,15±0,05 b	17,63±9,51 b
After	3,1±0,3 a	5,31±0,64 a	0,08±0,02 a	0,26±0,04 a	48,29±22,33 a
Tomate cv. Rio Grande					
Before	2,4±0,5 b	6,05±1,44 b	0,05±0,02 b	0,11±0,04 b	19,54±9,66 b
After	3,2±1,1 a	7,82±0,54 a	0,07±0,01 a	0,19±0,03 a	51,64±27,52 a

Different letters in the same parameter and horticultural species show significant differences (P≤0.05) through t-Student test.

Fungal, bacterial microbiota and oomycetes.

Greenhouse 1 vs. Greenhouse 2

Total fungal density grown in the greenhouse tomato monoculture soil in which during the last 14 years, organic amendments have been added (Greenhouse 2), showed significant differences (p-value<0,001 through Kruskal-Wallis test) compared with cucumber monoculture soil without supplies of organic amendments (Greenhouse 1), being the first case significantly higher (Figure 1A).

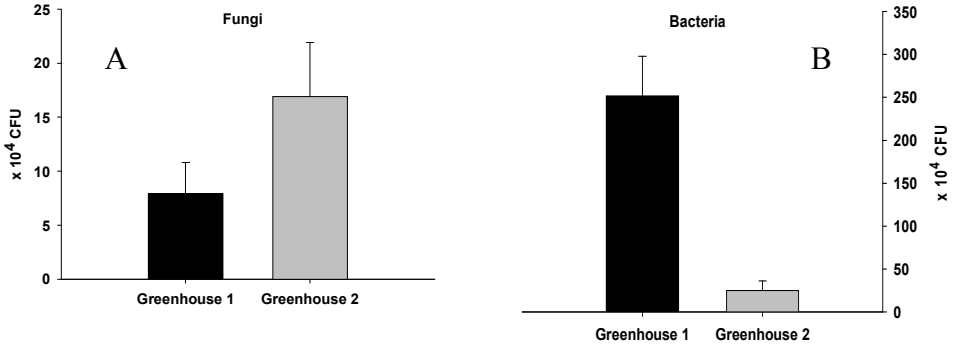

Fig. 1 - Total fungi (A) and bacteria (B) density in the soils of Greenhouses 1 (soil with low content of organic matter and cucumber monoculture) and 2 (soil with high content of organic matter and tomato monoculture). Values (average±typical deviation) expressed in 10^4 CFU·g^{-1} of dry soil. *** (p<0,001) through Kruskal-Wallis test

Likewise, fungal diversity in soil of Greenhouse 2 was also higher, it showed 13 different fungal genera compared with the 5 genera present in the Greenhouse 1 (Table 3) soil. In this respect, in Greenhouse 2 soil (soil with a high content of organic matter) population density of the different genera expressed was well-balanced, and showed total values that, in general, did not differ very much between genera, except for a possible exception. In this case, the *Acremonium* spp. and *Fusarium* spp. genera showed the highest populations, and *Gilmaniella* spp. and *Drechlera* spp. are the fungal genera that showed the lowest populations. However, in Greenhouse 1 soil (soil with low content of organic matter) the fungal population density belonging to *Fusarium* spp. genus stood out, followed by those belonging to *Penicillium* spp. genus, while the presence of *Acremonium* spp. and *Aspergillus* spp. was minimum. Also it must be considered that all the genera present in Greenhouse 1 soil were also found in Greenhouse 2 soil.

Table 3

Population density of fungi genera present in the soils of Greenhouse 1 (soil with low content of organic matter and cucumber monoculture) and of Greenhouse 2 (soil with high content of organic matter and tomato monoculture). Values (average±typical deviation) obtained from successive suspension-dilution technique and acidified malt extract agar, and expressed in 10^4 CFU·g^{-1} of dry soil.

	Greenhouse 1	Greenhouse 2
% Organic matter	0,54	4,46
Genera	x 10^4 CFU·g^{-1}	
Acremonium spp.	0,1±0,3	2,9±1,8
Alternaria spp.	--	0,4±0,7
Amblyosporum spp.	--	1,4±2,4
Aspergillus spp.	0,1±0,3	1,4±1,2
Drechlera spp.	--	0,2±0,6
Fusarium spp.	4,2±2,3	2,7±1,3
Gilmanella spp.	--	0,1±0,3
*Muco*r spp.	--	0,3±0,5
Paecylomices spp.	--	1,7±1,6
Penicillium spp.	1,4±1,1	1,9±1,4
Stachybotrys spp.	--	0,3±0,7
Stemphylium spp.	--	1,9±1,7
Non Identified	0,7±0,9	1,7±1,2
Total Genera	5	13

Likewise, the soil of Greenhouse 2 also showed a higher diversity of fungi species belonging to *Fusarium* spp. genus, it showed 4 species compared with a single species present in Greenhouse 1 soil (Table 5). In this respect, it must be highlighted that the species present in greenhouse 1, *Fusarium oxysporum*, which showed a very high population density and in this case it was identified as *F.oxysporum* f.sp. *radicis-cucumerinum* (the causing agent of root and crown rot in cucumber plants) was not present in the soil of Greenhouse 2. In this last soil, the species present depending on the population density in decreasing order were:

F. solani, F. equiseti, F. dimerum and F. sambucinum that did not show population values as high as *Fusarium oxysporum* in the soil of Greenhouse 1.

In the same way, the presence of oomycetes differs also depending on the soil considered (Table 7). In this case, in greenhouse 2 soil, which is rich in organic matter, there was presence of *Pythium* spp. (100% of presence) as well as *Phytophthora* spp. (40% of presence), while in the soil of Greenhouse 1, which is poor in organic matter, there was not presence of oomycetes.

However, on the contrary to the previous results shown, the total density of bacteria found in the soil of Greenhouse 1 (with low content of organic matter) was significantly higher (p-value<0,001 through Kruskal-Wallis test) than soil of Greenhouse 2 (with high content of organic matter) (Figure 1B).

Greenhouse 1 before and after the incorporation of organic amendments through biodisinfection

Total fungi density grown in greenhouse cucumber monoculture soil with low content of organic matter (Greenhouse 1) was significantly increased (p-value<0,001 through Kruskal-Wallis test) in the following season, after the first incorporation of organic amendments through biodisinfection (Figure 2A).

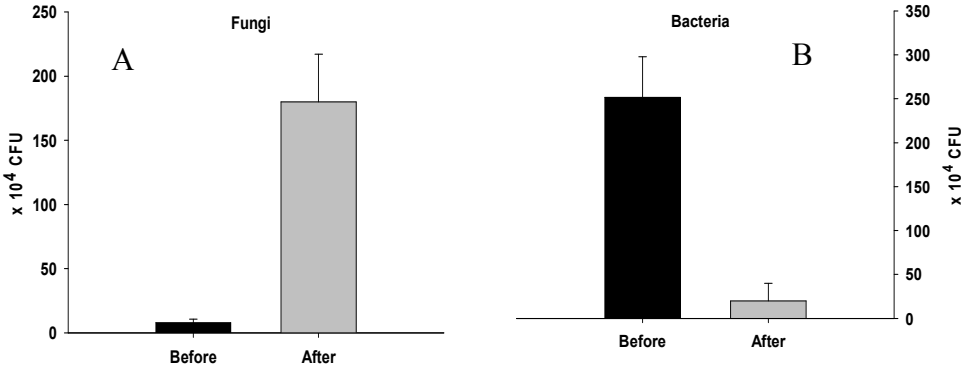

Fig. 2 - Total fungi (A) and bacteria (B) density in the soils of Greenhouses 1 (soil with low content of organic matter and cucumber monoculture) Before and After the incorporation of organic amendments through biodisinfection. Values (average±typical deviation) expressed in 10^4 CFU·g^{-1} of dry soil. *** (p<0,001) through Kruskal-Wallis test

In this case, fungal diversity was reduced from 5 genera present before the treatment to 3 genera detected after the treatment (Table 4). In this respect, it is noteworthy the shift of soil fungi genera which occurred after the treatment. In this sense, the genera that showed less importance before the treatment, *Acremonium* spp. and *Aspergillus* spp. stood out by their high population density after the treatment, and *Aspergillus* spp genus was the one stood out the most. On the other hand, after the treatment, there was no presence of *Fusarium* spp. genus fungi, which was the highest population density before biodisinfection was carried out.

Table 4

Population density of fungi genera present in the soils of Greenhouse 1 (soil with low content of organic matter and cucumber monoculture) Before and After the incorporation of organic amendments through biodisinfection. Values (average±typical deviation) obtained from successive suspension-dilution technique and acidified malt extract agar, and expressed in 10^4 CFU·g^{-1} of dry soil

Greenhouse 1	Before	After
% Organic matter	0,54	1,56
Genera	x 10^4 CFU·g^{-1}	
Acremonium spp.	0,1±0,3	12,0±10,3
Aspergillus spp.	0,1±0,3	166,0±37,8
Fusarium spp.	4,2±2,3	--
Penicillium spp.	1,4±1,1	--
Non Identified	0,7±0,9	2,0±4,2
Total Genera	5	3

Table 5

Population density of fungi species belonging to *Fusarium* spp. genera present in the soils of Greenhouse 1 (soil with low content of organic matter and cucumber monoculture) and of Greenhouse 2 (soil with high content of organic matter and tomato monoculture). Values (average±typical deviation) obtained from Komada's selective medium (1975) modified by Tello *et al.* (1991) and expressed in CFU·g^{-1} of dry soil

	Greenhouse 1	Greenhouse 2
% Organic matter	0,54	4,46
*Fusarium*sp.	CFU·g^{-1}	
F. oxysporum	17552,8±4215,0	--
F. solani	--	2622,0±424,3
F. equiseti	--	1645,2±170,3
F. dimerum	--	476,0±108,4
F. sambucinum	--	78,3±38,8
Total species	1	4

Table 6

Population density of fungi species belonging to Fusarium spp. genera present in the soils of Greenhouse 1 (soil with low content of organic matter and cucumber monoculture) Before and After the incorporation of organic amendments through biodisinfection. Values (average±typical deviation) obtained from Komada's selective medium (1975) modified by Tello et al. (1991) and expressed in CFU·g^{-1} of dry soil.

Greenhouse 1	Before	After
% Organic matter	0,54	1,56
*Fusarium*sp.	CFU·g^{-1}	
F. oxysporum	17552,8±4215,0	--
Total species	1	0

This last statement is corroborated by the results obtained in the assessments of the Fusarium flora through selective culture medium for *Fusarium* spp. genus (Table 6). Therefore, the high population of *Fusarium oxysporum,* which was present in the soil before the biodisinfection treatment, disappeared fully after the treatment.

The absence of oomycetes in greenhouse soil is clear after the treatment (Table 7).

Total density of bacteria borne by soil of Greenhouse 1 (with low content of organic matter) was significantly lower (p-value<0,001 through Kruskal-Wallis test) after biodisinfection treatment (Figure 2B).

Table 7

Presence of oomycetes (*Pythium* spp. and *Phytophthora* spp.) in the soil of Greenhouse 1 (soil with low content of organic matter and cucumber monoculture) Before and After the incorporation of organic amendments through biodisinfection, and in soil of Greenhouse 2 (soil with high content of organic matter and tomato monoculture). Values expressed as a % presence.

Oomycetes	Greenhouse 1 Before	Greenhouse 1 After	Greenhouse 2
Pythium spp.(% presence)	--	--	100
Phytophthora spp. (% presence)	--	--	40

The effect of soil fatigue on the decrease of plant development and/or production in horticultural crops is a topic that has not been dealt with very much in the current literature. For this reason, we have to turn to not very recent articles to find authors that, without referring to a specific pathogen action, confirm the existence of a fertility disorder of horticultural soils due to multiple reasons which can be accumulative, successive or simultaneous, and that it is translated into a complex syndrome that may cause yield losses, plant dwarfism, yellowing, underdevelopment and many others (Louvet, 1980; Bouhot 1983c, Tello *et al*. 2011). This phenomenon to which different authors linked to monoculture and/or repeated crops (Hoestra 1983; Massesse 1983; Sebillotte 1983; Messiaen *et al*. 1991; Cebolla & Maroto, 2004; Tello & Lacasa 2004), is not attributed to an exclusive cause due to its complexity, but it could have a close relationship with soil microbiota (Martínez *et al*. 2009; 2011), and therefore, with the levels of organic matter in cultivated soils, given that, the microbial community will depend on the available food. In this sense, the results obtained in the trials in a controlled environmental chamber with cucumber and tomato seedlings included in this study concluded higher vigour of plants grown in soils with supplies of organic amendments. In this case, in one of the studied soils (Greenhouse 1), the cucumber monoculture, which was repeated over time together, with the lack of supplies of organic matter, was translated into a deficient development of plants, possibly due to the adverse effects derived from the phenomenon of soil fatigue, which could be related with the nutritional state of the same (Bouhot 1983c, Chen *et al*. 1991). In this respect, since in this case the effects were not specific for cucumber plants (the lower development was also observed in tomato plants), in this soil, crop rotation, at least with tomatoes, would have not been enough to mitigate such effects. Likewise, in pepper monoculture developed in long-term cycles and very common in the fields of Cartagena, where organic amendments were not added to the soil, with the lack of pathogens, yield decreases were registered up to 60% of

the production. The reasons that caused these yield losses and were attributed to the phenomenon of soil fatigue were "covered up" for more than 15 years through the use of Methyl Bromide (Lacasa *et al*. 2002; Guerrero *et al*. 2004), an active matter that was withdrawn in Spain and the rest of developed countries in 2005, due to the commitments of the European Union with the Montreal Protocol.

On the other hand, the excellent development of the plants grown in the soil of the greenhouse where tomato monoculture was developed, but that added regularly organic amendments to the soil every year, make us think that the supplies of organic matter could be the reason of this "silencing" of the adverse effects derived from monoculture. Furthermore, this aspect is supported by the improvement observed in the plants grown in the first soil mentioned (very poor in organic matter) after the incorporation of different organic amendments through biosolarisation and by Guerrero *et al*.'s work (2014), in which the development of pepper plants grown in soil which showed specific fatigue on such crop, improved after applying biosolarisation treatments, reaching growths similar to those obtained in the treatments with Methyl Bromide. In this sense, it must also be taken into account that biosolarisation in greenhouse soils improves the physical and chemical characteristics of the same (Fernández *et al*. 2005; Núñez-Zofio *et al*. 2012).

Likewise, with reference to the microbiological content (fungi, bacteria and oomycetes) of the studied soils, soil with high content of organic matter, also had a higher amount and diversity of fungi than the soil in which the farmer did not add organic amendments. However, the bacterial population was significantly lower. In the same way, the incorporation of organic amendments through biosolarisation increased the total content of fungi in the soil with low content of organic matter, but a decrease was produced in the number of fungi genera as in the total content of bacteria. In this respect, the lower content of bacteria in the soils with higher amount of fungi, could be attributed to the inherent deficiencies of the analytical technique, since, probably the higher speed in the development and growth of some fungi genera compared with the bacterial community may limit the expression of this last one (Kirk *et al*. 2004). On the other hand, the decrease in the number of fungi genera after biosolarisation treatment may be attributed to the disinfecting power of the technique. In this sense, it is expected that only those genera with ability to resist such disinfecting power are present after the same. These same effects were found in Martínez *et al*.'s work (2009) in which general fungal microbiota, after different disinfection treatments (including biosolarisation), was reduced to *Aspergillus, Fusarium*, *Rhizopus and Penicillium* genera, being the most common and abundant *Aspergillus* and *Fusarium*. In this way, in our work, after the biosolarisation treatment, the presence of fungi belonging to *Aspergillus* spp is highlighted because it increased approximately 1000 times, compared with the population held before applying the treatment. Furthermore, it has to be considered that *Aspergillus* spp. are saprophytic fungi and decompose organic matter that participated actively in the nitrification

processes (Pateman *et al*. 1967), acting as the bacteria belonging to the *Nitrosomonas*, *Nitrosococcus* or *Nitrobacter* genera, among others, and played an important role in the leaching reduction of nitrates derived from the decomposition of organic matter. On the other hand, it is specially significant that the biodisinfection treatment carried out made completely inactive any *Fusarium oxysporum* propagule. In this way, during the campaign after the treatment, the cucumber plants grown in the greenhouse did not express any incidence of the disease caused by *Fusarium oxysporum* f. sp.*radicis-cucumerinum*, and showed higher vigour and yield (farmer's statement). Likewise, it must be highlighted that in the trials carried out in a controlled environmental chamber with the soil of that greenhouse before carrying out the biosolarisation treatment, disease was not expressed in the case of cucumber plants. Finally, the presence of oomycetes belonging to the genera *Pythium* spp. and *Phytophthora* spp. –recognised pathogens in horticultural crops, in the soil with high content of organic matter could be explained by a previous work (de Cara *et al*. 2009) carried out in the vicinity of the area in which the greenhouses, considered in this study, are located, and concluded that these microorganisms were introduced by irrigation water and passed from one greenhouse to another by contamination of the farm equipment. For this reason, farmers of this area use grafted plants, and therefore, these microorganisms appear in soil as common inhabitants.

CONCLUSIONS

Considering the complexity inherent to "soil environment" and in order to understand better the changes occurred after the supply of organic matter through biodisinfection, soil must be considered as a "living thing" in which the shift of its dynamic balance (Tello *et al*. 2011) caused by monoculture and the lack of organic matter must be restored until reaching its regeneration ability, which will be closely linked to the final microbial balance and, therefore, to the availability of organic matter in the soil. For this reason, it would be advisable to supply fresh organic matter to compost in the soil repeatedly, every year at the end of the horticultural season in order to reach a new balance to avoid soil fatigue in plant development.

In summary, the results showed that fatigue appeared in the soil with low content of organic matter, which showed at the same time the lowest density and diversity of fungal population. Unlike the Guerrero *et al*.'s study (2014), in which the authors advised crop rotation to mitigate the effects of specific fatigue in pepper, in the tomato and cucumber monoculture soils of this study, the addition of fresh organic matter seems to reconstitute its productive capacity, and this mitigates the fatigue and monoculture effects. It is very significant that this aspect has not been dealt with before in the specialised literature consulted.

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