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# Evaluation of agrochemicals and bioinputs for sustainable bean management on the Caribbean coast of Colombia

## Evaluación de agroquímicos-bioinsumos para el manejo sostenible del frijol en la costa Caribe colombiana

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### ABSTRACT

The sustainable expansion of bean cultivations requires technologies that do not limit their phyto-recovering properties. Therefore, the objective of this study was to propose agronomic management of conservation for bean cultivation considering the microbiological characteristics of two mega-environments of the Colombian Caribbean coast and the compatibility between agrochemicals and bioinputs. The methodology included rhizospheric microbe population counts, identification of phytopathogenic fungi in plant tissues and soils, compatibility studies of pesticides with biocontrollers, and determination of residual contents of pesticides in bean seeds. The microbial populations corresponded to those previously registered for the lower tropics, but with quantitative differences in the genera. *Phytophthora*, *Colletotrichum* and *Fusarium* were registered in the humid Caribbean, while *Colletotrichum* and *Curvularia* affected crops in the dry Caribbean. The *Beauveria* bioinput was not compatible with the evaluated agrochemicals, while *Trichoderma* was compatible with chlorpyrifos, thiabendazole and oxycarboxin. *Metarhizium* was compatible with glyphosate and oxycarboxin at 10% of the recommended dose. Lindane residues were found in the beans harvested at three of the studied locations. The combined use of agrochemicals and bioinputs on bean crops is feasible as long as the time of application of the latter is made according to the half-life of the chemical and the organic matter content of the soil is increased.

**Key words:** sustainable agriculture, entomopathogens, biocontrollers, *Phaseolus vulgaris* L.

### RESUMEN

La expansión sostenible del cultivo de frijol requiere el desarrollo de tecnologías que no limiten sus propiedades fito-recuperadoras. En consecuencia, el objetivo del estudio fue proponer un manejo agronómico de conservación del cultivo de frijol, considerando las características edafológicas y microbiológicas de dos mega-ambientes del Caribe colombiano y, la compatibilidad entre agroquímicos y bioinsumos. La metodología incluyó recuento de poblaciones microbianas rizosféricas; identificación de hongos fitopatógenos en tejido vegetal y suelo; estudio de compatibilidad de pesticidas con biocontroladores; y determinación del contenido de residuos de pesticidas en semillas de frijol. Las poblaciones microbianas correspondieron a los registrados previamente para suelos del trópico bajo, pero con diferencias cuantitativas de géneros. *Phytophthora*, *Colletotrichum* y *Fusarium* fueron registrados en el Caribe húmedo mientras se identificaron *Colletotrichum* y *Curvularia* en el Caribe seco. El bioinsumo *Beauveria* no fue compatible con los agroquímicos ensayados, mientras que *Trichoderma* fue compatible con clorpirifos, tiabendazol y oxicarboxin, y *Metarhizium* con glifosato y oxicarboxin al 10% de la dosis recomendada. Se identificó la presencia de residuos de lindano en semillas cosechadas en tres localidades. El uso combinado de agroquímicos y bioinsumos en el cultivo de frijol es factible, siempre y cuando el momento de aplicación del último sea acorde a la vida media del químico y se incremente el contenido de materia orgánica del suelo.

**Palabras clave:** agricultura sustentable, entomopatógenos, biocontroladores, *Phaseolus vulgaris* L.

## Introduction

The bean is one of the crops with the greatest direct consumption in Colombia and worldwide and has a national annual production of 71,467 t, cultivated in 34,032 ha (DANE, 2011). It plays an important role from

an environmental standpoint, given its nitrogen fixing properties, which is why it has been used extensively in sustainable agriculture, including the recuperation of degraded soils due to intensive agriculture, contamination by chemicals, and mining, among other anthropogenic interventions (García, 2013).

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However, the possible phyto-recuperative actions of the bean are limited by traditional agronomic management, characterized by the indiscriminate application of chemicals, socioeconomically justified by the necessity of increasing production and food security (Xie *et al.*, 2011). Such management deteriorates the physicochemical and biological properties of the soil, jeopardizing its quality and thereby being the third most common cause of desertification (Pava, 2011). This is problematic given the high use of agrochemicals in comparison to organic products on the Colombian Caribbean coast (DANE, 2011).

Additionally, the agrochemicals most frequently used in the region are nonselective and, therefore, have a low effect on pathogen and pest control. The lack of technical assistance for farmers limits the identification of causal agents, resulting in repeated applications of agrochemicals in order to control phytosanitary problems (Jojoa and Salazar, 2011; Tofiño *et al.*, 2012). Because of this, an unfavorable environmental impact is generated due to a reduction in the beneficial rhizospheric microbiota that is qualitatively and quantitatively sensitive to the action of diverse pesticides. This microbiota intervenes in the productive process of beans, such as growth promoters, entomopathogens, and antagonists, and alters the metabolites secreted by plants that constitute an important source of nutrients for diverse functional groups of microorganisms (Soto *et al.*, 2010).

In beans, up to 20 applications of insecticides and six to eight applications of fungicides have been reported, resulting in an increased risk of resistance to the active substances (Morros and Pire, 2003; Rodríguez *et al.*, 2003). Additionally, high application frequencies lead to elevated contents of pesticide residues in products that are highly carcinogenic, mutagenic and may affect the central nervous system (Roubos *et al.*, 2014). This justifies the development of alternative integral management systems for the sustainable production of food products (Gallego *et al.*, 2010; Santos *et al.*, 2013).

Especially on the Colombian Caribbean coast, the Integrated Pest and Disease Management (IPDM) is limited because, among other reasons, of the lack of compatibility studies between bioinputs and the agrochemicals commonly used in vegetables and the lack of information regarding the agroecological distribution of phytopathogenic fungus in the soil that may attack the bean (Santos *et al.*, 2013; Tofiño, 2013).

This study was developed with the objective of finding basic elements that contribute to the formulation

of a sustainable productive model for bean cultivation, based on the edapho-microbiological characteristics of the Colombian Caribbean coast and the compatibility between agrochemical and biological inputs, that will allow for an improvement in bean production as well as the quality of soils.

## Materials and methods

**Location.** Samples were collected from two commercial production zones, selected for their vocation for bean production and problems with desertification in the humid Caribbean (Cerete [department of Córdoba] and Carmen de Bolívar [department of Bolívar]), and from seven locations in the dry Caribbean (Codazzi, Manaure, La Jagua, Tamalameque and Valledupar [department of Cesar]; San Juan [department of La Guajira]; and Sevilla, [department of Magdalena]). The collection was done during the rainy season of 2013 for all of the samples except the samples from Codazzi, which were taken during the rainy season of 2014.

**Soil sampling.** Soil samples of 100 g were collected from each of five points, at 0 to 15 cm in depth, for a total of 500 g ha<sup>-1</sup> of sample in the cultivated locations. The samples were stored in airtight bags, refrigerated at 4°C, and transported to the Microbiology Laboratory at Universidad de Santander.

**Plant tissue sampling.** The plant tissue collection was done with convenience sampling by identifying the roots, stems and leaves of the bean plants with fungal symptomatology that corresponded to the genotypes developed by CIAT that were used in agronomic studies by Corpoica at the time. To eliminate microorganisms from the surface, the sampled material was cut into pieces and washed in distilled water for 20 min, disinfected with alcohol (30%) and hypochlorite (0.05%) solutions for 2 and 1 min, respectively, and dried in a laminar air-flow bench following the protocol of Tofiño *et al.* (2012).

**Quantification of rhizospheric soil microorganisms and identification of fungal genera.** Bacterial, fungal and actinomycete counts were carried out on each of the collected soil samples, measured as colony forming units per gram (cfu/g) of soil using 10 fold serial dilution with distilled water and subsequent inoculation by pour plate in nutrient agar for the bacteria and potato dextrose agar for the fungi. The fungal genera were identified by macro-microscopic observation and taxonomic keys and the relative frequency of each genus was compared to the number of samples collected per recorded mega-environment (Tofiño *et al.*, 2012).

**Characterization of phytopathogenic fungi.** The previously prepared plant tissue was incubated in oxytetracyclin glucose yeast agar at 28°C for 5 d. After the incubation, the macroscopic growth and microstructures of the isolated colonies were studied using lactophenol cotton blue staining, optic microscopy and taxonomic guides in order to determine their genera (Tofiño *et al.*, 2012). Thereafter, the incidence of phytopathogenic fungi in relation to the mega-environment cultivated with bean was calculated.

**Agrochemical-bioinput compatibility test.** The methodology proposed by Reyes *et al.* (2012) was modified (agrochemical doses higher than the recommended dose were not included) and applied. Pure cultures of *Trichoderma* (strain PB2011) from the strain collection of Universidad de Santander and formulations of bioinsecticides based on *Metarhizium anisopliae* (Metaril®) and *Beauveria bassiana* (Baubasil®) from the brand Fungicol® were used. This study was performed using the agrochemicals frequently used in bean cultivation in Cesar, according to previous surveys applied by technical assistants (Tofiño *et al.*, 2012). Three herbicides were applied: paraquat (Gramoxone®), glufosinate ammonium (Finale®), and glyphosate (Roundup® Activo SL). Additionally, one insecticide was used: chlorpyrifos (Lorsban™ líquido 4EC), as well as four fungicides: mancozeb (Invezeb® 80WP), carbendazim (Derosal® 500SC), oxycarboxin (PlantVax®75%), and thiabendazole (Mertect® 500SC). Three concentrations were applied: high (recommended dose), medium (50% of the recommended dose), and low (25% of the recommended dose for paraquat and glufosinate ammonium and 10% of the recommended dose for the remaining agrochemicals) (Tab. 1).

The mycelial growth and spore formation evaluation was done through inoculation of the active fungal growth zone in Petri dishes (50 mm) with oxytetracyclin glucose yeast agar (OGY), supplemented with the desired agrochemical.

The dishes were incubated in darkness at 28±5°C and observed every 72 h for mycelial growth and every 7 d for spore formation. Thereafter, the diameter of the colonies was measured to determine the effect of the agrochemicals on the growth compared to the control (OGY without agrochemicals). The three-level toxicity scale cited by Reyes *et al.* (2012) was used to classify the agrochemicals: level 1 - compatible, less than 10% effect on the mycelial growth (EMG); level 2 - moderately compatible, 10 to 30% EMG; level 3 - not compatible, more than 30% EMG.

**Weed control evaluation.** An application of glufosinate ammonium in combination with an organic herbicide based on fermented aromatic plant extracts (ecoredudoq) was done in Codazzi during the pre-seeding. The treatment combinations were as follows: control (manual weed control), 25F-75E (25% glufosinate ammonium + 75% ecoredudoq), 50F-50E (50% glufosinate ammonium + 50% ecoredudoq), 75F-25E (75% glufosinate ammonium + 25% ecoredudoq), 100F (100% glufosinate ammonium) and 100E (100% ecoredudoq) based on the recommended concentrations. After the treatments, the effectiveness of the weed control was evaluated and the rizospheric microorganism counts and fungal genera identification were performed following the previously described methodology.

**Pesticide residue analysis in the bean seeds.** Seeds from Codazzi, La Paz, Pueblo Bello and Manaure (Cesar, Colombia), as well as a control from Codazzi without an agrochemical application, were analyzed to evaluate the impact of agrochemical management in 2013. The samples were collected and prepared for analysis at the pesticide analysis laboratory, Fytolab (Cota, Colombia). Gas chromatography - tandem mass spectrometry (GC-MSMS) and liquid chromatography-tandem mass spectrometry (LC-MSMS) techniques were used, both methods are ISO 17025:2005 certified for fruits, vegetables, and cereals.

**TABLE 1.** Agrochemicals and applied doses for bean cultivations of the Colombian Caribbean coast.

Code	Agrochemical	Active ingredient	Dose		
			High (H)	Medium (M)	Low (L)
H1	Gramoxone®	Paraquat	1.00 L/200 L	0.50 L/200 L	0.25 L/200 L
H2	Finale®	Glufosinate ammonium	1.00 L/200 L	0.50 L/200 L	0.25 L/200 L
H3	Roundup®	Glyphosate	2.50 L/200 L	1.25 L/200 L	0.25 L/200 L
F1	Invezeb®	Mancozeb	450 g/200 L	225 g/200 L	45 g/200 L
F2	Derosal®	Carbendazim	250 mL/400 L	125 mL/400 L	25 mL/400 L
F3	Mertect®	Thiabendazole	2.50 mL L <sup>-1</sup>	1.25 mL L <sup>-1</sup>	0.25 mL L <sup>-1</sup>
F4	PlantVax®	Oxycarboxin	3.0 g L <sup>-1</sup>	1.5 g L <sup>-1</sup>	0.3 g L <sup>-1</sup>
I1	Lorsban™	Chlorpyrifos	400 mL/200 L	300 mL/200 L	200 mL/200 L

H: herbicide; F: fungicide; I: insecticide.

**Statistical analyses.** Descriptive statistics, such as the calculation of means and standard deviations for quantitative variables and frequencies for qualitative variables, were used for the characterization of the soil microbes. In the bioinput-agrochemical compatibility study, a completely randomized design with two factors (product and dose) and three repetitions were used. A randomized block design with three repetitions was used to evaluate the efficiency of the herbicides in the field. An analysis of variance followed by a Tukey test at the 5% level of probability was applied using SPSS v.17 for Windows.

## Results and discussion

### Microbiological analysis

In general, all of the locations cultivated with beans generated population densities, measured as ufc/g of soil, that were similar to those previously reported for tropical soils (Garau *et al.*, 2012). However, the diversity of the identified genera was low (between 2 and 6 genera with an average of 4.2), as compared to results obtained in other studies where more than 6 genera were identified (Tab. 2) (Arenas *et al.*, 2005).

The quantitative equilibrium of the rhizospheric microbe groups identified in the evaluated environments showed a direct positive relationship with the pH levels, which on average were  $6.02 \pm 0.5$ , being higher for the bacteria ( $r=0.7$ ) and lower for the fungi ( $r=0.3$ ). These results coincide with those found by Garau *et al.* (2012).

The low diversity of the fungal genera was associated with the inadequate levels of organic matter identified in some of the locations included in the studies. Concentrations of 0.81 to 2.9% were measured at locations where a maximum of four genera were identified, which, in combination with the indiscriminate application of herbicides, favors the

development of pathogens in the soil (Tofiño *et al.*, 2012; Ahemad, 2014).

**TABLE 3.** Frequency of fungal genera per mega-environment identified in the rhizospheric soil of the bean cultivations of the Colombian Caribbean coast.

Genera	Relative frequency humid Caribbean	Relative frequency dry Caribbean
<i>Aspergillus</i>	100	88
<i>Phytophthora</i>	50	88
<i>Penicillium</i>	75	50
<i>Trichoderma</i>	50	63
<i>Fusarium</i>	50	63
<i>Rhizopus</i>	50	50
<i>Macrophomina</i>	0	13
<i>Paecilomyces</i>	0	25
<i>Cladosporium</i>	13	0
<i>Mucor</i>	0	13
Mean	38.8 a	45.3 a
P	0.896	

Municipalities of humid Caribbean: Cerete and Carmen de Bolívar. Municipalities of dry Caribbean: Codazzi, Manaure, La Jagua, Tamalameque, Valledupar, San Juan and Sevilla.

Means with different letters indicate significant differences according to the Student's t-test ( $P \leq 0.05$ ).

In addition, no significant differences were found in the prevalence of genera between the mega-environments ( $P=0.896$ ) (Tab. 3). The genera *Penicillium*, *Aspergillus* sp., and *Rhizopus* have been identified previously as rhizospheric microbiota in bean cultivations, fulfilling functions as saprophytes and phosphate solubilizers and, in the case of *Aspergillus*, causing seed rotting (Peña-Betancourt and Conde-Martínez, 2012). Various species of *Phytophthora* and *Macrophomina* cause disease in the pod, root, and stem (Ulacio *et al.*, 2012). *Trichoderma* and *Paecilomyces* act as antagonists to pathogens and control pests (Mendoza *et al.*, 2013; Martínez *et al.*, 2013). *Fusarium* and *Cladosporium* have received attention due to their isolation in plant tissues with symptoms of radical wilting and anthracnose (El-Mohamedy and Abd Alla, 2013); the former can cause

**TABLE 2.** Rhizospheric microbiota count in soils cultivated with bean in nine locations of the Colombian Caribbean coast.

Location	Percentage of organic matter	pH	Bacteria	Fungi (ufc/g of soil)	Actines	Genera
Codazzi	2.27	6.07	$1.6 \cdot 10^7$	$1.8 \cdot 10^4$	$3.0 \cdot 10^5$	4
Valledupar	1.13	6.43	$4.4 \cdot 10^7$	$1.4 \cdot 10^5$	$2.7 \cdot 10^5$	4
Manaure	3.64	6.08	$3.5 \cdot 10^5$	$1.3 \cdot 10^4$	$1.5 \cdot 10^4$	6
Sevilla	2.37	6.26	$1.2 \cdot 10^7$	$1.3 \cdot 10^5$	$5.5 \cdot 10^4$	6
Cereté	2.9	6.85	$2.5 \cdot 10^8$	$4.5 \cdot 10^2$	$1.9 \cdot 10^4$	4
Carmen de Bolívar	3.2	6.14	$3.5 \cdot 10^6$	$1.5 \cdot 10^4$	$1.1 \cdot 10^5$	5
San Juan	1.07	5.30	$1.1 \cdot 10^6$	$1.5 \cdot 10^4$	$2.5 \cdot 10^5$	4
Tamalameque	1.07	5.58	$1.1 \cdot 10^6$	$1.3 \cdot 10^4$	$2.2 \cdot 10^4$	3
La Jagua	0.81	5.45	$1.9 \cdot 10^6$	$1.1 \cdot 10^4$	$1.7 \cdot 10^4$	2
Mean	$2.05 \pm 1.06$	$6.02 \pm 0.5$	$3.7 \cdot 10^7 \pm 8.0 \cdot 10^7$	$3.9 \cdot 10^4$	$1.2 \cdot 10^5$	4.2

production losses of between 17 and 45% (Garcés-Fiallos, 2013). In plant tissue samples of Sevilla beans of the varieties SCO487 and SCO611, *Curvularia* and *Colletotrichum* were found (Tab. 4), infecting the transition zone between the root and stem. *Colletotrichum* is found worldwide and has been reported to cause anthracnose, a disease that is considered to be the most limiting in bean cultivations and that can cause yield losses of between 38 and 95% (Gallego *et al.*, 2010).

**TABLE 4.** Phytopathogenic fungus identified in the plant tissues and rhizosphere of the bean cultivations of the Colombian Caribbean coast.

Code	Fungus identified in tissues and rhizosphere
CB1	<i>Colletotrichum</i> sp., <i>Fusarium</i> , <i>Phytophthora</i>
CB2	<i>Colletotrichum</i> , <i>Fusarium</i>
CB3	<i>Colletotrichum</i> , <i>Fusarium</i>
CB4	<i>Colletotrichum</i>
CB5	<i>Colletotrichum</i>
SCO487	<i>Colletotrichum</i> , <i>Curvularia</i>
SCO611	<i>Colletotrichum</i>

CB1- CB5: locality Carmen de Bolívar samples 1, 2, 3, 4 and 5, respectively; SCO487: tissue from the Sevilla variety SCO487; SCO611: tissue from the Sevilla variety SCO611.

The colonization of three phytopathogens, infecting individually and/or in cooperation, was observed in the bean tissue cultivated in Carmen de Bolívar (Tab. 4). For example, in sample CB1, *Colletotrichum* sp., *Fusarium*, and *Phytophthora* were observed. The cooperative infection of these phytopathogens causes damping-off and should be managed through selective fungicides (El-Mohamedy and Abd Alla, 2013).

The greater prevalence of pathogens identified in the bean plant tissue, the lower proportion of beneficial fungi in the rhizosphere in the humid environment, and a low average number of fungal genera in both mega-environments indicated the need to add bioinputs and increase beneficial microorganisms, such as *Trichoderma* and *Paecilomyces*. These biocontrollers have an antagonistic effect on the phytopathogens *Colletotrichum* sp., *Fusarium* and *Phytophthora*, identified in the dry as well as the humid Caribbean (Rojanet *et al.*, 2010; Taj and Kumar, 2012) and should be used as a component of IPDM, combined with selective agrochemicals with a low toxicity level, to avoid an inhibitory effect on the biocontrollers and other microbial genera found in beans (Herrera *et al.*, 2012). In general, the quantitative and qualitative characteristics of the biodiversity of microorganisms will improve and contribute to the development of a suppressive rhizosphere (Lang *et al.*, 2012; Yadav *et al.*, 2013), conserved through sustainable management with the addition of organic matter to reduce the environmental impact caused by the pesticides (Quintero, 2011; Tofiño *et al.*, 2012).

## Bioinput-agrochemical compatibility

The evaluated doses of paraquat and glufosinate ammonium affected the development of the biocontrollers, with significant differences in the radial growth ( $P=0.00$ ). However, according to the EMG scale, the glufosinate ammonium, at the concentration of 250 mL/200 L, was moderately compatible with *Trichoderma* and *Beauveria* with an EMG lower than 30%. This compatibility level is associated with a lower toxicity level of the product according to categories of the Environmental Protection Agency. Although this dose is not effective in the field, the specifications regarding the product's half-life (MAVDT, 2011) indicate the possibility of applying the higher dose and, after 6 to 11 d, applying the bioinput. The inhibitory effect of paraquat on *B. bassiana* at more than 50% has been shown previously (Pandey *et al.*, 2008) (Tab. 5).

No significant differences were found in the growth of the three microorganisms between the low dose of glyphosate and the control ( $P=0.85$ , and  $P=0.32$ ). Moreover, the low dose corresponded to level 1 compatibility. Therefore, applications of glyphosate and bioinputs should be done with a 20 to 60 d difference (UNL, 2010).

It was found that thiabendazole and chlorpyrifos allowed for the growth of *Trichoderma* in all of the concentrations with a compatibility corresponding to level 1 or 2 (Tab. 5). However, similar to the findings of Muhammad *et al.* (2010), the growth of *Beauveria* and *Metarhizium* was inhibited by these two agrochemicals.

The fungicides mancozeb and carbendazim inhibited the growth of the fungi with significant differences between them and the control ( $P=0.00$ ), with a level 3 compatibility. Oxycarboxin presented a high level of compatibility with *Metarhizium* according to the toxicity scale and did not show a significantly different growth between the low concentration and the control ( $P=0.646$ ). However, the growth of *B. bassiana* was inhibited by the evaluated fungicides but with growth at the low dose of oxycarboxin.

Herrera *et al.* (2012) evaluated the growth of various strains of *Trichoderma* using recommended concentrations of mancozeb for the control of *Phytophthora*, finding that both the native and mutant strains were highly sensitive to the fungicide. Likewise, its inhibitory effect on *B. bassiana* growth has been reported previously (Gangwar, 2013).

## Management plan based on microbiological analyses and compatibility tests

According to the obtained results and previous reports regarding the inhibition of herbicides on the saprophytes

**TABLE 5.** Bioinput-agrochemical compatibility at different doses of the bean cultivations of the Colombian Caribbean coast.

Type	Product	Dose	<i>Trichoderma</i> growth (mm)	EMG	<i>Metarhizium</i> growth (mm)	EMG	<i>Beauveria</i> growth (mm)	EMG
Control		0	50.0 f	-	35.0 f	-	40.0 h	-
Herbicide	Paraquat	Low	4.0 b	3	0 a	3	8.0 abc	3
		Medium	0 a	3	0 a	3	4.0 ab	3
		High	0 a	3	0 a	3	0 a	3
	Glufosinate ammonium	Low	37.0 e	2	18.0 cd	3	28.0 gh	2
		Medium	33.0 d	3	5.0 b	3	23.0 gh	3
		High	5.0 b	3	5.0 b	3	14.0 cdef	3
	Glyphosate	Low	48.3 f	1	35.0 f	1	42.0 h	1
		Medium	40.0 e	2	0 a	3	22.0 efgh	3
		High	25.0 c	3	0 a	3	20.0 efgh	3
Insecticide	Chlorpyrifos	Low	40.3 f	2	20.0 e	3	0 a	3
		Medium	37.1 e	2	16.0 cd	3	0 a	3
		High	37.1 e	2	16.6 cd	3	0 a	3
	Mancozeb	Low	0 a	3	0 a	3	0 a	3
		Medium	0 a	3	0 a	3	0 a	3
		High	0 a	3	0 a	3	0 a	3
Fungicide	Carbendazim	Low	0 a	3	0 a	3	0 a	3
		Medium	0 a	3	0 a	3	0 a	3
		High	0 a	3	0 a	3	0 a	3
	Thiabendazole	Low	50.0 f	1	20.0 d	3	11.0 bcd	3
		Medium	50.0 f	1	0 a	3	7.0 abc	3
		High	48.0 f	1	0 a	3	4.0 ab	3
	Oxycarboxin	Low	50.0 f	1	27.3 f	2	13.0 bcd	3
		Medium	50.0 f	1	14.0 cd	3	0 a	3
		High	47.3 f	1	8.0 c	3	0 a	3

EMG: effect on mycelial growth; 1, compatible with less than 10% of EMG; 2, moderately compatible between 10 and 30% of EMG; 3, not compatible with more than 30% of EMG. Means with different letters in each column indicate significant differences according to the Tukey test ( $P \leq 0.05$ ).

*Mucor*, *Aspergillus* and *Penicillium* (Zain *et al.*, 2013), which are frequently found in the study areas, the order of inhibition of the beneficial rhizospheric microbiota and bioinputs by herbicides can be established as asparaquate>glufosinate ammonium=glyphosate (Santoro, 2014). *Trichoderma* was compatible with glyphosate at concentrations 10 times lower than the recommended dose and moderately compatible with glufosinate ammonium at the same concentration. However, the efficacy of the agrochemicals toward weeds was greatly reduced when a low concentration was applied; therefore, it is advisable to use 100% of the recommended dose, considering its half-life, when programming applications of bioinputs. Biological products should therefore be applied 20 and 11 d after the application of glyphosate and glufosinate ammonium, respectively, in order to avoid effects on the growth of entopathogenic and antagonistic fungi. *Metarhizium* was only compatible with glyphosate and oxycarboxin at 10% of the recommended dose, which suggests that this bioinput can be applied 20 and 44 d after the application of the respective agrochemicals.

Given the above, the field application can be 1 L of glufosinate ammonium/200 L water per ha or 2 L of

glyphosate/200 L water per ha and the addition of the bio-inputs *Trichoderma* and *Metarhizium* can occur after 6 to 11 d in the case of glufosinate ammonium and after 20 d in the case of glyphosate.

Likewise, given their high compatibility, it is recommended to apply chlorpyrifos and oxycarboxin together with bio-inputs based on *Trichoderma* (ANLA, 2011; MAVDT, 2007).

*Beauveria bassiana* only showed compatibility with low doses of glyphosate, which limits its inclusion in the IPDM of beans. Therefore, it is recommended to use *Metarhizium* given its high effectiveness and compatibility with the mentioned agrochemicals.

### Field trials

No statistical difference ( $P=0.069$ ) was found between the manual weed eradication (99.3% efficacy) and the application of glufosinate ammonium at 100% of the recommended dose (97.3% efficacy). However, significant differences were found between these and the combinations of the two herbicides, where the individual application of ecoredudoq had the lowest efficacy (9.3%). In order to

**TABLE 6.** Herbicide treatment efficacy and effect on the soil microflora of the bean cultivations of the Colombian Caribbean coast.

Treatment	Efficacy (%)	Bacteria (ufc/g)	Fungi (ufc/g)	Actino-bacteria (ufc/g)	Identified fungal genera
MWC	99.3 a	1.3·10 <sup>5</sup>	7.0·10 <sup>2</sup>	1.5·10 <sup>5</sup>	<i>Aspergillus</i> , <i>Trichoderma</i> , <i>Rhizopus</i>
25F-75E	53.3 b	1.7·10 <sup>4</sup>	2.0·10 <sup>3</sup>	3.0·10 <sup>4</sup>	<i>Rhizopus</i> , <i>Aspergillus</i>
50F-50E	60.0 b	2.3·10 <sup>4</sup>	3.4·10 <sup>3</sup>	4.7·10 <sup>4</sup>	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Fusarium</i>
75F-25E	71.0 ab	1.0·10 <sup>5</sup>	5.5·10 <sup>2</sup>	3.0·10 <sup>5</sup>	<i>Aspergillus</i> , <i>Penicillium</i>
100F	97.3 a	2.0·10 <sup>5</sup>	2.2·10 <sup>3</sup>	2.5·10 <sup>4</sup>	<i>Aspergillus</i> , <i>Trichoderma</i> , <i>Rhizopus</i> , <i>Fusarium</i>
100E	9.3 c	1.0·10 <sup>2</sup>	1.1·10 <sup>3</sup>	2.2·10 <sup>4</sup>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Phytophthora</i>

MWC, manual weed control; 25F-75E, 25% glufosinate ammonium + 75% ecoredudoq; 50F-50E, 50% glufosinate ammonium + 50% ecoredudoq; 75F-25E, 75% glufosinate ammonium + 25% ecoredudoq; 100F, 100% glufosinate ammonium; 100E, 100% ecoredudoq.

Means with different letters in each column indicate significant differences according to the Tukey test ( $P \leq 0.05$ ).

**TABLE 7.** Pesticide residues in the bean seeds cultivated in Codazzi, Manaure, La Paz, and Pueblo Bello (municipalities of Colombian Caribbean coast) in 2013 and 2014.

Location	Chemical (mg kg <sup>-1</sup> )	
	Crop of 2013	Crop of 2014
Codazzi-Motilonia	Chlorpyrifos 0.007	Chlorpyrifos 0.050
	Lindane 0.016	Thiabendazole 0.013
Manaure	Chlorpyrifos 0.006	Chlorpyrifos 0.021
	Lindane 0.021	
	Carbendazim 0.30	
La Paz	Chlorpyrifos 0.009	Not applied
	Lindane 0.035	
Pueblo Bello	Compounds not detected	Carbendazim 0.024
		Carboxine 0.029
		Lindane 0.011
Codazzi-Miraflores Farm	Not applied	Compounds not detected

Maximum residue levels (mg kg<sup>-1</sup>): chlorpyrifos and thiabendazole 0.050, carboxine 0.20, carbendazim 0.5, lindane 0.0100.

reach weed control, the combination of at least 50% of the recommended dose of glufosinate ammonium is needed as ecoredudoq by itself does not present an herbicidal effect on succulent weeds such as *Portulaca oleracea*, which was predominant in the evaluated field (Tofiño, 2000). Additionally, there was a reduction in the bacterial population in the treatments that included ecoredudoq, which may favor the growth of *Phytophthora*.

### Pesticide residue detection in the bean seeds

Concentrations of lindane surpassing the maximum residue level (MRL) were found in samples obtained in Codazzi, Manaure, and La Paz in the 2013 crop (MRL = 0.0100 mg Kg<sup>-1</sup> according to the Ministerio de la Protección Social, 2007) (Tab. 7). This product was not included in the management plan of the cultivation during this study, which indicates its high residual effect. The use of lindane is prohibited in 52 countries, including Colombia, because it is bioaccumulative and a carcinogenic persistent toxin, detected worldwide in the air, water, snow, tissues and soil with a half-life in the latter of one to two years (Quintero, 2011; Maliszewska-Kordybach *et al.*, 2014). The

accumulation of pesticide residues in the analyzed bean seeds is associated with a plant's capacity to extract the chemical from the soil and the low levels of organic matter in the area (Robles-González *et al.*, 2006).

Chlorpyrifos, thiabendazole, and carboxine were identified at levels lower than the maximum permitted and, due to their low residual levels and high compatibility with the studied bioinputs, were included in the management plan.

### Conclusions

In general, all of the evaluated samples showed favorable microbial populations for bean growth, corresponding to the principal population groups of tropical soils. However, on a qualitative level, the average diversity of the fungal genera was low and in direct proportion to the decrease of the physicochemical quality of the soil in terms of organic matter and pH, regardless of the mega-environment. As a consequence, these unfavorable soil characteristics promote susceptibility to disease because of the lack of a suppressive rhizosphere. This was shown by the higher prevalence of



pathogenic microorganisms over beneficial ones, indicating the need to use pesticides, such as chlorpyrifos, thiabendazole, oxycarboxin, and glyphosate, that have a low effect on the development of the microbiota present in the soils used for bean cultivation in the Caribbean region. Such pesticides permit the use of biocontrollers such as *Trichoderma* and *Metarhizium*, applied according to the half-life of the chemical, in order to avoid major deterioration of vulnerable Caribbean soils. In addition, organic fertilizers are recommended in order to increase the organic matter content of soils, which favors the suppressive rhizospheric population and the development of the crop.

The deficient health of soils dedicated to bean cultivation on the Caribbean coast justifies the previous recommendations, including techniques used in organic agriculture and IPDM with compatible chemical and biological products, in order to benefit the productivity and the phyto-recovering properties of the plants.

*Beauveria bassiana* was not compatible with the evaluated agrochemicals and should not be used in integrated management. This indicates the need of further studies on native strains that are better adapted to the climatic and edaphic conditions and the agronomic management of the studied area.

The accumulation of highly toxic pesticides in bean seeds, favored by the phytoextractive properties of the plants and the physicochemical properties of the soil, is an indicator of the need of applying sustainable management that includes agrochemicals with a low residual effect.

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