

Scientometric analysis of Colombian research on bio-inoculants for agricultural production

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Received: 11-02-2015

Accepted: 13-11-2015

Published on line: 26-02-2016

Citation: Zambrano-Moreno DC,
Avellaneda-Franco L, Zambrano G,
Bonilla-Buitrago RR. Scientometric
analysis of Colombian research on
bio-inoculants for agricultural
production,
Universitas Scientiarum, 21 (1): 63-81, 2016.
doi: 10.11144/Javeriana.SC21-1.saoc

Funding: N/A

Electronic supplementary material:
N/A



Abstract

The excessive use of synthetic chemical inputs in agricultural production has led to the disruption of biogeochemical cycles. One of the alternatives that arose within the systems of sustainable agriculture was the partial or total replacement of chemicals by biological substances. The analysis of relevant scientific literature has become a tool for assessing the quality of knowledge generation and its impact on the environment. A scientometric analysis was conducted of Colombian research on bio-inoculants from 2009 through 2014 in journals added to the Web of Sciences™ in order to identify the characteristics of the main target crops, the microorganisms used, and the beneficial effects on agriculture. In this work, 34 articles were identified: 24 (71 %) were research on bio-fertilizer development and 10 (29 %) on bio-pesticides. Articles mainly focused on the study of Gram-negative bacilli affecting the area (77 %), while others focused on issues and topics surrounding vegetables (30 %). The analysis of co-occurrence of keywords identified: i. several genera of microorganisms (e.g. *Azotobacter* sp., *Bradyrhizobium* sp.) and sustainable agriculture as issues that have a leading role in this scientific field, ii. plant growth promoting rhizobacteria (PGPR) as an emerging issue, iii. biological nitrogen fixation (BNF) as a subject which has risen in a complementary manner and iv. endophytic bacteria and biodiversity as issues in growth. This study showed that research in Colombia could be targeted on issues such as endophytic bacteria, diversity and productivity.

Keywords: Bioinoculants, scientometrics, microbial inoculants, sustainable development.

Introduction

The world's population is expected to grow from 6.8 billion to 9.1 billion by 2050. Feeding these people would be easy if natural resources, such as land and water, were inexhaustible (Godfray et al. 2010). However, resources are limited and to continue with environmentally destructive agriculture and livestock farming is a poor option. The intensive use of chemical inputs in agricultural production has led to a disruption of biogeochemical cycles. Moreover, agriculture and livestock contribute to climate change by releasing greenhouse gases such as nitrous oxide, mainly due to organic

nitrogen fertilizers and methane from the Ruminant's digestion processes and storage of animal manure. Paradoxically, agricultural productivity is very sensitive to climate change (Hatfield et al. 2011). These issues generate a challenge to ensure food security in the world.

According to Sanjuán & Moreno (2010), reevaluating agricultural practices to improve their productivity of feeding billions of people worldwide without damaging the soil is made possible by biotechnology techniques and the use of microorganisms. Agricultural microbiology is a field responsible for the transfer of knowledge from general microbiology and microbial ecology to agricultural biotechnology research. (Tikhonovich & Provorov 2011). Within the scope of agricultural microbiology is the production of bio-inoculants with standards of quality and efficiency that improve yield of crops.

In the last two decades, the use of microbial inoculants in agriculture and agricultural research in the public and private sector have increased (Hayat et al. 2010) in the search for solutions that contribute to the sustainable intensification of agriculture. The bio-inoculants mainly include free-living bacteria, fungi, and arbuscular mycorrhizal fungi (AMF) (Dodd & Ruiz-Lozano 2012) which are isolated from a variety of environments including soil, plants, and waste (e.g. water and composted manure). Among the bio-fertilizers that have been studied in depth are the plant growth promoting rhizobacteria (PGPR). The bio-inoculants are classified as bio control agents (also called bio-pesticides) and bio-fertilizers (Bashan & Holguin 1998).

The bio-fertilizer or biological products containing live microorganisms that, when applied to the surface of the seed, plants, or soil promotes plant growth by various mechanisms, such as increased nutrient supply, increased biomass or root area, and increased nutrient uptake ability by the plant (Vessey 2003). Also, microorganisms are able to mitigate the impact of abiotic stresses (salinity, drought and acidity) on the plant (Cheng et al. 2007).

The research and policies supporting sustainable agricultural production have improved the efficiency and quality of microbial inoculants by influencing the creation of the commercial register of several products based on microorganisms; both for biological control as biofertilization with mycorrhizae and PGPR preparations that are commercially available in several countries. However, the spreading use of microbial inoculants, especially those who act like phyto-stimulants or bio-fertilizers, have been hampered due to their variability and inconsistent results obtained in laboratory, greenhouse, and field. Moreover, the lack of adequate formulations and the long commercial registration process are among the factors that limit the use of bio-inoculants on a wider scale (Malus et al. 2012) and restricts the bio-inoculant's potential as an important tool in the sustainable intensification of agriculture.

In general, crop response to inoculation with PGPR may be affected and present differences between the results of laboratory and field applications because after the suspensions of microorganisms are inoculated into the soil without adequate support, the bacterial population decreases rapidly. This phenomenon is due, according to Bashan et al. (2014), to the inherent heterogeneity of the soil; the main obstacle to the introduction of PGPR. Unprotected inoculated microorganisms

must compete with better adapted native microflora and maintain the enzyme activity in the rhizosphere under conditions not corresponding to their optimal growth temperature. Consequently, an important part of the formulation of inoculants is to provide an adequate number of microorganisms combined with physical protection to prevent rapid decrease down PGPR introduced.

One key tool to guarantee the quality of bio-inoculants is the analysis of scientific publications about their development because this allows assess to its coherence and statistical support of the process, and its impact on the environment (Rueda-Clausen et al. 2005). The aim of this study was to identify the characteristics of bio-inoculants as main target crops, their microorganisms used, and the beneficial effects on the Colombian Agriculture by mean of a scientometric analysis of research on bio-inoculants during the years 2009 through 2014 in journals indexed in Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index, KCI-Korean Journal Database and Scielo Citation Index.

Methodology

Type of study

A descriptive scientometric study was carried out, through the review of original articles published about bio-inoculants by Colombian authors during the years 2009 to 2014 indexed in the principal collection of the Web of Science™ (WoS) from Thomson Reuters Institute of Scientific Information (ISI) that includes the subscribed databases Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index, KCI-Korean Journal Database and Scielo Citation Index.

Materials analysis and its origin

Search criteria

In this study, the search terms used in the basic-research field were: promoting rhizobacteria, nitrogen fixation, bio-fertilizer, bio-inoculants, bio-pesticides and biological control. The search was for articles published between 2009 and 2014, without making any topic filter. Studies published in English, Spanish, and Portuguese were consulted. Only original research articles where at least one of the authors was affiliated to a Colombian institutions were considered in the scientometric analysis, due to the inclusion of non-original papers (grey literature) analysis could generate bias in the study (Perez et al. 2002). Original articles were considered as those who contribute original results (i.e. those that have not been published previously) have evaluated by scientific peers, and have the typical structure of introduction, materials and methods, results, discussion, and references (Villar et al. 2007).

The aforementioned search criteria was entered into the WoS showing a total of 14 429 documents. The process of refining the search filter was applied exclusively to present original articles and found 12 792 items. Then, it was refined by country, where 414 documents were obtained and examined. Only 34 articles were selected, taking into account the definition of “bioinsumo” (i.e. defined by the Instituto Colombiano Agropecuario (ICA) as a substance or mixture of substances made of organic or

natural, sustainable and safe source.) Only bio-inoculants with microorganisms as active principle were included, namely biological inoculants and biological control agents. We excluded articles in which the organism was isolated from waste because the safety of the microorganism is not guaranteed. Additionally, the microorganism used and research should be obtained from different ecosystems from Colombia because it was in our interest to review the use of the native biodiversity and their use in agricultural production.

Data collection and processing

The obtained information was organized in a two-way table on Microsoft Excel (2013) to control the quality. In these tables; the title, journal name, language, names of authors, year of publication, keywords, and abstract are registered. Subsequently, 34 articles were read in full to supplement the Excel database with information such as objective plant, used microorganisms, biological activities tested, and the beneficial effect of obtained results in the agriculture. This information was used to generate descriptive analysis.

Analysis of co-occurrence

The co-occurrence analysis is a method that identifies the most frequently associated words contained in the scientific publications in a database (Solleiro, 2002). From the generated Excel database, we extracted an $m \times n$ matrix where m is the number of keywords analyzed, and n is the number of keywords (matrix keyword-article) (i.e. applying the methodology described by Vargas & Castellanos (2005)).

The matrix was normalized by calculating Jaccard index (Pelc 2000), which is defined by Equation 1.

$$J(ij) = \frac{C_{ij}}{C_i + C_j - C_{ij}} \quad (1)$$

Where: C_{ij} is the number of occurrences of the words i and j , C_i frequency of word i and C_j word frequency j .

From the results obtained using the Jaccard index, a multidimensional scaling technique was used. This technique generates a two-dimensional graph representing the numerical relation between different variables found in the 42 x 42 matrix obtained from the keywords (Cox & Cox 2001). The horizontal and vertical axis of the graph represents the centrality and the density, respectively. A strategic diagram was created using the SPSS 21.0 software with the information of the matrix.

The strategic plot was interpreted as Longar & Rivers (2014) suggested; based on the appearance of words in each quadrant of the Cartesian axis, as shown in **Figure 1**.

- First quadrant (located at the above right): the topics are characterized by main issues and high development. Also, the topics are supported by well-structured research groups, which play a driving role in the evaluated scientific field.

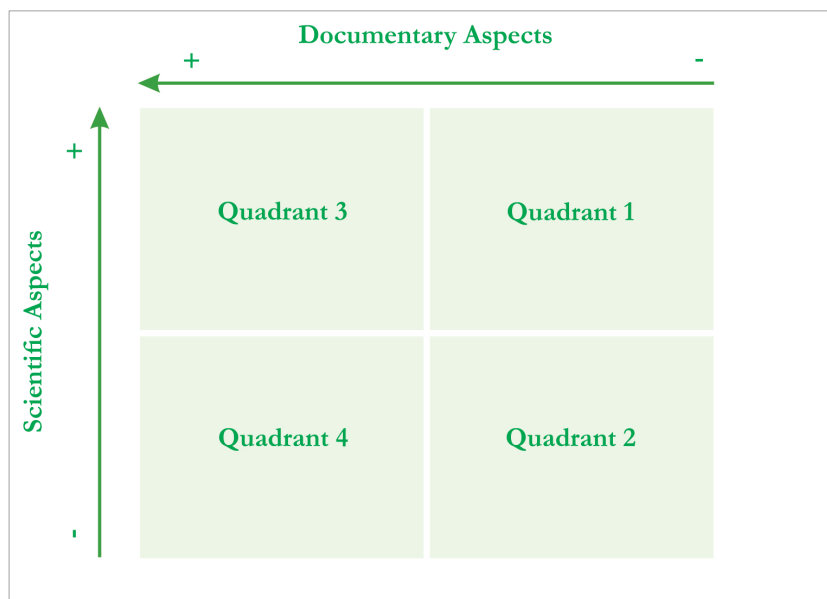


Fig. 1. Position of the quadrants in the strategic diagram.

- Second quadrant (located at the bottom right): Presents the topics considered well-connected but underdeveloped; these can be seen as emerging or bridge issues.
- Third quadrant (located at the top left): the topics are considered as representative of a highly specialized with a high activity, but these are isolate or in peripheral fields of scientific study; are subjects of other fields of study who venture into it.
- Fourth quadrant (located at the bottom left shows): the topics that appeared for the first time and in many cases definitely disappear; this includes underdeveloped peripheral topics.

Results and discussion

The search on WoS with the above mentioned criteria showed a total of 14 429 documents (**Table 1**) of which 12 792 corresponded to original articles. Only 3.24 % (414/12 792) of the original articles found have at least one author with Colombian

Table 1. Search results in number of articles.

Search terms	Total	Article	Colombian articles	% Colombian articles	Selected articles
Promoting bacteria	1 603	1 432	35	2.44	4
Nitrogen fixation	5 858	5 316	36	0.68	15
Biofertilizer	557	532	11	2.07	3
Bioinoculants	80	78	3	3.85	2
Biopesticides	752	628	21	3.34	2
Biological control	5 579	4 806	308	6.41	8
	14 429	12 792	414	3.24	34

affiliation. According to this result, the Colombian scientific production is barely visible, similar to the result found between 1995 and 2007 by Baquero et al. (2007); a total of 15 Colombian articles indexed in standard journals.

414 original articles were found with membership to Colombian institutions. However, after review and application of the search criteria, 34 original articles were obtained that it were included in this study. The results of searching on WoS found 34 Colombian authors published between 2009 and 2014. Twenty four articles (71 %) were investigated for the development of bio-fertilizers and ten (29 %) for bio-pesticides. During the period between 1996 and 2007, Baquero et al. (2007) reported that Colombia had 15 publications on bio-inoculants, specifically on biological control and insects, contrary to what was seen in recent years, where the trend of research has been directed towards the issue of bio-fertilizers.

Ten of the Colombian articles were published in 2014, seven in 2013, two in 2012, six in 2011, six in 2010, and three in 2009. The annual frequency of publication shows a growing trend since 2009. This increase is consistent with research done by Baquero et al. (2007), who identified that publications related to the topic of bio-inoculants presented an upward trend on a global scale, with an average of 72 publications, of which the production of Latin American countries reached only 1 % in total.

The journals where the articles were published are showed in **Table 2**. Ten of eighteen journals are Colombian. It is noteworthy that 74 % of articles were published in the Spanish language; generating that the impact factor of these publication to decline. According to Téllez et al. (2007), an important aspect that reduces the diffusion of Latin American research published in journals is the language of publication.

Table 2. Number of articles published by journal.

Journal	Number of articles
Revista Colombiana de Biotecnología	5
Acta Biológica Colombiana	4
Pastos y Forrajes	3
Universitas Scientiarum	3
African Journal of Biotechnology	2
Agronomía Colombiana	2
Acta Agronómica	2
Applied Soil Ecology	2
Revista Facultad Nacional de Agronomía Medellín	2
Actualidades Biológicas	1
Bosque	1
Brazilian Journal of Microbiology	1
Journal of General Plant Pathology	1
Plant Soil	1
Revista Colombiana de Ciencias Químico - Farmacéuticas	1
Revista Mexicana de Ciencias Agrícolas	1
Revista Corpoica y Tecnología Agropecuaria	1
Journal of Visualized Experiments	1

Application main crops: The advances in bio-inoculants during the period analyzed in the country are mainly focused in vegetables (30 % of articles), followed by research on cereals (16 %), and herbaceous crops (14 %). Lesser percentage progress for tree species (14 %), perennes (11 %), shrubs (7 %), tubers (5 %), and ornamental (3 %).

The highest percentage in research on vegetables may be given to the issue of organic products, which has had a special interest in growing demand in market segments. The National Horticultural Plan (CCI 2012) argues the sales of organic products in the world are growing between 20 % and 50 % annually, depending on the type of food. This growth is supported by [Dias et al. \(2013\)](#); it has been established as a component of a healthy diet, leading to a growing interest in developing new approaches to improve vegetable production.

Type of microorganisms: Filamentous fungi, mycorrhizal fungi, Gram positive bacilli, and Gram negative bacilli are the more used morphological groups of microorganisms. In the analyzed studies, Gram negative bacilli was predominate with 77 %, followed by filamentous fungi at 11 %, Gram positive bacilli with 7 %, and finally, mycorrhizal fungi with 5 %. It is likely that the highest percentage was Gram negative bacilli because in the rhizosphere, Gram negative bacteria predominates with high growth rates ([Beneduzi et al. 2012](#)). The most evaluated activity was biological nitrogen fixation. According to [Francis et al. \(2010\)](#), the interaction with the plant has been investigated mainly with Gram negative bacteria because they are easily isolated from the tissues of plants, easy to handle, and susceptible to genetic analysis.

The trend of biological control research showed orientation to filamentous fungi, especially *Trichoderma*. In efficiency, as biological control of pests and plant pathogens, *Trichoderma* has been studied extensively on the benefits for farmers and its ability to protect the environment ([Cuevas et al. 2013](#)). However, in recent years, an increase in studies regarding the characteristics of *Trichoderma* have risen out of concern about the risks of introducing an invasive alien species to environmental biodiversity is widely recognized. Due to the high reproductive rate of *Trichoderma* sp., the strains that have not been introduced from other countries can do parasitism or predation by non-target species, competition, and movement of species or disturbance of the functions of native ecosystems.

Beneficial effect on agriculture: The development and use of bio-inoculants based microbes has been increasing worldwide due to the recognition of the harmful effects on the environment of excessive or inappropriate use of chemical inputs and the requirement of strategies to feed the world's population by 2050 with sustainable agriculture. Also, the newest technologies used to perform experiments to understand the relation and the interaction between the plants, soil, and microorganisms that occur in the rhizosphere ([Malusá & Vassiley 2014](#)) are an important tool to improve the formulation of bio-inoculants.

The increase of crop yield or quality caused by bio-inoculants has been linked, in some cases, to the increased availability and uptake of nutrients, particularly biological nitrogen fixation (**Table 3**). For example, [Valencia & Ligarreto \(2010\)](#) reported that some soybean varieties showed a positive response with strains of *Bradyrhizobium japonicum* in oxisols of the Colombian Orinoquia and that the symbiotic relationship and grain yield were somehow affected the most important factors of genotype-environment interaction: variety, water shortages, soil compaction and diseases, particularly the presence of *Cercospora sojina*.

Table 3. Summary of the articles analyzed and reported profits in agriculture. FBN: Biological Nitrogen Fixation, AIA: production of indole acetic acid.

Crop	Type microorganism	Activity evaluated	Reference	Beneficial effect on agriculture
Cowpea (<i>Vigna unguiculata</i>)	<i>Rhizobium</i> sp., <i>Bradyrhizobium</i> sp.	Antibiotic resistance, Zn + 2, Al + 2 and Co+ 2, temperature, and pH tolerance, NaCl concentrations.	Cuadrado et al. 2009	Different strains from the same node show promiscuity of <i>V. unguiculata</i> that gives an advantage over other legume species.
Tomato (<i>Solanum lycopersicum</i>)	<i>Trichoderma koningiopsis</i>	Biological control of <i>Fusarium</i> sp.	Jaimes et al. 2009	Reducing the incidence of neck rot and roots by 35%, compared to the absolute control.
Patula pine (<i>Pinus patula</i>)	<i>Azotobacter chroococcum</i> , <i>Bacillus macerans</i> , <i>Enterobacter agglomerans</i> , <i>Pseudomonas</i> sp. and <i>Suillus luteus</i>	FBN	Orozco & Martínez 2009	Stimulation of longitudinal growth and improved nitrogen nutrition of <i>P. patula</i> .
Guinea grass (<i>Panicum máximum</i>)	<i>Azospirillum</i> sp.	FBN, AIA	Cárdenas et al. 2010	<i>Azospirillum</i> sp. maintained its viable population in conditions of water stress.
Clover (<i>Trifolium</i>)	<i>Actinomycete strains</i>	Solubilizing/mineralizing sparingly phosphate sources, N ₂ -fixation and/or siderophore production.	Francis et al. 2010	Selected actinomycetes improve arbuscular mycorrhizal formation by clover plants and <i>Glomus mosseae</i> .
Guinea grass, Anglenton grass (<i>Dichanthium aristatum</i>) y Common grazing (<i>Brachiaria decumbens</i>)	<i>Azospirillum</i> sp., <i>Herbaspirillum</i> sp., <i>Burkholderia</i> sp., <i>Gluconacetobacter</i> sp., <i>Derrxia</i> sp., <i>Beijerinckia</i> sp. and <i>Azotobacter</i> sp.	Microaerophilic population diazotrophic and free life diazotrophic.	Garrido et al. 2010	In times of drought, it reduced the number of bacteria in the roots observed and soil.
Eucalyptus (<i>Eucalyptus</i>)	<i>Beijerinckia</i> sp., <i>Azotobacter</i> sp., <i>Derrxia</i> sp., <i>Herbaspirillum</i> sp., <i>Azospirillum</i> sp., <i>Gluconacetobacter</i> sp. and <i>Burkholderia</i> sp.	FBN, AIA	Obando et al. 2010	The population of the species diazotrophic effect of climatic periods were not affected.
Tomato	<i>Bacillus thuringiensis</i>	Evaluated five bioassay methods measuring three commercial products toxicity on <i>Tuta absoluta</i> larvae	Ramirez et al. 2010	The three commercial products produced high mortality in Lab conditions.
Soybean (<i>Glycine max</i>)	<i>Bradyrhizobium japonicum</i>	Symbiotic association	Valencia & Ligarreto 2010	The symbiotic association had gains in grain yield and disease tolerance.
Stevia (<i>Stevia rebaudiana</i>)	<i>Azotobacter nigricans</i>	Microbiological and physicochemical analysis	Borda et al. 2011	The application of bio-fertilizer showed a positive correlation with mineralization of compost and synthesis of glycosides.
Quickstick (<i>Gliricidia sepium</i>)	<i>Rhizobium</i> sp.	FBN and AIA	Cubillos et al. 2011a	Rhizobiol© stimulates growth and development of seedlings.
Passion Fruit (<i>Passiflora edulis</i>)	<i>Trichoderma harzianum</i>	Biocontrol capacity of <i>T. harzianum</i> Rifai against <i>Fusarium solani</i> (Mart.).	Cubillos et al. 2011b	Biocontrol effect of <i>F. solani</i> using concentrations of 106 and 108 conidia/ mL of both native and commercial strains.

Crop	Type microorganism	Activity evaluated	Reference	Beneficial effect on agriculture
Tomato, cabbage (<i>Brassica oleracea</i>), carrot (<i>Daucus carota</i>), spinach (<i>Spinacia oleracea</i>) and pumpkin (<i>Cucurbita pepo</i>)	<i>A. chroococcum</i> , <i>A. nigricans</i>	FBN	Jiménez et al. 2011	Usefulness of molecular and Bioinformatics techniques as tools to support the conventional techniques of microbial characterization.
Pasto	<i>Klebsiella</i> sp., <i>Enterobacter</i> sp. and <i>Micrococcus</i> sp.	FBN	Kelemu et al. 2011	The total content of N in stems and leaves was significantly higher in inoculated plants.
Tomato	<i>Trichoderma koningiopsis</i>	Candidate genes	Simbaqueba et. al. 2011	Candidate genes can be used as markers for assisted selection of induced systemic resistance (ISR).
Cotton (<i>Gossypium hirsutum</i>)	<i>Azotobacter</i> sp. and <i>Azospirillum</i> sp.	FBN, AIA	Guzmán et al. 2012	Promoting plant growth <i>in vitro</i> .
Corn (<i>Zea mays</i>)	<i>A. chroococcum</i>	FBN, AIA, phosphate solubilization	Rojas et al. 2012	Bacteria improved resistance to salt stress in maize plants.
Potato (<i>Solanum tuberosum</i>)	Community microbiota rhizosphere	Microorganisms N cycle	Flórez et al. 2013	Agricultural management practices governing the distribution and activity of functional groups associated with the nitrogen cycle.
Cocoa (<i>Theobroma cacao</i> L.)	<i>Paecilomyces</i> sp. and <i>Lecanicillium</i> sp.	Pathogenicity of native strains <i>Paecilomyces</i> sp. and <i>Lecanicillium</i> sp. on larvae of <i>Carmenta foraseminis</i>	Medina et al. 2013	The lowest concentration of inoculum to remove 50 to 90% of the treated population, indicating it is more effective against larvae.
Corn	<i>Stenotrophomonas maltophilia</i> and <i>Azotobacter vinelandii</i>	Greenhouse test	Moreno & Galvis 2013	Improved plant emergence, stem diameter and length of the leaves and stems.
Bean (<i>Phaseolus vulgaris</i>)	<i>Beauveria bassiana</i>	Evaluations of endophytic biological control	Parsa et al. 2013	Specific outcome may depend on the target crop species or variety, the fungal entomopathogen species strain or isolate used, and the plant's growing conditions.
Eucalyptus	<i>A. chroococcum</i> and <i>A. vinelandii</i>	FBN	Rojas et al. 2013	Lyophilization with S/BSA best technique for preserving the viability and activity of the strains.
Lettuce (<i>Lactuca sativa</i>), Golden berry (<i>Physalis peruviana</i>)	<i>Trichoderma</i> sp.	Biological control of <i>Sclerotinia sclerotiorum</i>	Smith et al. 2013	Prevents 70 % germination of sclerotia of <i>S. sclerotiorum</i> . In bioassays, no relationship between the rate of mycelial growth and parasitism was found.
Cacao	<i>Burkholderia</i> sp., <i>Gluconacetobacter</i> sp., <i>Herbaspirillum</i> sp.	FBN, biological activity	Arguello & Moreno 2014	Further development in inoculated plants stem length, root and leaf blade; and higher concentration of nitrogen in plant tissue.
Guinea grass	<i>Azospirillum</i> sp., <i>E. agglomerans</i>	Biological activity	Cárdenas et al. 2014	<i>Azospirillum</i> sp. co-inoculated with <i>Enterobacter agglomerans</i> recorded the highest values in plant growth.
Tomato	<i>Candida guilliermondii</i>	Biological control of <i>Rhizopus stolonifer</i> in postharvest tomatoes	Celis et al. 2014	Increase the shelf life of tomatoes improving the distribution of the product and preventing postharvest losses

Crop	Type microorganism	Activity evaluated	Reference	Beneficial effect on agriculture
Chrysanthemum (<i>Chrysanthemum</i>)	<i>Purpureocillium</i> sp.	Potential control symbionts (<i>Scutigerella</i> sp.)	<u>Gallego et al. 2014</u>	In vitro compatibility with most biocontrol fungi except <i>T. viride</i> .
Pea (<i>Pisum sativum</i>)	<i>Trichoderma</i> spp.	Antagonistic capacity to control of <i>F. oxysporum</i>	<u>Insuasty et al. 2014</u>	Showed antagonistic activity in the greenhouse.
Cowpea		FBN, AIA, nodulation tests	<u>Mendoza & Bonilla 2014</u>	Under greenhouse conditions the biological fertilization was more efficient than chemical.
Soybean, Pea	<i>Rhizobium</i> sp., <i>Bradyrhizobium</i> sp.	Biological activity	<u>Moreno et al. 2014</u>	The temperature did not affect the survival and rhizobia concentration after 180 days of storage ensuring quality inoculant.
Lettuce	<i>Pseudomonas fluorescens</i> , <i>Pseudomonas</i> sp.	Solubilization of P, AIA, synthesis of siderophores	<u>Sánchez et al. 2014a</u>	Increase biomass and plant development.
Golden berry	<i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	Root exudates (malic acid) and AIA	<u>Sánchez et al. 2014b</u>	The strains generated higher growth promotion under greenhouse conditions using 50% tricalcium phosphate.
Rice (<i>Oryza sativa</i>)	Consortia unidentified	FBN, AIA	<u>Vanegas & Uribe 2014</u>	Continuous enrichment in NFB medium is a good strategy for the isolation of PGPR on rice plants.
Unmentioned	<i>A. chroococcum</i>	Optimization of culture medium for production, FBN, AIA.	<u>Gutiérrez et al. 2011</u>	Optimizing culture medium allowing 150% increase in biomass production.

Moreno & Galvis (2013) performed an experiment in maize plants testing the effects of inoculation on *A. vinelandii* ATCC 9046 and six isolates of native microorganisms compared with chemical fertilization treatment, and treatment without fertilization. They found that the inoculated corn plants with *Stenotrophomonas maltophilia* showed better results compared with the control and in chemical fertilization variables such as: emergence, diameter and length of the stem and , and dry weight.

In rice, Vanegas & Uribe (2014) reported that 72 microbial consortia analyzed increased, 41.7 % noted the nitrogenase activity increased, and 50 % the production of indole compounds showed an increase in plant growth promoting activity. Mendoza & Bonilla (2014) mention that the direct relation between biological nitrogen fixation (BNF) and the photosynthesis process improved the productivity and profitability per hectare of cowpea through the mutual relationship between rhizobia and legumes.

One of the most used techniques to measure the indirect benefits of hormones for plant used by the authors were the quantification of indole-3-acetic acid (IAA-indole acetic acid) that is consistent with reports by Spaepen et al. (2007) and mentioned that it is the quantified and more common in the scientific literature activity. Obando et al. (2010) found that a strain of *Azotobacter vinelandii* presumptively had the capacity to produce 49.57 g AIA / ml, compared with the control strains Sp7 (40.17 g AIA / ml) and AC1 (38.26 g AIA / ml) because of its potential activity in promoting plant growth was selected to develop tests at the level of emissions.

Another activity that was evaluated in the found articles was the synthesis of a range of different antibiotics associated with the ability of bacteria to prevent the growth of pathogens in plant. In spite of the specificity and mode of action studied in detail around the world, it was not a topic frequently investigated in Colombia. This topic was only found in one of the publications, Cuadrado et al. (2009), who reported that fast-growing rhizobia are inherently more sensitive to streptomycin than slow growth; the resistant to gentamicin and amikacin confers an advantage on the strains of rapid growth versus slow growth.

Analysis of co-occurrence

The obtained results suggest an important dynamic in the field of study; this type of structure is very complex and has central, peripheral, and diverse levels of development (Figure 2). For example, quadrant 1 shows highly specialized topics in the country and which Colombian researchers have had a high impact. In this quadrant, several kinds of microorganisms occur, such as *Azotobacter* sp. and *Bradyrhizobium* sp. Several plant species have become part of the research due to its inclusion to keywords in quadrant 1. In addition, there were more keywords associated: salt stress and sustainable agriculture; and later added, bio-fertilizer and biological control.

It is important that the term “sustainable agriculture” is in the first quadrant because, internationally, it has been recognized as the practice in which organic farming is based and considered one of the tools for sustainable development and poverty reduction in developing countries (Martínez et al. 2012). This should be done in accordance with the notions of appropriate land use and its feasibility based on objective welfare, quality of life, and environmental assessment.

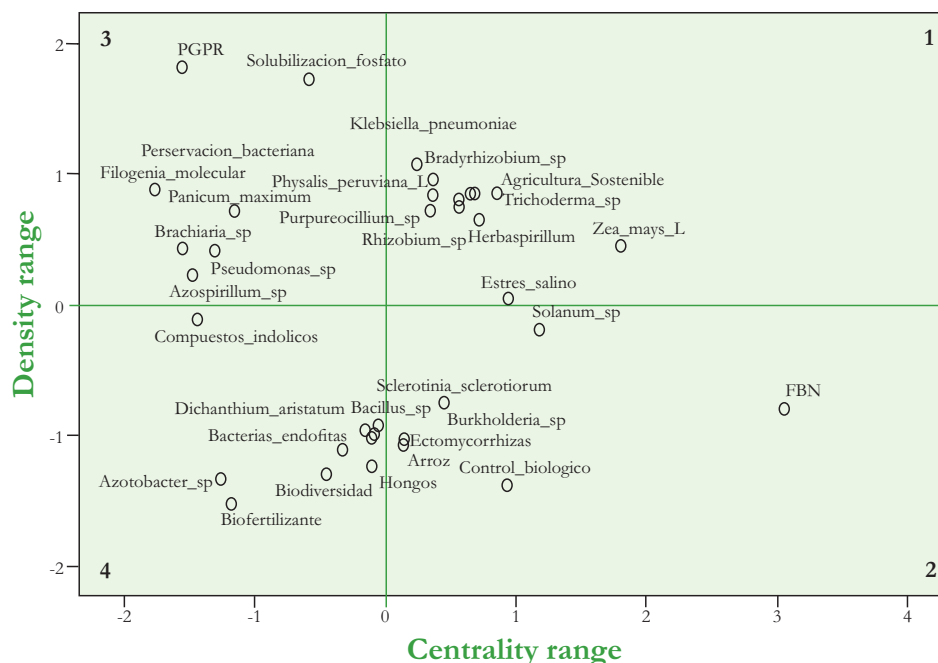


Fig. 2. Quadrant 1: the topics are characterized by main issues and high development. Quadrant 2: Presents the topics considered well-connected but underdeveloped. Quadrant 3: the topics are considered as representative of a highly specialized with a high activity. And quadrant 4: the topics that appeared for the first time and in many cases disappear.

Quadrant 2 shows highly specialized topics in the country and where the Colombian researchers have a weak impact. These topics could become promising subjects. Quadrant 3 shows the specialized topics that have established methodologies for their research, but they are issues of the other fields and have dabbled in a complementary manner on the issue of bio-products. In these, we found that PGPR was a specialized topic because of its integral part in biofertilizers; bacterial preservation is critical to maintaining the genetic stability of developed products. Finally, molecular phylogeny was used to have confirmation at the molecular level of genus and species of microorganisms, allowing subsequently ensure their identity. In these, we found PGPR, an integral type of bio-fertilizers; bacterial preservation is critical to maintaining the genetic stability of the developed products; molecular phylogeny for confirmation at the molecular level of genus and species of microorganism, allowing subsequently maintaining their identity

PGPR are bacteria that may be free or associative life, aerobic, anaerobic or facultative bacteria that produce hormones, vitamins, and growth factors that enhance plant growth and increase the anaerobic plant performance. In Colombia, it was identified that have conducted research related to solubilization of phosphates, nitrogen fixation and production of auxin. According to Malus et al. (2012), future advancements in the PGPR selection should be aimed at improving abiotic stress on crops, namely, tolerance to drought, salinity, inorganic and organic contaminants. In Colombia, it became clear that some investigations are focusing on these areas of interest. For example, Cárdenas et al. (2010) reported that the population of *Azospirillum* tolerates water stress, and Rojas et al. (2012) mention the bacteria improved resistance to salt stress in corn plant caused by high levels of NaCl in the soil.

Quadrant 3 shows the specialized topics that have established methodologies for their research, but relate to issues of other fields and have dabbled in a complementary manner on the issue of bio-products. An important issue is the biological nitrogen fixation (FBN) which is presented as a cross-cutting issue in the developed research. Likewise, the presence of topics associated with biological control research and development in ectomycorrhizas were observed. The use of FBN in agricultural crops is done through some rhizosphere bacteria that have the ability to fix N₂ in organic form, and supply this at the plants. These bacteria can be in a symbiotic or non-symbiotic relation with the plant. Legumes form symbiosis with *Rhizobium* or related genera and the inoculation of these bacteria is made to improve plant establishment. In this study, we found the use of these bacteria in cowpea (Cuadrado et al. 2009), soybean (Valencia & Ligarreto 2010, Moreno et al. 2014), and quickstick (Cubillos et al. 2011). In the case of non-leguminous plants, there is no symbiotic relation, therefore the plant is inoculated with free-living N₂ fixing bacteria, such as *Azotobacter* and *Azospirillum*. These were inoculated in patula pine (Orozco & Martínez, 2009), pastures (Garrido et al. 2010, Cárdenas et al. 2010, Cárdenas et al. 2014), stevia (Borda et al. 2011), vegetables (Jiménez et al. 2011), *eucalyptus* (Obando et al. 2010, Rojas et al. 2013), and maize (Rojas et al. 2012). In high efficiency plants having photosynthesis (C₄ plants) sugar cane and corn, successful results have been obtained when inoculated into the rhizosphere.

Quadrant 4 shows low specialization in the country, but these topics contained are growing in research; for example, endophytic bacteria and biodiversity. The endophytic

bacteria are recognized as organisms residing in plant tissues: mainly intercellular spaces, rarely in intracellular spaces, and within vascular tissues without causing disease symptoms in the plant, according to Perez et al. (2010) Despite the importance of this type of bacteria, this study showed lack of published papers in Colombia on topics such as the presence of endophytic bacteria, diversity, and productivity in relation to agro-ecosystems in different parts of the country.

Conclusions

This study was based in the analysis of scientific and technology publications produced by Colombian researchers about the bio-inoculants issue in the period between 2009 and 2014. It was performed to identify the characteristics of main target crops, microorganisms used, and the beneficial effects on agriculture of the bio inoculants by the analysis of scientific literature.

In Colombia, there have been advances in research in bio-inoculants, especially PGPR; showing that this issue is known by researchers and developed with established methodologies. This allows them to work in a laboratory, greenhouse and field experiments for the and development bio-inoculants efficiently and of good quality. PGPR issues showed thrust in Columbia, however, requires strategies for successful technology transfer of this biotechnology to producers, to allow potential users to participate in development processes and research.

The results show lack of research on topics that expand the application and efficiency in agriculture, such as consistency between regulators in different countries regarding the requirements and conditions for release into the environment of strains. Additionally, no research has been done on understanding of the advantages and disadvantages of using rhizosphere microorganisms with endophytes, the interactions of them released with native microbiota, and selecting strains of PGPR to function optimally under specific environmental conditions found, as pH, salinity or water stress.

Finally, the results show that Colombian scientific influence on bio-inoculants is barely visible in indexed journals as a worldwide standard. The number of publications found may be limited to achieving intellectual property protection and in many cases protect trade secrets, this situation requires that the patent applications and filings with the ICA these bio-products must be analyzed.

Conflict of interest

We declare no conflict of interests.

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Análisis cientométrico de investigación colombiana en bio-inoculantes para producción agrícola

Resumen. El uso excesivo de insumos sintéticos en la producción agrícola ha llevado a la disrupción de los ciclos biogeoquímicos. Como alternativa a este problema, los sistemas de agricultura sostenible han reemplazado parcial o totalmente los químicos por sustancias biológicas. El análisis de literatura científica relevante se ha convertido en una herramienta para determinar la calidad de la generación de conocimiento en este campo y su impacto en el ambiente. Se llevó a cabo un análisis cientométrico de la investigación en Colombia sobre bioinoculantes entre 2009 y 2014. Se tuvieron en cuenta revistas indizadas en la Red de Ciencias®. Se buscó identificar las características de los principales cultivos, los microorganismos utilizados y sus efectos benéficos en la agricultura. Se seleccionaron 34 artículos: 24 (71%) sobre desarrollo de biofertilizantes y 10 (29%) sobre biopesticidas. Los artículos se enfocaron principalmente en el estudio de bacilos Gram-negativos (77%), mientras que otros se enfocaron en los temas relativos a hortalizas (30%). El análisis de co-ocurrencia de palabras claves identificó: i. Algunos géneros de microorganismos (*Azotobacter* sp., *Bradyrhizobium* sp.) y la agricultura sostenible como temas relevantes en este campo, ii. Rizobacterias promotoras del crecimiento en plantas (PGPR) como un tópico emergente, iii. Fijación biológica de nitrógeno (FBN) como un tema emergente y complementario y iv. Bacterias endofíticas y biodiversidad como tópicos en crecimiento. Este estudio mostró también que la investigación en Colombia podría enfocarse en temas como bacterias endofíticas, diversidad y productividad.

Palabras clave: Bioinoculantes; cientometría; inoculantes microbianos; desarrollo sostenible.

Análise cientométrica das pesquisas colombianas em bioinoculantes para a produção agrícola

Resumo. O uso excessivo de produtos químicos sintéticos na produção agrícola levou a uma perturbação dos ciclos biogeoquímicos. Uma das alternativas que surgiram dentro dos sistemas de agricultura sustentável foi a substituição total ou parcial de compostos químicos por substâncias biológicas. O estudo de literatura científica relevante se tornou uma ferramenta para avaliar a qualidade da geração de conhecimento e seu impacto no meio ambiente. A análise cientométrica foi realizada em investigações colombianas sobre bioinoculantes de 2009 a 2014, em revistas disponíveis em Web of Science™, com o intuito de identificar as características das principais culturas alvos, os microorganismos utilizados, e o efeito benéfico na agricultura. Neste trabalho, 34 artigos foram identificados: 24 (71 %) eram pesquisas sobre o desenvolvimento de biofertilizantes e 10 (29 %) sobre biopesticidas. Os artigos eram focados principalmente no estudo de bacilos Gram-negativos afetando a área (77 %), enquanto que outros eram voltados a questões e temas de vegetais (30 %). A análise de coocorrência de palavras-chave identificou: i. diversos gêneros de microorganismos (Ex. *Azotobacter* sp., *Bradyrhizobium* sp.) e agricultura sustentável como questões fundamentais nesta área científica, ii. rizobactérias promotoras de crescimento de plantas (PGPR) como um tema emergente, iii. fixação biológica de nitrogênio (BNF) como um assunto que tem surgido de maneira complementar e iv. bactérias endofíticas e biodiversidade como temas em crescimento. Este estudo mostrou que a pesquisa na Colômbia poderia ser dirigida a questões como bactérias endofíticas, diversidade e produtividade.

Palavras-chave: Bioinoculantes, cientometria; inoculantes microbianos; desenvolvimento sustentável.

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