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Effect of agricultural inputs and essential oils on the soil of vegetables in Colombia's Caribbean region

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Efecto de agroinsumos y aceites esenciales en el suelo de hortalizas en el Caribe colombiano

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Efeito de agroinsumos e óleos essenciais no solo de hortaliças no Caribe colombiano

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Abstract

The contribution of vegetables to food security and economic development in Colombia, as well as to environmental problems worldwide, justifies the interest to design sustainable production strategies for the agro-chain. This study was developed to evaluate the effect of the application of essential oils and agricultural inputs in chili peppers, beans and eggplants in Codazzi, Cesar, Colombia. The methodology included the compatibility analysis between pesticides used in these vegetables, and *Lippia alba* and *Cymbopogon citratus* oils in relation to their biocidal effect *in vitro* on native strains of *Macrophomina phaseolina*, *Phytophthora capsici* and *Colletotrichum gloeosporioides*. Applications of thiabendazole in an individual basis and combined with oils were carried out in the field. Physicochemical and microbiological indicators of soil, pests and diseases incidence and crop yield were measured. Oils controlled up to 97% of plant pathogens *in vitro* and exhibited compatibility with carbendazim and thiabendazole. In the field, up to 67 % of disease control was observed with *C. citratus* + thiabendazole compared to the control ($p = 0.00$), yields were close to the regional average, and better microbiological and physicochemical soil conditions were observed. In conclusion, there are differences in the edaphic effect between treatments, as the agrochemical and the oil combinations were more favorable than the individual effect of each product on the variables evaluated. The above exhorts to continue soil evaluations with oils to elucidate the duration of the described effects.

Keywords: Biological control, *Capsicum*, Cesar (Colombia), Codazzi, essential oils, *Phaseolus vulgaris*, *Solanum melongena*

Resumen

El aporte de las hortalizas a la seguridad alimentaria y al desarrollo económico de Colombia, así como a la problemática ambiental mundial, justifica el interés de diseñar estrategias productivas sostenibles para la agrocadena. Se desarrolló un estudio con el fin de evaluar el efecto de la aplicación de aceites esenciales y agroinsumos en cultivos de ají, fríjol y berenjena en Codazzi, Cesar. La metodología incluyó el análisis de compatibilidad de pesticidas utilizados en estas hortalizas y aceites de *Lippia alba* y *Cymbopogon citratus*, en lo que se refiere al efecto biocida *in vitro* en cepas nativas de *Macrophomina phaseolina*, *Phytophthora capsici* y *Colletotrichum gloeosporioides*. En campo, se aplicó tiabendazol de manera individual y combinado con los aceites. Se midieron indicadores fisicoquímicos y microbiológicos del suelo, la incidencia de plagas y enfermedades, y el rendimiento de los cultivos. *In vitro,* los aceites controlaron hasta el 97 % de los fitopatógenos y mostraron compatibilidad con carbendazim y tiabendazol. En campo, se observaron un control de enfermedades de hasta el 67 % con *C. citratus* + tiabendazol respecto al testigo ($p = 0,00$), rendimientos cercanos al promedio regional, y mejores condiciones microbiológicas y fisicoquímicas del suelo. En conclusión, existen diferencias en el efecto edáfico entre tratamientos, ya que el agroquímico y la combinación de aceites fueron más favorables que el efecto individual de cada producto en las variables evaluadas. Lo anterior exhorta a continuar realizando evaluaciones con aceites en campo, para dilucidar la duración de los efectos descritos.

Palabras clave: aceites esenciales, *Capsicum*, Codazzi, Cesar (Colombia), control biológico, *Phaseolus vulgaris*, *Solanum melongena*

Resumo

A contribuição das hortaliças para a segurança alimentícia e para o desenvolvimento econômico da Colômbia, bem como para a problemática ambiental mundial, justifica o interesse de elaborar estratégias produtivas sustentáveis para a agrocadeia. Desenvolveu-se um estudo com o objetivo de avaliar o efeito da aplicação de óleos essenciais e agroinsumos em cultivos de *ají* (*Capsicum annuum*), feijão e berinjela em Codazzi, Cesar. A metodologia incluiu a análise de compatibilidade de pesticidas utilizados nessas hortaliças e óleos de *Lippia alba* e *Cymbopogon citratus*, no que se refere ao efeito biocida *in vitro* em cepas nativas de *Macrophomina phaseolina*, *Phytophthora capsici* e *Colletotrichum gloeosporioides*. Em campo, aplicou-se tiabendazol de maneira individual e combinado com os óleos. Foram medidos indicadores físico-químicos e microbiológicos do solo, a incidência de pragas e doenças, e o desempenho dos cultivos. *In vitro,* os óleos controlaram até 97 % dos fitopatógenos e mostraram compatibilidade com carbendazim e tiabendazol. Em campo, foi observado um controle de doenças de até 67 % com *C. citratus* + tiabendazol a respeito do grupo controle ($p = 0,00$), desempenhos próximos à média regional, e melhores condições microbiológicas e físico-químicas do solo. Em conclusão, existem diferenças no efeito edáfico entre tratamentos, já que o agroquímico e a combinação de óleos foram mais favoráveis que o efeito individual de cada produto nas variáveis avaliadas. Isso leva a continuar realizando avaliações com óleos em campo, para dilucidar a duração dos efeitos descritos.

Palavras chave: *Capsicum*, Codazzi, Cesar (Colombia), controle biológico, óleo essencial, *Phaseolus vulgaris*, *Solanum melongena*

Introduction

The horticultural chain indicators show that the world market for vegetables has a growing tendency. According to the Food and Agricultural Organization of the United Nations (Organización de las Naciones Unidas para la Agricultura y la Alimentación [FAO], 2014) an annual production in 2014 of 276 million tons of vegetables. It appears that the consumption of vegetables have been benefited by consumer preferences for healthy products with nutraceutic properties (Corporación Colombia Internacional [CCI], 2015; Quipo, Rojas, Ramírez, & Ordóñez, 2013).

Likewise, in Colombia, this chain comprises an important economic line with a gross production of 1,495,835 t in 2015 (Departamento Administrativo Nacional de Estadística [DANE], 2016). Within the agricultural subsector in the Caribbean region, products as common bean (*Phaseolus vulgaris* L. [Fabaceae]), chili pepper (*Capsicum* spp. [Solanaceae]) and eggplant (*Solanum melongena* L. [Solanaceae]) make a special contribution to food security.

In year 2013, the production of these three-important species increased; for example, the production for chili pepper increased to 7,735 t; for eggplant it increased to 2,496 t and for common bean it rose to 5.560 t; furthermore, this increase has been supported by the fact that the Plan Nacional Hortícola [National Horticultural Plan] promotes productivity increase in vegetables to encourage export (CCI, 2015). However, market projections towards very selective markets as the United States and the European Union require the use of good agricultural practices (GAP) (Gaviria, Patiño, & Saldarriaga 2013).

However, in the Caribbean region of Colombia, the productive systems with sustainable alternatives are scarce and the impact that traditional agriculture causes on many livelihood aspects are stronger each day (Tofiño, Velásquez, & Zapata, 2016). In the region, integrated crop management is limited, among other reasons, by the reduced number of compatibility studies between bioinputs and agrochemicals that are currently being used in vegetable production systems (Melo, Ariza, Lissbrant, & Tofiño, 2015), as well as by the producer's lack of knowledge in relation to relevant aspects as the use of bioinputs as management strategies.

The aforementioned is aggravated by climate change effects that decrease the time period between the normal occurring pattern that is called El Niño-Southern Oscillation (ENSO), that affects the normal dynamic, causing the emergence of pests and increases in production costs (Lau, Jarvis, & Ramírez, 2013).

Biotic stresses and its traditional control affects negatively the productivity and safety of agricultural products, especially for small- and medium-scale producers; this is mostly due to the use of slightly selective and highly residual products that causes huge agricultural losses to these producers, affecting also public health and food security (Criollo, Lagos, Piarpuezan, & Pérez, 2011).

Among the sustainable practices found to improve the crop's health state, we find the associated crops and the use of bioinputs and essential oils for pathogen control (Villa et al., 2015). Alzate, Mier, Afanador, Durango, and García (2009) have found that essential oil of lemongrass (*Cymbopogon citratus*) has antifungal properties against *Colletotrichum acutatum* with an effectiveness of more than 60 %. Likewise, the use of bioinputs as *Trichoderma* spp. is a feasible alternative for pathogen control (Landero et al., 2015).

However, although in the tropical dry Caribbean area in Colombia there are few studies on the use of bioinputs in vegetables, various authors have recorded that non-fungicidal agrochemicals as herbicides and insecticides influence changes in the soil's microbial population and pathogen prevalence (Gaviria et al., 2013; Tofiño, Cabal, & Gil, 2012).

In addition, previous research on *in vitro* effects of *C. citratus*, agrochemicals and *Trichoderma* spp. to control *Colletotrichum gloeosporioides* show that these biocides are effective when used individually (Tofiño, Chinchilla, & Ortega, 2016).

Considering the aforementioned, integrated essays to evaluate both the individual, as well as the combined application impact of fungicides used in the traditional regional technological package together with essential oils in chili pepper, common bean and eggplant crops were carried out in Codazzi, Cesar, Colombia.

The aim of this study was to generate knowledge regarding the effect of chemical products used together regarding vegetable health and yield, to validate the use of essential oils to control pathogens of concern, and to verify the possible effect of the controls used in soil variables sensitive to agronomic management. This information may contribute to the development of GAP strategies for the studied ecoregion.

Materials and methods

Essential oils

The plants harvested for the essential oil extraction was carried out manually using healthy foliar material that previously had not been subject to fertilization plans or any kind of agrochemical application. These were classified in the herbarium José Cuatrecasas of Universidad Nacional de Colombia, Palmira campus as: *C. citratus* (DC.) Stapf. (Poaceae) (code 4952 20/10/2014) and *Lippia alba* (Mill.) N. E. Br. ex Britton & P. Wilson (Verbenaceae) (code 4863 18/10/2014).

The essential oil of *C. citratus* was extracted in the indigenous reservation of Kankuamo in Atánquez, Valledupar, Colombia. This location has a relative humidity between 56 % and 74 %, and the crops were cultivated in loamy and slightly acid soil, with a high fertility and 2 % of organic matter; the yield during extraction was 0.71 %.

The essential oil of *L. alba* was extracted in the natural products laboratory of Universidad de Córdoba, from plants harvested at Motilonia Research Center (RC), of the Colombian Corporation for Agricultural Research (Corporación Colombiana de Investigación Agropecuaria, Corpoica); the relative humidity of this location was 70.1 %, the soil is clay-loamy and slightly acid, with a moderate fertility and 1 % of organic matter; the yield during extraction was 0.5 %.

The extraction of both essential oils was carried out using 1 kg of fresh plant material and through steam distillation without solvents during three hours; the extract was conserved in amber colored vials with airtight sealed screw caps that were stored in a cooler at 4 °C.

Both oils were analyzed in the natural products laboratory of Universidad de Córdoba through phytochemical assessment using gas chromatography (GC) and mass spectrometry (MS), with a selective detector and presumptive identification in Apolar DB-5MS (60 m) and Polar DB-WAX (60 m) columns, using the NIST library with the MassLab program.

Pathogen isolation and inoculum production

In the microbiology unit of Motilonia RC, the phytopathogenic fungi *Macrophomina phaseolina* (Tassi) Goid., *Phytophthora capsici* Leonian and *Colletotrichum gloeosporioides* (Penz.) Sacc., were isolated from symptomatic roots, leaves and fruits of common beans, eggplant and chili pepper, respectively, and placed in potato and dextrose agar (PDA) at 25 °C.

These were then suspended at a concentration of 1.0 × 10^6 UFC/ml in sterile distilled water and identified according to the taxonomic keys published by Gañán, Álvarez and Castaño (2015); the identification was verified through molecular analyzes carried out in the laboratories of Corporación para Investigaciones Biológicas [Corporation for Biological Studies] (CIB) in Medellin, with DNA extractions using hexadecyltrimethylammonium bromide (CTAB). Amplification in the internal transcribed spacer (ITS) region with a polymerase chain reaction (PCR) technique was carried out using universal *primers* and sequencing.

For *Colletotrichum* and *Macrophomina* ITS1 and ITS4 were used, and for *Phytophthora* ITS4 and ITS6 were employed (table 1). The reactions were incubated at 95 °C during five minutes; then 30 cycles at 94 °C were carried out during one minute (denaturation), at 55 °C during one minute (alignment), and at 72 °C during one minute and a half (extension); one cycle of 72 °C during five minutes (final extension), and then a last cycle for an indefinite amount of time at 8 °C were carried out.

The PCR products were sent to Macrogen (Korea) for sequencing. The forward and reverse sequences were purified, edited and aligned using the Geneious software, version 9.1.5. The consensus sequences were compared to the ones available from the Genebank database with BLAST nucleotides to identify the isolates.

In vitro efficiency and compatibility

To evaluate the biocidal effect in the phytopathogens *M. phaseolina, P. capsici* and *C. gloeosporioides*, their growth in PDA was measured in concentrations between 50 and 1,200 µg/ml in each pure essential oil, according to the microorganism and compared to the PDA without essential oil as a negative control (Barrera & Bautista, 2008).

Fungi colonies of eight days of age in 1 cm slices were planted. Radial growth was measured on its diameter after seven days of incubation at 28 °C in triplicate. It was expressed as mycelial growth inhibition (MGI) percentage using the formula established by Broekaert et al. (1990, cited by Díaz et al., 2011): MGI (%) = [(dc - dt)/dc] × 100, where *dc* is the mycelial growth diameter in PDA without essential oil, and *dt* is the same diameter with oil. The lowest concentration was established as the oil's minimum inhibitory concentration (MIC) that produced an 80 % inhibition.

A compatibility analysis was carried out following the work of Melo et al. (2015) and modified due to differences in the agrochemical product used. Each pathogen's isolate grew in a PDA media with the insecticide chlorpyrifos, the herbicide glyphosate and the fungicide carbendazim and thiabendazole with recommended doses of 5 ml/L, 13.9 ml/L, 0.8 ml/L and 0.9 ml/L, respectively.

These four agrochemicals were mixed with the biocontrol *Trichoderma* spp. (Trichol®) and the MGI percentage of each pathogen and of this biocontrol agent were established in a similar way as with the essential oil treatments. Furthermore, the MIC of the essential oil with the highest MGI was used and then its compatibility with the agrochemical products was assessed.

Crop planting and application of treatments in the field

Seeds of the biofortified common bean Corpoica 39 (*Phaseolus vulgaris*), of chili peppers (*Capsicum* spp.) and of the C029 eggplant variety (*Solanum*

Table 1. PCR conditions to identify pathogens

Name	Primer	Sequence	Expected size (pb)
C. gloeosporioides	ITS4	TCCTCCGCTTATTGATATGC	500-700
	ITS1	TCCGTAGGTGAACCTGCGG	
M. phaseolina	ITS4	TCCTCCGCTTATTGATATGC	500-700
	ITS1	TCCGTAGGTGAACCTGCGG	
P. capsici	ITS4	TCCTCCGCTTATTGATATGC	700-1,000
	ITS6	GAAGGTGAAGAAGTCGTAGTAACAAGG	

Source: Prepared by the authors

melongena) were planted in the facilities of Motilonia RC in Codazzi, Cesar (Colombia) located at 10°00'03.0" N and 73°14'53.3" W, and 105 meters above sea level. These were planted with regular intervals between plants of 0.25, 0.50 and 0.80 m, respectively, and one meter between rows and in 5 × 5 m plots.

The experimental plot was fertilized according to the balance between the soil analysis and the specific requirements for each crop. For common bean 92 kg/ha of Agrimins, 100 kg/ha of diammonium phosphate, 750 cc/ha of Nutrifoliar, and 50 kg/ha of potassium chloride (KCl) were added; in the chili pepper plots doses of urea (200 kg/ha), phosphoric acid (150 kg/ha), KCl (400 kg/ha), calcium nitrate (60 kg/ha), magnesium sulphate ($MgSO_4$) (60 kg/ha) and Micronutrex (40 kg/ha) were added; and in the eggplant plots 135 kg/ha of urea, 310 kg/ha of diammonium phosphate and 565 kg/ha of KCl were added.

The plots were distributed in complete randomized blocks with four replicates in a factorial arrangement. Each block comprises a phytopathogen control treatment plus a control without treatment. Treatments were as follows: T0: without phytopathogen control; T1: essential oil of *L. alba*; T2: essential oil of *L. alba* + thiabendazole; T3: essential oil of *C. citratus*; T4: essential oil of *C. citratus* + thiabendazole, and T5: thiabendazole. The concentration of essential oils in all treatments was 1,200 µg/ml, and the recommended dose for thiabendazole was 0.9 ml/L.

Essential oils were applied by direct spray on the foliage and in the soil close to the roots, and thiabendazole was used according to the fact sheet 45 days after germination and when each crop's reached flowering stage. After being left to act for three days on the ground, a sampling to make an analysis of response variables was carried out. Additionally, correspondence between the strains evaluated *in vitro* and the one that were controlled in the field were verified through macroscopic and microscopic characterizations compared with taxonomic keys and molecular studies.

Physicochemical, biological and yield criteria measurements

Five hundred grams of pre-planting soil samples were collected in five sampling points within plots to obtain a composite sample of each experimental unit, from a depth of 15 cm for the microbiological analysis and 30 cm for the physicochemical analysis. Given the soil's microspecific variability these sampling points were marked to carry out samplings during cultivation with the same method.

Bacteria, fungi and actinomycetes counts were carried out for each sample and expressed in colony forming units (CFU) per gram of soil, using the serial dilutions technique and draining in Petri dishes according to the methodology published by Tofiño et al. (2012) and Gañán et al. (2015). Likewise, soil quality indicators as pH, organic matter, cation exchange capacity (CEC) and electric conductivity (EC) were measured.

An electronic precision balance was used to measure fruit production in each plot and expressed in t/ha, using damage scale tables in 20 plants per plot for the incidence of diseases and pests as the leafhopper (*Empoasca kraemeri* Ross & Moore [Hemiptera: Cicadellidae]), the silverleaf whitefly (*Bemisia tabaci* [Gennadius] [Hemiptera: Aleyrodidae]), and the mite *Tetranychus urticae* C. L. Koch (Acari: Tetranychidae), in common bean, chili pepper and eggplant.

Statistical analysis

The average and the standard deviation for growth and the phytopathogen inhibition variables were quantified. Further, a lineal regression bivariate model for essential oil and the phytopathogen's studied were used considering the inhibitory dose. Hereinafter, a variance analysis followed by the Tukey test with an alfa equal to 0.05 between treatments, considering the soil's physicochemical and microbiological variables and also in crop yield was carried out.

The reaction variables to pests and diseases expressed in percentage of plants with higher degree of severity and assessed visually were analyzed through a non-parametric frequency distribution evaluation by degree of reaction, followed by a chi-square test (Lagarde, Medina, Ramis, & Maselli, 2010).

A principal component analysis (PCA) was applied to these variables to identify the effect of each treatment in those properties, as well as to identify the most sensitive ones to the application of biological or chemical fungicides. The program SPSS version 20 was used.

Results and discussion

Composition of essential oils

In the sample of essential oil of *L. alba*, the most abundant compounds were identified: neral (16.2 %), geraniol (8.2 %), geranial (20.7 %), and β-caryophyllene (9.0 %), i.e. citral chemotype (36.9 %) (geranial + neral). Likewise, in the samples of the essential oil of *C. citratus* the most abundant compounds are myrcene (12.0 %), neral (23.1 %) and geranial (34.9 %), belonging to the citral chemotype (58.0 %).

Celis, Escobar, Isaza, Martínez, and Stashenko (2007) reported superior levels of these chemotypes in essential oil of *L. alba* extracted through microwave radiation-assisted hydrodistillation, whose mixture of alpha- and beta-unsaturated aldehydes- comprises 42 %.

Likewise, the concentration of the essential oil of *C. citratus* was found to have levels slightly below the level referred in studies carried out by Alzate et al. (2009), with 65 % of the citral chemotype extracted by steam distillation of the plant material cultivated in the municipality of Guarne, department of Antioquia, Colombia.

This heterogeneity was expected as the oil's composition and quality are conditioned by environmental factors, by the agronomic management and the extraction methods (Astani, Reichling & Schnitzler, 2010; Hennebelle, Sahpaz, Dermont, Joseph, & Bailleul, 2006). A biocidal effect of the citral chemotype in fungi of agricultural importance as *C. acutatum* (Alzate et al., 2009), *Aspergillus flavus* (Shukla, Kumar, Singh, & Dubey, 2009) and *C. gloeosporioides* (Anaruma et al., 2010) has been reported.

In vitro effect of essential oils of *L. alba* and *C. citratus* in phytopathogens and their compatibility with fungicides

After seven days with 400 µg/ml of essential oils there was no inhibition of *M. phaseolina,* while with double the amount (800 µg/ml) of *C. citratus* there was an inhibition of 92.9 % that exceeds the 80.0 % required for the MIC ($r = 0.988$). Therefore, this value was chosen to carry out compatibility tests with agroinputs and with commercial *Trichoderma* spp. products.

Regarding *C. gloeosporioides*, the essay with 1,000 µg/ml of the essential oil inhibited 100 % ($r = 0.994$), that is equivalent to the MIC (table 2). For *P. capsici,* the MIC was obtained with 109 µg/ml of *C. citratus* ($r = 0.949$), compared to the 150 µg/ml found for *L. alba.* As a consequence, in the following stage of this research, essential oil of *C. citratus* was used, as in sustainable strategies the producer's economy is the ultimate goal, and therefore, lower dosage decreases treatment costs (Alzate et al., 2009; Villa et al., 2015).

Nevertheless*, L. alba* is a promissory species due to its capacity to be cultivated in warm areas as the dry Caribbean region with a quick development and higher biomass values, as well as with essential oil volumes compared to *C. citratus* (Rivera, Cardozo, & García, 2004).

Therefore, we recommend its assessment with other pathogens of regional interest as *Fusarium* and *Pythium*. The biocidal effect of *C. citratus* on the three fungi studied agrees with the study carried out by Alzate et al. (2009) where the high fungicidal ability of the citral component is highlighted, as it inhibits germination and mycelial development.

The agrochemicals and their mixture have a significant inhibitory effect ($p = 0.00$) in the growth of the phytopathogens evaluated (table 3), except for glyphosate in *M. phaseolina* and *C. gloeosporioides*. On the other hand, carbendazim and thiabendazole showed and inhibition percentage ranging from 85.7 % to 88.1 %.

Table 2. Minimum inhibitory concentration of essential oils of *C. citratus* and *L. alba*

Essential oil	µg/ml	*M. phaseolina* (inhibition percentage)	µg/ml	*C. gloeosporioides* (inhibition percentage)	µg/ml	*P. capsici* (inhibition percentage)
C. citratus	400	0.0 ± 0.0c	473.5	9.8 ± 0.9c	67	32 ± 1.0b
	800	92.9 ± 1.3a	750.0	44.8 ± 1.5b	81	45 ± 1.0b
	1,200	97.5 ± 0.7	1,000.0	100 ± 0.0a	109	88 ± 0.5a
L. alba	400	0.0 ± 0.0c	600.0	50 ± 0.6b	50	11 ± 0.0c
	800	38.9 ± 0.5b	750.0	50 ± 0.5b	100	37 ± 0.5b
	1,200	90.5 ± 1.0	900.0	92.2 ± 0.8	150	86 ± 1.0a
Control	0	0.0 ± 0.0c	0.0	0.0 ± 0.0c	0	0.0 ± 0.0c

Values in the column followed by the same letter do not differ significantly according to the Tukey test ($p = 0.05$).
Source: Prepared by the authors

Table 3. Compatibility test among agrochemicals, *Trichoderma* spp. and essential oils to control *M. phaseolina, C. gloeosporioides* and *P. capsici*

Treatments	Dosis	*M. phaseolina* (inhibition percentage)	*C. gloeosporioides* (inhibition percentage)	*P. capsici* (inhibition percentage)
Control in PDA		0,0 ± 2,4a	0.0 ± 0.0a	0.0 ± 0.0a
Chlorpyrifos	5 ml/L	33.3 ± 7.1b	57.1 ± 2.4b	64.3 ± 2.4b
Glyphosate	13.9 ml/L	0.0 ± 2.4a	0.0 ± 4.8a	85.7 ± 2.4d
Carbendazim	0.8 ml/L	88.1 ± 2.4c	88.1 ± 2.4c	88.1 ± 2.4d
Thiabendazole	0.9 ml/L	85.7 ± 4.8c	88.1 ± 2.4c	88.1 ± 2.4d
Mixture of chemicals	sac	92.9 ± 2.4cd	90.5 ± 2.4c	64.3 ± 4.8b
Trichol® (*Trichoderma viride*)	1 g/L	100.0 ± 0.0d	100.0 ± 0.0d	100.0 ± 0.0e
Mixture of chemicals + EO	SAC + MIC	92.9 ± 0.0cd	92.9 ± 0.0d	85.7 ± 2.4d
Mixture of chemicals + Trichol®	SAC + Tri	92.9 ± 2.4cd	90.5 ± 2.4cd	78.6 ± 4.8c
Trichol® + EO	Tri + MIC	90.5 ± 2.4cd	90.5 ± 0.0c	100.0 ± 0.0e
EO of *C. citratus*	mic	92.9 ± 0.0cd	92.9 ± 0.0cd	88.1 ± 2.4d

SAC: sum of agrochemical concentrations; F: fungicide; EO: essential oil; MIC: minimum inhibitory concentration; Tri: Trichol® concentration. The letters in the inhibition column refer to the level of significance according to the Tukey test ($p \leq 0.05$).
Source: Prepared by the authors

Likewise, a mixture of agrochemicals potentiates fungicides even with a 92.9 % inhibition, as well as the mixture of agrochemicals with essential oils. In the case of *P. capsici*, this combination inhibited 85.7 %, compared to the mixture without oil that only inhibited 64.3 %. The aforementioned underpins the alternative use of essential oils to control phytopathogens as *M. phaseolina, P. capsici* and *C. gloeosporioides,* and shows their capacity to be integrated with phytosanitary chemicals used in horticultural systems in the dry Caribbean.

On the other side, although treatments with Trichol® as well as those with combinations with agrochemicals and essential oils inhibited the pathogen, it is important to underline that fact that essential oils inhibited a 100 % of the growth of the pathogen as well as of the biocontrol *Trichoderma* spp.

This indicates an incompatibility in the simultaneous use of a biocontrol agent and essential oils; therefore, this essay in the field was not continued further, as it requires a previous development of a Trichol® and essential oils rotation scheme, depending on the duration of the effect of the volatile compounds that are related to their biocidal ability.

In contrast, there was compatibility among Trichol®, chlorpyrifos and thiabendazole, but there was incompatibility with carbendazim, i.e. it was not further used for the field trials. Similar results were registered by Melo et al. (2015) for common bean in the dry Caribbean region, where the essential oil of *C. citratus* inhibited the pathogen *M. phaseolina,* as well as other oils in different concentrations (Khaledi, Taheri, & Tarighi, 2015; Sánchez et al., 2008).

Soil and crop yield response to the phytosanitary control

In general terms, the agronomical yield response to chili pepper, common bean and eggplant to different control treatments for pathogens as *M. phaseolina, P. capsici* and *C. gloeosporioides* and identified through molecular analysis, was significantly positive compared with the absolute control without treatment ($p < 0.05$).

In turn, the treatments where essential oils of *L. alba* and *C. citratus* were used, the microbiological activity of the soil showed significant differences among treatments compared to the control, as well as a higher value in organic matter content and electrical conductivity (table 4).

Preplanting analyses showed better microbiological conditions but a lower quality in physicochemical properties, compared to the data obtained in the crops in which treatments with essential oils were applied. Namely, in general, there is a slight inhibitory effect of the bacteria and fungi populations in soils where vegetables are grown and that have phytopathogen control, both with essential oils as well as with chemical treatments, obtaining the highest inhibition in the latter ($p < 0.05$).

In any case, crop productivity was not affected, but on the contrary, it was favored by the control of diseases (table 5). The previously mentioned is relevant as in the study area phytosanitary practices are deficient, and in some cases, no type of phytosanitary control practices are carried out to crops belonging to small-scale producers (Tofiño, Velásquez, & Zapata, 2016).

Soil pH in treatments in which vegetables were cultivated varied from having a strong to a moderate acidity (4.4 and 6.0), according to the scale for agricultural soils described by Ibarra, Ruiz, González, Flores, and Díaz (2009), similar to the preplanting condition, meanwhile in the control without applications a moderate to neutral level (6.3 to 6.8) was observed.

The acidifying tendency could be associated to factors as the acidic nature of the chemicals (pH 5.0) and of the oils (pH 3.0) applied (Rodríguez, Castro, Sánchez, Gómez, & Correa 2015); therefore, the addition of these kinds of products might have affected the soil`s pH compared to the control. This also generates reactions in macro-elements due to the decrease in the phosphorous and the potassium available in all treatments (Balta et al., 2015) (table 4).

Regarding the organic matter in soil cultivated with common bean and eggplant, all the treatments showed significant differences compared to the

Table 4. Response of soils and vegetable crops to different treatments for the control of *M. phaseolina, P. capsici* and *C. gloeosporioides*

	Code	pH	Organic matter (%)	Phosphorous (mg/kg)	Potassium (cmol/kg)	CEC (cmol/kg)	Electrical conductivity (dS/m)	Bacteria (ufc/g)	Fungi (ufc/g)	Yield (t/ha)
Common beans	Tx: previous to planting	5.3a	0.9a	203.1e	0.60d	6.8a	1.59c	1.6×10^6e	1.8×10^4c	-
	T0: without control	6.8e	0.8a	51.7d	0.30c	10.4c	0.48b	1.3×10^5d	7.0×10^2a	0.43a
	T1: *L. alba*	5.7bc	3.4d	1.4a	0.20b	19.2g	0.17a	5.9×10^4b	2.7×10^4d	0.49b
	T2: *L. alba* + thiabendazole	5.8bc	2.9c	1.9b	0.20b	12.6d	0.16a	7.2×10^4c	1.2×10^3a	0.65d
	T3: *C. citratus*	5.7b	3.0c	1.5a	0.20b	15.1f	0.16a	8.3×10^4c	3.7×10^4e	0.63c
	T4: *C. citratus* + thiabendazole	6.0cd	2.9c	1.7ab	0.10a	13.4e	0.16a	2.6×10^4a	4.4×10^3b	0.77e
	T5: thiabendazole	6.03d	2.1b	4.2c	0.30c	8.9b	0.15a	2.0×10^4a	2.2×10^2a	0.85f
Chili pepper	Tx: previous to planting	5.3b	0.9a	203.1f	0.60e	6.8a	1.59d	1.6×10^6d	1.8×10^4d	-
	T0: without control	6.6e	2.2ab	4,9d	0.70e	27.1e	0.19c	3.9×10^5c	6.8×10^3c	35.7a
	T1: *L. alba*	5.2b	2.8d	5.1d	0.30c	10.9b	0.16b	8.4×10^4b	$6{,}1 \times 10^4$f	44.0c
	T2: *L. alba* + thiabendazole	5.9cd	2.4bc	11.8e	0.20b	12.0c	0.16b	8.5×10^4b	5.1×10^4e	45.8cd
	T3: *C. citratus*	5.6c	2.1a	1.4a	0.10a	11.8c	0.13a	8.0×10^4b	6.1×10^3c	40.1b
	T4: *C. citratus* + thiabendazole	6.0d	2.0a	3.0c	0.50d	28.5f	0.16b	6.3×10^4a	4.1×10^3b	47.0d
	T5: thiabendazole	4.4a	2.5cd	1.8b	0.10a	15.5d	0.15ab	6.5×10^4a	1.2×10^3a	47.6d

	Code	pH	Organic matter (%)	Phosphorous (mg/kg)	Potassium (cmol/kg)	CEC (cmol/kg)	Electrical conductivity (dS/m)	Bacteria (ufc/g)	Fungi (ufc/g)	Yield (t/ha)
Eggplant	Tx: previous to planting	5.3a	0.9a	203.1f	0,60f	6.8a	1.59e	1.6×10^6d	1.8×10^4d	-
	T0: without control	6.3d	1.9a	6.7d	0.12a	13.5b	0.51d	5.0×10^6e	1.3×10^4d	15.7a
	T1: *L. alba*	6.0c	2.5b	8.5e	0.34e	17.4f	0.18a	3.9×10^5c	6.0×10^3c	17.6b
	T2: *L. alba* + thiabendazole	5.8bc	3.3c	3.1c	0.32e	16.1e	0.23c	2.0×10^5ab	4.4×10^3b	18.0c
	T3: *C. citratus*	5.6a	3.1c	1.0a	0.18c	8.2a	0.19a	2.9×10^5bc	2.7×10^3a	18.0c
	T4: *C. citratus* + thiabendazole	5.7ab	3.3c	1.2a	0.28d	15.1d	0.22bc	3.2×10^5c	2.8×10^3a	18.9d
	T5: thiabendazole	5.9bc	2.1a	2.5b	0.15b	14.2c	0.20ab	9.2×10^5a	2.1×10^3a	19.1d

pH: potential hydrogen; CEC: cation exchange capacity. Values (by crop) in one column, followed by the same letter, do not differ significantly according to the Tukey test (5 %).
Source: Prepared by the authors

Table 5. Evaluation of the frequencies in plants per treatment in each disease and pest scale

Treatments	Reaction to diseases*			Pest incidence**		
	Common beans	Chili pepper	Eggplant	Common beans	Chili pepper	Eggplant
T0: without control	5.5	98.0	0.0	11.5	10.0	0.0
T1: *L. alba*	4.6	60.0	0.0	19.5	0.0	0.0
T2: *L. alba* + thiabendazole	4.7	59.0	0.0	4.3	0.0	0.0
T3: *C. citratus*	4.4	47.0	0.0	12.2	0.0	0.0
T4: *C. citratus* + thiabendazole	5.0	31.0	0.0	5.0	0.0	0.0
T5: thiabendazole	4.0	49.0	0.0	18.2	0.0	0.0
$p > 0.05$	0.22	0.00	-	0.36	-	-

*Diseases prevalence percentage in level 5; **Percentage of plants susceptible to pest attacks; P: value of the Chi-square test.
Source: Prepared by the authors

control (without application) and the soil conditions during preplanting. The highest value was observed in individual essential oils application treatments, i.e. T1 and T3.

In chili pepper plots levels of organic matter were similar in treatments with *C. citratus* and the control, with adequate values for crop development in all treatments, although these were higher than the ones found in preplanting conditions. This indicator has been reported by Camacho, Luengas and Leiva (2010), due to its sensitivity to agricultural intervention.

The CEC response was highly sensitive to the management, with significant differences among treatments, including the control. It is probable that this is due to the nature of the molecules of the essential oils and of the chemical fungicide that caused a different CEC influenced by the soil's solubility, temperature and pH.

Despite sensitivity in management, the CEC was positive in the vegetable crops under study and this contributed to nutrient assimilation supplied during fertilization; this is reflected in a yield according to the one reported for the region (Camacho et al., 2010; Sacchi, Campitelli, Soria, & Ceppi, 2015).

Furthermore, electrical conductivity did not show statistical differences among treatments, but they were found between these and the control; this was higher in all the soils assessed and classified as non-saline. In other words, these are qualitatively equal as they have the same salinity range, and therefore, the plant's development is not affected in none of the treatments in relation to this variable (Mogollón, Martínez, & Torres, 2015).

Toxicity and residuality has been found in soils that integrate the horticultural productive system of the dry Caribbean in Colombia caused by chlorpyrifos, glyphosate, thiabendazole and carbendazim. According to Sebiomo, Ogundero, and Bankole (2011), negative effects on the soil's cultivable bacteria and fungi microbiota have been caused by chlorpyrifos and glyphosate; this is an extremely relevant finding due to the effect of the microbial dynamics on plant nutrition and pathogen attack resistance.

Works of authors as Yunlong, Xiaoqiang, Guohui, Yueqin, and Hua (2009), Srinivasulu, Mohiddin, Madakka, and Rangaswamy (2012) and Tortella et al. (2013) report low contamination values or damages associated to fungicides as carbendazim when applied in lower doses to the ones recommended commercially.

Therefore, it is necessary that these and other chemical products in the study zone are managed in the framework of the GAP strategy, based on the monitoring and the control of infectious outbreaks; moreover, also on the controlled use of these to reduce the effect in the soil that corresponds to the root's influence area (Melo et al., 2015).

In addition, the study showed that the bacterial population had decreased and that there were significant differences compared to the control without applications, and to the soil features previous to planting. Fungi quantification was higher in treatments where only essential oils of *L. alba* or *C. citratus* were applied, while with the individual chemical treatment T5 the lowest values were found.

In all cases, the cultivable microbial population showed values beneath the values reported by Melo et al. (2015) for tropical soils; this is associated with acid soils, as this was found to occur in a more pronounced way in treatments that included fungicide control. This could be linked with the biocidal nature of the chemical compound that can generate an indiscriminate lethal effect on beneficial microorganisms as well as in pathogens (Carbonell et al., 2000; Melo et al., 2015).

A similar situation was seen in treatments with essential oils but in a higher magnitude, as the residuality is lower in volatile biocidal components as citral that prevents the germination of fungal cells (Quintana, González, Plascencia, & Cortez, 2010).

However, once the citral's average lifespan is exceeded, the microbiota is restored (Alzate et al., 2009; Jaurixje, Torres, Mendoza, Henríquez, & Contreras, 2013). In all cases, the magnitude of the essential oils and the fungicide effects must be verified on the soil's micribiota through high sensitivity techniques as metagenomics (Dober et al., 2016).

In relation to yield, values were higher compared to the crops with treatments, and significant differences were found among them: in common beans, the chemical treatment T5 (thiabendazole) gave the best results with a production of 0.85 t/ha, followed by T4 (essential oil of *C. citratus* + thiabendazole), with 0.77 t/ha, that has a value close to the regional average, i.e. 0.8 t/ha. A similar effect in eggplant was observed with treatment T5 (thiabendazole) obtaining a yield of 19.1 t/ha, that is higher to the regional average 18 t/ha (CCI, 2015).

Regarding the phytosanitary behavior (table 5) there were only significant differences in the diseases reaction as the attack was higher in the control for all crops; however, it was higher in chili pepper with 98 % damage by stains in fruits, but the yield was not affected as it exceeded the regional average of 6.6 t/ha. Further studies should evaluate the treatment effect in storage durability (CCI, 2015; Guigón & González, 2001; Silva et al., 2014).

In relation to plague presence responses an incidence between 4.3 % and 19 % was observed. In common bean, all the treatments showed a 5 % leafhopper incidence while the control treatment in eggplant showed a 10 % incidence level of the silverleaf whitefly.

Principal component analysis of rhizosphere and yield variables in the control of fungi in chili pepper, common bean and eggplant

In general terms, the principal component analysis (PCA) results, that explained in average the 98.7 % of the variance among physicochemical, microbiological and yield variables in response to the control of phytopathogens as *M. phaseolina, P. capsici* and *C. gloeosporioides*, show different edaphological and agronomical responses according to the type of treatment used compared to the control without applications.

The above mentioned is associated with higher pests and diseases incidence, but with a lower change in the soil's physicochemical and microbiological indicators. Furthermore, there are also differences ($p < 0.05$) between the use of essential oil on its own and when combined with the chemical fungicide.

Thus, considering the current study conditions, the treatments applying exclusively essential oils as well as those with chemical products caused negative impacts on the soil, but when combined, these were reduced ($p = 0.00$) or they promoted the behavior of some beneficial edaphological variables for the crops.

It is in the case of cultivable soil bacteria in common bean, in the degree of acidity in soil where chili pepper was cultivated and in the eggplant's organic matter, that superior values were found when combined treatments were carried out, i.e. T2 (essential oil of *L. alba* + thiabendazole) and T4 (essential oil of *C. citratus*+ thiabendazole), compared to those that employ essential oils or chemicals in an individual basis (table 4).

This condition responds to different factors. Synthetic pesticides are based in a sole product, in contrast to essential oils that are a complex mixture of components that includes minor constituents that act in synergy inside the plant as a defense strategy (Batish, Singh, Kohli, & Kaur, 2008).

However, the volatile nature requires compensation with stabilizing agents. Different studies have documented that benzimidazoles have an antioxidant function in oleic bases of plant origin and hydrocarbons (Basta et al., 2016; Komatsu, Souza, Carvalho, de Campos, & Totten, 2010); therefore, it is important that further assessments of this effect that thiabendazole causes on essential oils are carried out.

In this essay, the bacteria and fungi under chemical treatments decreased in more than 80 % compared to the control without phytopathogen control applications, meanwhile with a combined management a maximum of 50 % was achieved. This suggests that there is the possibility that the above mentioned can be included in GAP, due to the level of toxicity that thiabendazole shows (Organización Mundial de la Salud [World Health Organization, WHO], 2010) and the need to evaluate dosages that are lower than the traditionally recommended in combination with essential oils; this, in order to decrease its use intensity, as the soil's microbiota reestablishes very quickly in natural environments compared to the ones with an exclusive chemical management (Gaviria et al., 2013).

This agrees with authors as Yunlong et al. (2009) that recorded similar tendencies and mentioned a recovery of the microbiota in 21 days after the use of chemicals was suspended, compared to the control without application. Whilst, studies with fungicides show a recovery after 90 days after the last application was carried out, when using natural extracts this begins after only 10 days (Monkiedje, Olusoji, & Spiteller, 2002; Zhao et al., 2016). Moreover, the organic or chemical nature of the fertilizer used affects the pH and the bacterial composition (Ling et al., 2014).

Specifically, in the case of common bean, the two components explained 96.5 % of the essay's variance (figure 1). The first one with 73.2 % contributes mostly to variables as pH, organic matter and bacterial count. Furthermore, the second with 23.3 % is associated with the CEC and the incidence of the pest evaluated, i.e. the leafhopper *E. kraemeri*.

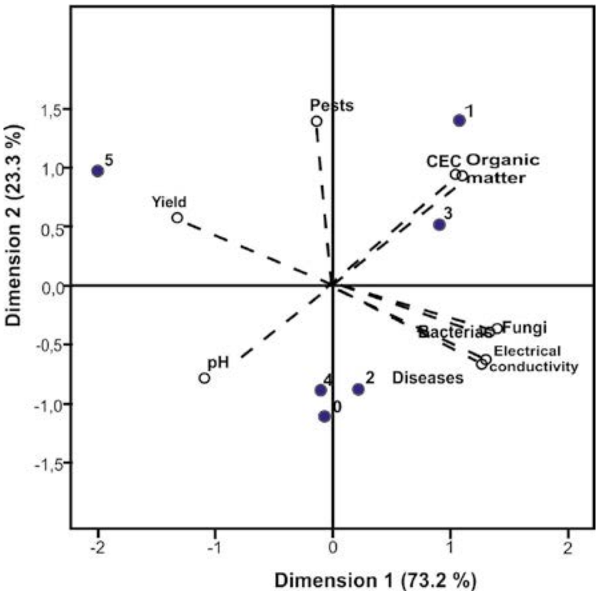

Figure 1. Biospatial dispersion diagram of physicochemical, microbiological and yield variables for common bean with diverse phytopathogen management. The numbers show the treatment used: 0: without control; 1: *L. alba*; 2: *L. alba* + thiabendazole; 3: *C. citratus*; 4: *C. citratus* + thiabendazole; T5: thiabendazole.
Source: Prepared by the authors

This is due to the fact that the soil's organic matter, pH and microbial population are sensitive to common bean cultivation given its symbiotic nitrogen fixation activity, that is in turn influenced by the crop's agronomic management (Ángeles & Cruz, 2015; Esquivel, Lamadrid, Díaz, Torres, & Pérez, 2014).

Differences among treatments have been found consisting exclusively of essential oils and those in which these are combined with chemicals. However, all could be differentiated from the control treatment in which some favorable properties for the crops cultivated were conserved, but there is also a higher diseases incidence.

The first group comprised only by essential oils favored CEC and the adequate development of fungi groups in the soil; however, it also showed a higher pest incidence. This suggests that the combination with chemical fungicides favors mostly the crop's yield. This would be influenced by the phytoprotective effect of the essential oils and their components (Batish et al., 2008) that act in synergy with the high fungicidal activity of thiabendazole. Although the fungicide controlled the phytopathogens' pressure in the crop in a more effective way, it is necessary to develop further studies on the sustainability during the time the phytoprotective effect occurs, given the volatile nature of the oils and their high biodegradability (Batish et al., 2008; Vera, Olivero, Jaramillo, & Stashenko, 2010).

These results are consistent with those referred in the case of soybean, and in relation to the evaluation of the fungicide Vitavax in association with *Rhizobium*, that affected the microbial dynamics in the soil and nodulation, as well as the reaction to diseases and even to crop yield (Vozniuk, Tytova, Lyaska, & Lutynska, 2015).

Regarding the PCA in chili pepper, the two components explained 99.8 % of the essay's variance. The first one with 64.6 % of the variance contributed mostly to variables as pH, electrical conductivity and bacteria count per gram of soil. The second one with 35.2 % was associated with the CEC, organic matter and fungi count per gram of soil; this is similar to what was found in common bean, indicating the high sensitivity of the chemical and microbiological variables to the treatments in the soils of the study area.

Moreover, a different behavior was found among the control and all other treatments, and the one with only essential oil of *L. alba* (T1), that was related positively with the organic matter content and the adequate fungi development in the soil (figure 2). These results are consistent with the ones registered with the application of mefenoxam and metalaxyl in sandy-loam soils, where the microbial activity index as well as the microbial population density in specific microbial systems, were more sensitive indicators during change (Monkiedje, Ilori, & Spiteller, 2002).

Consistent with what was found in common bean, the control without applications conserves various favorable properties for the crop, but it also shows a higher prevalence of diseases and a lower yield, that was higher with T4 and T5.

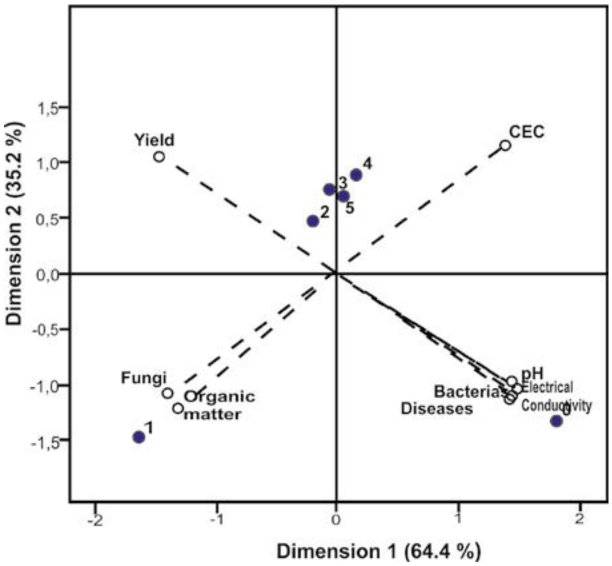

Figure 2. Biospatial dispersion diagram of physicochemical, microbiological and yield variables for chili pepper with diverse phytopathogen management. The numbers show the treatment used: 0: without control; 1: *L. alba*; 2: *L. alba* + thiabendazole; 3: *C. citratus*; 4: *C. citratus* + thiabendazole; T5: thiabendazole.
Source: Prepared by the authors

Likewise, Carvalho et al. (2008), cited by Stangarlin, Kuhn, Assi, and Schwan (2011) carried out an *in vivo* essay with plant extracts of *C. citratus* and *Cymbopogon martini* to control *C. gloesporioides* in chili pepper; they found significant difference in diseases control in relation to the control, although at a concentration of 10 %, a minor weight reduction in fruits with *C. martini* was found.

The PCA for eggplant explained 99.9 % of the essay's variance (figure 3). The first component with 83.7 % was associated with pH, bacterial count and crop yield (negative relation), that could be explained by the soil's acidity conditions favorable for bacteria development with a low influence of the biogeochemical cycles or in the eggplant's yield (Sapundjieva, Kostadinov, Kartalska, Shilev, & Naidenov, 2009).

Likewise, the second component with 16.2 % of the variance included the CEC. Differences among treatments that contained essential oil of *L. alba*, the control and the other treatments were found. The control, just as in the cases of common bean and chili pepper conserved various edaphic properties favorable for the crop, but there was also a higher prevalence of diseases.

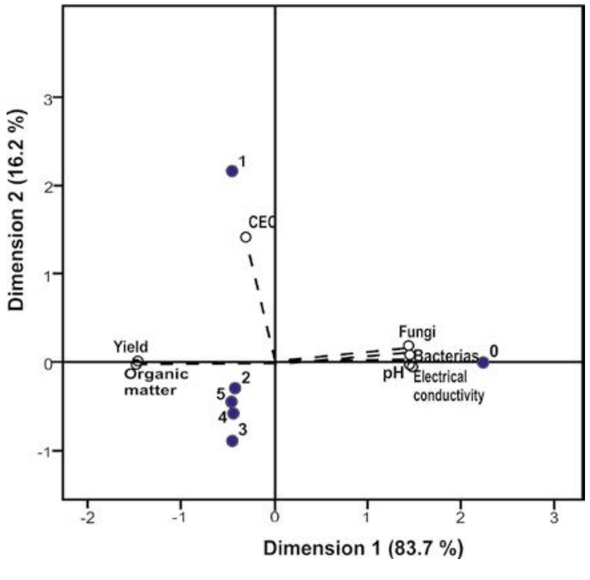

Figure 3. Biospatial dispersion diagram of physicochemical, microbiological and yield variables for eggplant with diverse phytopathogen management. The numbers show the treatment used: 0: without control; 1: *L. alba*; 2: *L. alba* + thiabendazole; 3: *C. citratus*; 4: *C. citratus* + thiabendazole; T5: thiabendazole.
Source: Prepared by the authors

Treatment T1 (*L. alba*) was related positively with the CEC, and T2 (*L. alba* + thiabendazole) with the organic matter content and yield. The aforementioned could be associated, in addition to its fungicide activity, to the protective effect in vegetable roots that was reported by Vera et al. (2010) for onion (*Allium cepa* L.); this is demonstrated with the decrease in chromosome aberrations as well as with an increase in the length and weight of roots exposed to mercurochrome in 10 and 500 μm, plus essential oil of *L. alba* at 100 μm, with significant differences in relation to the chemicals applied individually.

In combination with chemicals, these treatments reached similar yield values to the regional averages and better physicochemical and microbiological conditions. The treatment applied exclusively with fungicide was more aggressive for the soil's microbiota and promoted its acidity, although it obtained higher results both regarding diseases control as well as crop yield.

In general terms, the PCA for common bean and chili pepper associated to the variance among treatments showed relations with pH, level of organic matter and microbial population (bacteria and fungi) reactions.

Regarding eggplant, the soil's organic matter was similar among treatments, except the control without application. The previously mentioned suggest that the agronomic management effect on the biogeochemical and edaphic cycles influences in a greater or lesser degree the yield according to each vegetable species.

Therefore, work must be carried out in the standardization of the combined use of chemical fungicides and essential oils through the measurement of their impact on various aspects as the properties associated to health and quality of the agricultural soil, as well as on its long-term conservation, among others.

In addition to the variables assessed in this study, other biological criteria of special importance due to their crop management sensitivity —as the stability of microaggregates, the relation between annelids and ants, the study of the mesofauna and macrofauna, among others—, must be included (Altieri & Nicholls, 2001; Tofiño, Velásquez, & Zapata, 2016).

Conclusions

In general terms, essential oils controlled effectively the phytopathogens *M. phaseolina, P. capsici* and *C. gloeosporioides,* with a 100 % *in vitro* inhibition and 67 % efficiency in the field. The results suggest that the treatments that consist in the combination of essential oils and fungicides (T2 and T4) showed the best vegetable crop yields in comparison to the control treatment without applications.

Seemingly for the vegetable crops evaluated, this tendency has a lower impact in the soil in relation to pH, microbiological activity for bacteria, rhizosphere fungi and nutrient availability indicators, in comparison to the treatments with essential oils or chemical fungicides. In these last ones, an influence in nutrient dynamics was observed but must be verified in further works according to specific crop needs.

In consequence, it has been recommended to verify the control in the field for fungicide subdosages associated with the concentrations of essential oils validated *in vitro* for the pathogens evaluated in this study.

Additionally, we suggest to carry on with more specific tests as measuring the impact in all the properties associated to the soil`s health and degradation schemes, as well as in the natural elimination of essential oils in an individual or in a combined manner together with chemical fungicides.

Likewise, the results obtained can be refined through further studies with metagenomic strategies in order to define the effects of the treatments in the general operation of the agricultural soils.

On the other hand, the use of essential oils of *L. alba* and *C. citratus,* together with the use of bioinputs based on *Trichoderma* spp., must be analyzed in the field in a rotation scheme with fungicides or essential oils individually and in combination, due to the incompatibility that was found in *in vitro* essays when combined.

For the bioprospection of aromatic plants and the regional strengthening of the agrochain, it is of utmost importance the design of agroproductive strategies for species as *L. alba* and *C. citratus,* that optimize their essential oil productivity with higher phytochemical quality in the dry Caribbean region of Colombia.

Finally, phytotoxicity and cytotoxicity studies of essential oils applied as aqueous foliar solutions should be carried out in order to move forward in formulations of fungicide products in the framework of GAP for horticultural production.

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