ARTÍCULOS DE REVISIÓN

Biological Control: A tool for sustainable agriculture, a point of view of its benefits in Ecuador

Control Biológico: Una herramienta para una agricultura sustentable, un punto de vista de sus beneficios en Ecuador

JOURNAL OF THE Selva Andina Biosphere. Official Journal of the Selva Andina Research Society.

Viera-Arroyo, William Fernando; Tello-Torres, Cristina Margarita; Salinas Aníbal, Arturo; Navia-Santillán, Daniel Fernando; Medina-Rivera, Lorena Anabel; Delgado-Parra, Alex Gabriel; Perdomo-Quispe, Cynthia Estefanía; Pincay-Verdezoto, Ana Katherine; Báez-Cevallos, Francisco Javier; Vásquez-Castillo, Wilson Arturo; Jackson, Trevor

Viera-Arroyo, William Fernando *
william.viera@iniap.gob.ec
National Institute of Agricultural Research.(NIAR), Ecuador

Tello-Torres, Cristina Margarita
cristina.tello@iniap.gob.ec
National Institute of Agricultural Research.(NIAR), Ecuador

Salinas Aníbal, Arturo
anibal.martinez@iniap.gob.ec
National Institute of Agricultural Research.(NIAR), Ecuador

Navia-Santillán, Daniel Fernando
daniel.navia@iniap.gob.ec
National Institute of Agricultural Research.(NIAR), Ecuador

Medina-Rivera, Lorena Anabel
lorena.medina@iica.int
Inter-American Institute for Cooperation on Agriculture. (IICA), Ecuador

Delgado-Parra, Alex Gabriel
alex.degado7521@yahoo.com
Inter-American Institute for Cooperation on Agriculture. (IICA), Ecuador

Perdomo-Quispe, Cynthia Estefanía
stefyq-perdomo@hotmail.com
Inter-American Institute for Cooperation on Agriculture. (IICA), Ecuador

Pincay-Verdezoto, Ana Katherine
anapincay3475@gmail.com

Abstract: Agriculture is passing through a global crisis; productivity and sustainability are two challenges for farmers as they strive to produce more food, preserve the environment and maintain the health of living beings. Developed and developing countries need to adopt new productive systems to improve the quality of food, preserve the environment and ecosystems. Currently, the abuse and misuse of chemical products has caused a series of problems including contamination of soil and water and pest resistance to active ingredients. Climate change and other transformations that agriculture is going through, have generated over recent decades the emergence of an industry of biological inputs, which are environmentally friendly products with little risk to human health. These biological products have characteristics and modes of action that provide pest control with fewer risks than from the use of chemical pesticides. This review covers the importance, use and applications of biological products based on beneficial fungi in Latin America, highlighting the project "Biocontrol for Sustainable Farming Systems" carried out in Ecuador, funded by MFAT New Zealand, which has demonstrated the efficiency of microbial products in agriculture through the research results obtained during the project. In addition, aspects related to quality control of bioproducts and regulations for their registration are also mentioned.

Keywords: Agricultural production, beneficial microorganism, biological control, Ecuador, quality control, regulation, sustainable agriculture.

Resumen: La agricultura atraviesa una crisis mundial, la productividad y la sostenibilidad son dos factores que los agricultores están desafiando para producir más alimentos, preservar el medio ambiente y la salud de los seres vivos. Los países desarrollados y subdesarrollados necesitan adoptar nuevas alternativas productivas, que mejoren la calidad de los alimentos, preservando el medio ambiente y los ecosistemas. Actualmente, el abuso y mal uso de los productos químicos ha causado una serie

Inter-American Institute for Cooperation on Agriculture. (IICA), Ecuador

Báez-Cevallos, Francisco Javier
ranciscobaez86@hotmail.com
National Institute of Agricultural Research.(NIAR), Ecuador

Vásquez-Castillo, Wilson Arturo
wilson.vasquez@udla.edu.ec
University of the Americas. (UDLA), Ecuador

Jackson, Trevor
trevor.jackson@agresearch.co.nz
AgResearch Ltd., Nueva Zelanda

Journal of the Selva Andina Biosphere
Selva Andina Research Society, Bolivia
ISSN: 2308-3867
Periodicity: Bianual
vol. 8, no. 2, 2020
directoreditorbiosphere@gmail.com

Received: 01 March 2020
Accepted: 01 September 2020
Published: 01 November 2020

URL: http://portal.amelica.org/ameli/jatsRepo/71/712031012/index.html

DOI: https://doi.org/10.36610/j.jsab.2020.080200128x

Selva Andina Research Society

de problemas como contaminación (suelo y agua) y resistencia de las plagas a los ingredientes activos. El cambio climático y otras transformaciones por las que atraviesa la agricultura, generaron hace décadas la aparición de la industria de insumos biológicos, que son productos amigables con el medio ambiente y la salud humana. Estos productos tienen características y modos de acción que ayudan al control de plagas con menos riesgos que el uso de pesticidas. Esta revisión recopila la importancia, el uso y las aplicaciones de productos biológicos basados en hongos benéficos en América Latina, destacando el proyecto "Biocontrol para Sistemas Agrícolas Sustentables" ejecutado en Ecuador, financiado por MFAT Nueva Zelandia, el cual ha demostrado la eficiencia del uso de microorganismos en la agricultura a través de los resultados de las investigaciones llevadas a cabo durante la ejecución de este Proyecto. Además, se mencionan aspectos relacionados con el control de calidad de productos biológicos y las regulaciones para su registro.

Palabras clave: Agricultura sustentable, control biológico, control de calidad, microorganismos benéficos, Ecuador, normativa, producción agrícola.

Introduction

The growing global concern about the damage that excessive use of synthetic pesticides is causing human health, environment, biodiversity and food security, has provoked a general rejection of chemical control of pests (CCP) in agricultural production[1]. In this context, contemporary agriculture faces the challenge of intensifying agricultural production, while ensuring the protection of the environment, human health, with sustainable solutions, in which the safe and rational use of pesticides may be a key factor, to guard against future food shortages[2].

The soil, which provides the base for agriculture, is one of the habitats with the greatest biodiversity of microbes in nature with about 10^4 to 10^9 microorganisms of different species per gram of soil. To maintain

Author notes

* **Dirección de contacto:** National Institute of Agricultural Research.(NIAR) Av. Eloy Alfaro N30-350 y Amazonas. Quito. Ecuador. Casilla postal: 170401 Tel: +593 2 256 7645
William Fernando Viera-Arroyo E-mail address: william.viera@iniap.gob.ec

soil health, environmentally friendly technologies such as the use of biological control (BC) within integrated pest management (IPM)[3] have been promoted.

The last decades have seen a worldwide trend to restrict the application of agrochemicals to reduce their negative impacts on the environment[4] and reduce residues in harvested products[5]. At the same time, resistance has been generated by pests and pathogens to many pesticides preventing effective control and inducing farmers to overuse them[6]. This has stimulated the search and implementation of control alternatives friendly to human health and the environment, as is the case of the BC.

Biological control was conceived at the beginning of the 19th century, when naturists from different countries observed the important role of entomophagous organisms in nature and following the example of these "biological controllers" attempted to restore the disturbed ecological balance created by agriculture by using living organisms or their metabolites to eliminate or reduce the damage caused by harmful organisms[7].

Biological control agents (BCAs) are being developed worldwide, including organisms such as fungi, bacteria, viruses and insects that reduce the populations of pests that affect crops. Fungi in particular, have aroused the interest of companies and research organizations due to their role in controlling insects and plant diseases, without damaging the environment or human health. Sustainable development must guarantee future generations natural assets equivalent to those that present generations inherited and the advancement towards environmental sustainability will require behavioral changes in people as consumers, which will have consequences on the traditional roles of the family, the community, government agencies and the market, as well as require a complete reorganization of traditional farming techniques[8].

Pests are the main limitation of agricultural production, and pest control (PC) can represent up to a third or more of the cost of production, depending on the severity of the attack. Traditionally, PC has been carried out with the use of synthetic chemical products (CP), many of which cause deterioration of the environment, human health, the evolution of more aggressive pest populations, the loss of pollinators and natural predators. The negative effects of pesticides have driven the search for new control strategies that prioritize recovery of natural environmental regulation mechanisms within IPM[9,10].

BC is a friendly and natural alternative for the environment, which can involve the use of beneficial microorganisms (BM) such as fungi, bacteria, nematodes and viruses that act to reduce the population of pests to lower, manageable densities, either temporarily or permanently[11]. The fundamental purpose of biological management of agricultural pests is to miti gate the harmful effects and economic losses of insect pests and plant diseases, reduce or replace the use of chemical pesticides, and integrate compatible and synergistic strategies to optimize the effectiveness of IPM[12].

The advantages of BC are clear since it has no negative effects on workers in the field, it acts permanently within the natural biodiversity and the agricultural products marketed are perceived of the highest quality because they do not have chemical residues, concepts that are closely related to IPM[13]. Additionally, there are advantages with little competition for products in the market and the global trend towards the preservation of the environment and the consumption of foods free of pesticide chemicals[11]. In general, for effective implementation of a BC strategy, the ecological and social components must be considered from the moment a BCA is discovered and throughout the development process[12].

The microorganisms used for BC must present specific characteristics such as having rapid growth, high capacity for reproduction and survival, different levels of dormancy, be free of natural antagonists, have high competitive ability, adaptability to the treated plant and high versatility in the environment. There are a great variety of potentially useful microorganisms which can be used as BCAs, among the most studied and of which a greater number of products have been developed are *Trichoderma* spp., Bacillus thuringiensis, *Bacillus subtilis, Paecilomyces lilacinus* and *Verticillium lecanii*[11].

The most common BC strategies are[12]: i). Conservation, defined as modifying the environment or existing practices to protect and enhance the activity of specific natural enemies (NE) or others that reduce the harmful effect of pests, ii). Classic, which is the international introduction of an exotic BCA, usually coevolved, for an invasive pest leading to its permanent establishment and long-term PC, iii). Augmentative, involving supplemental release of NE, iv). Inoculation, defined as the intentional inoculation of a BCA with the expectation that the agent will multiply and control the pest for a prolonged period of time, but not permanently, and v). Inundative, which refers to the release or application of BCA in large quantities to decimate pests when their population increases in such a way that the crop is put at risk.

The objective of this review is to describe the importance of BC as a vital component of sustainable agriculture that preserves natural resources and the environment through the use of microorganisms selected for their high efficiency and safety. Successful cases in Latin America, mainly in Ecuador, will be reported.

Development

Biocontrol can be defined as the use of living organisms to suppress pest populations[14]. There are several historical references to the use of BC worldwide. Subsistence agriculture has presented practices that suppressed pest populations, but rather than being based on scientifically proven knowledge, these practices were based on experience and tradition[15]. The same author mentions that despite the lack of interest in improving the effectiveness of NE, some progress was made in the mid-20th century. In recent years, around 2700 NE have been introduced in 196 different countries or islands for PC, as well as more than 440 species are being produced and sold commercially worldwide[16]. In the past two decades, BC scientists have studied the risks of the introduction of exotic species. Foreign companies that have justified effectiveness for this type of control and have developed risk assessment to reduce the risks[17], consequently the development of products with native species is a recommended alternative. The science behind BC has made remarkable progress in the last 50 years, shifting from trial and error to more predictive approaches based on theories of controller-pest interactions and population dynamics[16]. The BC has a long history of success in developed and developing countries. In Ecuador BC was studied and applied before 1937, however, there are not many detailed reports or reports on this issue[18].

Beauveria used as an entomopathogen. *Beauveria* sp., belongs to the Division Ascomycota, Order Hipocreales, it is the most widely used entomopathogenic fungus in biological formulations for control of pests in the orders Lepidoptera, Coleoptera, Hemiptera, Diptera and Hymenoptera[19-22].

Infection by this fungus begins with the adhesion of the conidia to the host's cuticle, followed by germination, which fixes it in the cuticle producing a hypha that allows it to penetrate the insect with the help of hydrolytic enzymes (proteases, lipases, chitinases), mechanical pressure and other factors[23]. Within the hemolymph, hyphal bodies produce blastospores that disperse throughout the body, destroying tissues, evading the immune system, and producing toxins that contribute to the death of the host[24]. After the host dies and with favorable environmental conditions, the hyphae emerge, sporulate on the surface of the corpse, and action of wind, rain or other abiotic and biotic factors will spread the spores to new hosts and a new cycle of infection may start[25].

Efficacy of infection is mediated by factors environmental such as humidity, temperature, precipitation, solar radiation, biotic factors such as age of the host and pathogenicity of the biological control agent[26-28]. Therefore, for BC products propagules must be formulated with additives, such as inert diluents, dispersants, adherents, protectants, among others to increase the useful life time, and ensure its efficiency in the field[29,30]. The species of the genus *Beauveria* used as insecticides are *B. bassiana* and *B. brong* niartii in the most common presentations, wettable powders followed by suspension concentrates and emulsifiable suspensions[22].

These formulations have been used in various crops to control mites (*Tetranychus urticae*), whiteflies (*Bemisia tabaci*), beetle (*Gonipterus scutellatus*), coffee borer (*Hypothenemus hampei*), banana borer (*Cosmopolites sordidus*), defoliator worm (Dione juno), thrips, aphids, bed bugs, grasshoppers[22,25,31-33].

Trichoderma is the main fungal genus used in biological control. Due to the efficiency and ease of production, *Trichoderma* is among the most successful BCAs in agriculture. Throughout the world, countless phytosanitary products based on this fungus are marketed, which acts as a biopesticide, biofertilizer, growth promoter and inducer of natural resistance, whose application can be foliar, before sowing in seeds or propagation material, treatments after pruning, incorporation into the soil during sowing or transplanting, irrigation, among others[34]. The most common formulations available on the market are wettable powders, granules, emulsifiable concentrates and concentrated suspensions[35].

Trichoderma is the most studied and used biological fungicide in agriculture. It is a genus of fungi that live freely in soil and root ecosystems. Its antagonistic properties are based on the activation of very diverse mechanisms. *Trichoderma* can exert the biocontrol of phytopathogenic fungi (PF) indirectly competing for space and/or nutrients, producing antibiotics or stimulating the growth of plants and their defense mechanisms, on the other hand, it can carry out biocontrol directly by means of mycoparasitism[36].

Some members of this genus have symbiotic associations with plants such as legumes, grasses, nightshades and others, while other species are used as biocontrol agents against PF such as *Rhizoctonia, Pythium, Sclerotium* and *Phytophthora*, among others, which affect many crops of commercial interest such as rice, corn, onion, tomato, beans, wheat, cocoa, etc.[37]. They are also considered able to degrade highly persistent pesticides in the environment, serving as a soil decontaminating agents[38], as well as having antibiotic action and acting as a plant growth promoters (PGP).

Among *Trichoderma* commercial products the species reported are: *T. harzianum, T. asperellum, T. viri de, T. atroviride, T. gamsii, T. hamatum, T. koningii, T. lignorum, T. polysporum* and many labels indicate a generic *Trichoderma* (sp. or spp.)[39]. Many products consist of a single species of *Trichoderma* and others with combinations of two or more species, or a mixture with other species of fungi and even bacteria that, when combined with inert materials such as powders, microtalcs, solvents, emulsifiers and other additives, improve the stability of the microorganism, protect it from environmental conditions, giving it a longer viability time during storage, ensuring its effectiveness when applied in the field[40].

According to one study[41], *T. inhamatum* has an insecticidal and enzymatic against the fruit fly *Drosophila melanogaster*, colonizing 12 flies and maintaining chitinolytic enzymatic activity for 24 days after inoculation. Other authors[3], argue that species such as *T. harzianum* are not only used as BCAs, but are also excellent PGPs, increasing the germination of seeds such as sunflower and papaya by 30%, which allows us to deduce that the seeds easily assimilate the products synthesized by the microorganisms, accelerating the germination process.

It has been noted[42] that endophytic *Trichoderma* strains of a plant species such as quinoa produce positive effects on the crop, improving the plant growth and the ability to reduce the severity of *Peronospora variabilis*, due to the production of substances that directly attack the pathogens or by inducing systemic resistance, which in turn reduces the incidence of the pathogen in the plant.

In crops of high economic importance such as cocoa, the need to change the chemical control strategies against *Moniliophthora roreri* and *M. perniciosa* for others more friendly with the environment such as the use of *Trichoderma* spp. has been recognized because of their ability to inhibit growth of these diseases due to their myco-parasitic activity and the production of various metabolites active against the disease[43]. Other authors[44] have noted that application of *T. koningiopsis* and *T. stromaticum* improved cocoa crop health, reducing the incidence of diseases and increasing the production of healthy pods. Furthermore, in this study, *T. koningiopsis* was the most effective organism for control, adapting to the agroecosystem and surviving with the plants during the dry season.

Studies in roses[45] have indicated that *Trichoderma* spp., being an antagonist fungus, can be combined with other BCAs such as *Penicillium* sp., to combat diseases such as *Sphaeroteca pannosa*, and present a positive cost benefit, obtaining a positive return of USD 1.54 with a recovery of USD 0.54 for every dollar invested. Research in Ecuador with the MD-2 pineapple showed that the height, number of leaves and the fresh weight of the fruit increased positively with the application of *T. harzianum* pre-sowing[46].

Trichoderma spp., can be formulated in different formats and applied using different technologies. The field application of *T. koningiopsisTh003* formulated as dispersible granules significantly reduced plant mortality by 35% within an integrated management program of the neck and root rot of tomato, an important result given the difficulty in controlling the disease with fungicides[47].

The use of different types of biological formulations (powders, granules, liquids) based on microorganisms has consistently increased in response to the need to reduce the use of agrochemicals in PC, but it is necessary to evaluate the quality of these products by determining their biological efficacy, viability and purity which are important characteristics to ensure success in the field[48-50]. Research on bioformulations based on *T. asperellum* showed that the microorganism had greater stability during storage in dry solid release systems compared to liquids[51].

However, more detailed studies based on conidia of *T. asperellum* carried out with four prototype bioformulations (wettable powder, emulsifiable concentrate, covered and dispersible granule) have determined the main physicochemical (water activity, percentage humidity, pH, rammed density, suspensibility, particle size, wettability, stability of the emulsifiable concentrate) and microbiological (viability, percentage germination, purity, efficiency of the formulation process) parameters necessary for an effective quality control[52].

Research on Trichoderma in Latin America. In Bolivia, one of the methods for BC of insects that has gained importance in recent years is the use of microorganisms such as entomopathogenic fungi which produce secondary metabolites and enzymes: proteases, amylases, chitinases, etc., - key factors in biocontrol[41].

The application of *Trichoderma* as a biocontrol agent of *Helminthosporium solani* for three years, produced a partial effect, reducing the severity of the disease between 10 to 22%, which although not sufficient for commercial purposes is a biological alternative for the construction of IPM in potato cultivation[53].

In Chile, the efficiency of *Trichoderma* sp. strains on different pathogens has been evaluated, observing different degrees of control depending on the pest, in addition to specificity of action and the safest use of the biocontrol agent. For example, one strain showed excellent behavior in the control of *Fusarium solani, F. oxysporum, Phythophtora* spp., and *Venturia* inae qualis in commercial apple and tomato orchards, while the Sherwood strain exhibited efficient control of *Botrytis cinerea* both in laboratory and in experimental lettuce cultures[54].

In Argentina, the antagonistic behavior *in vitro* of 15 native strains of *Trichoderma* isolated from soil samples was tested against strains of *Sclerotinia sclerotiorum* using the dual culture methodology. Four isolates were selected as the most efficient in inhibiting the growth and formation of sclerotia of *S. sclerotiorum*. One of the isolates expressed a superior behavior, managing to cancel the, in addition to having registering the maximum growth in the dual culture and preventing formation of sclerotia. On the basis of this study, the most promising strains are being produced on cheap substrates (broken corn and wheat grains) and formulated before evaluation of their effects *in vivo* as a biocontrol agents and growth promoters[55].

In Colombia, isolates of *Trichoderma* sp., have shown effects on the phytopathogenic fungi *Fusarium* sp. and *Rhizoctonia* sp. producing different degrees of antagonism. The greatest impact was noted on the growth of *Fusarium* sp., with 78.30% inhibition compared to 18.67% of *Rhizoctonia* sp. Additionally, the research showed that banana flour with 5% and 2% yeast without buffer is an efficient medium as a substrate for the multiplication of *Trichoderma* sp., producing high numbers of conidia, with a final concentration of $1.1x10^9$ conidia/mL, and pH 5.7 seven days after processing[56].

In Venezuela, the inhibitory effect of three native isolates of *T. harzianum* was tested against phytosanitary problems caused by *Sclerotium rolfsiiin Aloe vera* plantations. The isolates produced a good antagonistic effect, inhibiting the mycelial growth of the pathogen (50-90%) and reducing sclerotia formation (86%)[57].

In Peru, *Peronospora variabilis* affects the quinoa crop and can cause a reduction in the yield of up to 99%. A BC strategy for this pest has been the use of *Trichoderma* because it has an endophytic capacity of up to 60% and produced an increase in yield (3127.30 $kg.ha^{-1}$) compared to the control (1141.27 $kg.ha^{-1}$)[42].

Trichoderma has also been used as a phosphate solubilizer, an effect proven in Brazil through studies carried out to determine the increase in rice biomass under greenhouse conditions. The influence of *Trichoderma* inoculation on rice was determined after 30 and 45 days from sowing, measuring different growth parameters, including height, root length and dry matter. Plant growth increased significantly (24.9 cm) in the presence of natural phosphate stimulated by *Trichoderma* in comparison with the control (18 cm). In general, the relative efficiency of biomass (RE) was significantly higher, with a high content of available P and higher P utilization efficiency (P-UEF), raised from 10 to 61% and from 12 to 62%, for the treatments respectively[58].

Regulations for the control of bio-inputs. The stability and quality of the microbial active ingredient are the main requirements for the registration and commercialization of bio-products. Each country has its own agency that is responsible for developing regulations and procedures that allow for registration and ensure the quality of the products that are marketed in their territory[59].

The national market for BC in the Andean countries is growing, however, there is no established regulation in most of these countries for the development, elaboration, production and commercialization of biological products (BP)[13].

At the Latin American level, Colombia is the country whose regulations can serve as an example for the registration of bioformulations. The agency in charge of regulating and guaranteeing the quality of agricultural inputs is the Instituto Colombiano Agropecuario (ICA) through Resolution 000698 of February 4, 2011[35]. Until December 2018, 303 bio-products have been registered in this country, of which 38 are based on *Trichoderma*[60].

However, in countries such as Argentina, Chile, Bolivia, Peru, Ecuador and Venezuela, the regulations and procedures for the regularization of bioproducts are in the process of being improved. It is estimated that only 38% of the bio-products marketed are registered[39].

In Argentina, the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), through Resolution 350/99, has so far registered 27 BP, of which 3 are based on *Trichoderma*[61].

In Bolivia, the Servicio Nacional de Sanidad Agropecuaria e Inocuidad Alimentaria Unidad Nacional de Sanidad Vegetal (SENASAG) through Administrative Resolution No. 055/2002 and No. 012/2006 has registered 10 BP of these 3 are based on *Trichoderma* until June of 2011[62,63].

In Chile, the Servicio Agrícola y Ganadero (SAG), through Resolution 3670/1999 and 2229/2001, has registered 21 products based on microorganisms, of which 9 have *Trichoderma* as an active ingredient[64].

In Peru, the Ministerio de Agricultura Servicio Nacional de Sanidad Agraria (SENASA), has registered around 120 products based on microorganisms, of which 17 are based on *Trichoderma*[65].

In Ecuador, on August 5, 2019, the Agencia de Regulación y Control Fito y Zoosanitario (AGROCALIDAD) issued the regulations for the registration of biopesticides and related materials, through Resolution 143 and issued the document called "Manual of Procedures for the Registration and Control of Biological Control Agents, Vegetable Extracts, Mineral Preparations, Semiochemicals and

Andean Products Related to Agricultural Use". Previously, commercial BP were registered in the category of fertilizers and of 13 products that appear in the database up to April 2019, 4 are based on *Trichoderma*[66]. Through the new resolution, it is expected to formalize the commercialization of biopesticides, guarantee quality inputs in the national market, provide agricultural inputs for all productive contexts, regulate the registration based on requirements according to new technologies and generate tools for the benefit of the agricultural sector.

Experiences in Ecuador: Project "Biocontrol for Sustainable Farming Systems". In Ecuador, the use of BCAs has begun to gain more space with the conduct of research by public and private companies that have tested this type of input in various crops such as flowers, bananas, sugar cane, coffee, potatoes, broccoli, blackberries, among others. Favourable results in pest control and growth promotion have led to the adoption of biopesticides and other beneficial microbes reducing the negative impact of overuse of agricultural chemicals on the environment and obtaining food free of contaminants[67-70].

In 2009, the "Biocontrol for Sustainable Farming Systems" Project began in Ecuador, executed by the Instituto Nacional de Investigaciones Agropecuarias (INIAP) and the Instituto Interamericano de Cooperación para la Agricultura (IICA), with financing from MFAT-New Zealand. It had the advice of specialists from AgResearch, Plant and Food Research, Lincoln University, INIFAP (Mexico), CORPOICA (Colombia) and USDA (USA), with the aim of generating beneficial microbial formulations and biocontrol technologies that could be implemented in agricultural crops under the environmental conditions of Ecuador[71]. The main achievements achieved through this project during the last ten years are presented below, among which are:

i). Creation of a biological control laboratory for research in INIAP. A Biological Control Laboratory was created at the INIAP Santa Catalina Research Site as part of the Department of Plant Protection. This laboratory has developed several research processes, mainly on producing microbial formulations based on beneficial fungi and prospecting, isolation and multiplication of beneficial microbes from soil samples and infected pests. In addition, methods for quality control of bio-products based on fungi have been standardized. The quality control carried out includes the implementation of microbiological tests of the product (concentration, viability, percentage of germination and purity) and physicochemical analysis (water activity, percentage of humidity, pH, rammed density, suspension, particle size, wettability of dry products and stability of emulsifiable concentrates)[72]. On For the implemented methods, continuous training has been provided for technicians from bio-input production laboratories of the Ministry of Agriculture (MAG), private companies, as well as students and teachers from agricultural schools and universities.

In addition, a Biocontrol Laboratory Technicians Network was formed, which is made up of researchers from INIAP, laboratory technicians from private companies, and government technicians from MAG and AGROCALIDAD. Various workshops have been held on methods for the multiplication, production and analysis of the quality of bio-inputs based on beneficial fungi, in order to generate high-quality bio-inputs that favor sustainable agriculture in Ecuador[72]. It is expected to formalize this Network of Biocontrol Technicians to give it continuity and greater representation.

ii). Evaluation of the quality of commercial bio-inputs. During the first years of the project, the need to evaluate the quality of bioproducts available in the Ecuadorian market was identified, in order to understand the situation and establish a baseline. In 2011, a survey of commercial bio-inputs was carried out including quality control, through microbiological tests, to determine the concentration, viability and purity of microbes in the product. Eighteen commercial products were evaluated, of these 64% of the products did not have viable microorganisms as declared on the label, and 42% showed a high percentage of contamination. Thirty five percent contained viable conidia but the quantified concentration of the microorganism was lower than that reported on the label. Based on the results obtained in the analyzes, the commercial products evaluated were clearly deficient in their quality.

Once the limitation in quality of the commercial products had been determined and recognized as a fundamental factor in their inefficiency, a training program and dissemination of results began, integrating the different actors (AGROCALIDAD, MAG, bio-input producers, farmers and universities) involved in the production of bio-inputs. Ongoing training was carried out in the course of this project on the methods that were defined in INIAP to unify criteria and standardize processes for evaluating the quality of bio-inputs and improve production methods.

In 2019, a new evaluation was carried out to determine impact of the training component of the project in influencing improvement of production systems and quality of bio-inputs available in the Ecuadorian market. Of 13 products evaluated based on *Trichoderma* spp., 46% complied with what was established on the product label. Technicians from the companies producing the products reaching standards had attended project workshops but the continued presence of sub-standard, un-registered products on the market shows that it is still necessary to reach more producers with training workshops. In this evaluation, a new parameter called "water activity" was considered, which represents the amount of water available for microorganisms to develop their metabolism, which influences their growth and viability. To maintain the viability of a biological product based on fungi, water activity should be between 0.2 to 0.7 aw[72], 61%of the evaluated products showed optimal values of this parameter.

iii). Diagnosis of laboratories for quality control of bio-inputs in Ecuador. An evaluation of public sector laboratories and private companies that could perform analysis of quality of bio-inputs was carried out. These included biological control agents (66.67%), plant extracts (14.29%) and related products for agricultural use (19.04%). BCAs analyzed included fungi and bacteria (43.75%), predators and parasitoids (43.75%) and entomopathogenic nematodes (12.5%). Ninety percent of the laboratories reported that they have experience and facilities to carry out research bioassays and 71.43% of the laboratories claimed to have the capacity to evaluate the quality of bio-inputs. The 80.95% of the surveyed laboratories were located in the Sierra region and 19.05% in the Coast. Ninety percent of the laboratories expressed interest in being part of the Bio-Input Laboratory Network coordinated by AGROCALIDAD to carry out quality control analysis; an activity providing a service that will generate an economic income.

iv). Development of prototypes of bioformulations based on beneficial fungi. The concentration of active ingredient, feasibility of production and purity of the final product are important characteristics to ensure the success of a formulation. Several formulations: dispersible granule, covered granule, wettable powder and emulsifiable concentrate, were evaluated to determine the stability and survival of *T. asperellum* and *Purpureocillium lilacinum* spores. The bioformulations were made after mass production of spores on rice substrate and use of formulation additives available nationally. Storage stability tests showed that dry formulations of *T. asperellum*, covered granules and soluble granules, were the most stable with viabilities of $1.6x10^8$ and $1.7x10^8$ CFU/g, respectively and purities greater than 98%, after storage at room temperature (16 °C) for two months. In the P. lilacinum formulations, the wettable powder showed best stability after two months storage, with a viability of $1.1x10^8$ CFU/g and purity of 99%[51]. In addition, physicochemical and microbiological parameters of prototype *T. asperellum* formulations (wettable powder, emulsifiable concentrate, coated granule and dispersible granule) were evaluated under ambient (30 °C) and refrigerated (4 °C) temperature conditions during six months of storage. The wettable powder and covered granules stored at 4 °C stood out with conidial viability exceeding 85% germination and colony concentration maintained above $1x10^8$ spores/g. However, the wettable powder at 30 °C maintained a germination of 81% after 6 months storage[52]. In general, the results of the evaluation of the physicochemical characteristics (stability, wettability, moisture percentage, water activity and pH), determined the stability of these prototypes. Currently, the scaling-up of these technologies is being developed in Ecuador in collaboration with private companies, which is the scaling model adopted by other countries such as New Zealand.

These studies showed quality could be maintained for powder and granule formulations, which preserved the stability of microorganisms at ambient temperatures without the need for refrigeration during transport and storage.

v). Investigations in agricultural crops. The Andean potato weevil (*Premnontrypes vorax*) is considered one of the most important pest insects of potatoes due to the damage it causes feeding on the tuber. To control this pest, farmers have applied insecticides of high toxicity, in some cases in excess, which have negative impacts on the environment and the health of producers and consumers[73]. In a study of alternatives to chemical insecticides, the efficacy of two bioformulations of *B. bassiana* (clay and rice substrate) and the system of application (traps with plants bait with the bioformulate, foliar applications of the bioformulate at 40, 60 and 80 days; application of granules to the base of the plants at 40, 60 and 80 days) were studied. It was observed that with a population of 17 adults of Andean potato weevil/m^2, in a clay-silty soil, with organic matter content of 9.8%, treatment with a clay based granular formulation of *B. bassiana* in foliar applications and at the base of the plants at 40, 60 and 80 days, resulted in 8.7% weevil damage in tubers, compared with 23.7% tuber damage in the control without application. This entomopathogen, therefore, reduced the percentage of damage in harvested tubers, and could provide a biological alternative for incorporation into management programs for this pest, provided there are good humidity conditions and presence of high levels of organic matter in the soil[74].

Broccoli (*Brassica oleracea*) is Ecuador's second most important non-traditional export product and *Trichoderma* sp. has been applied for seedling protection and crop management. *Trichoderma* sp. fungus has been applied to the substrate of broccoli seedlings and monitored to determine its effect from the sowing of the seed until before transplantation. At 32 days, and prior to transplanting, the seedlings treated with *Trichoderma* sp., had higher fresh weights than treatments without application. By quantifying the concentration of the fungus in the substrate, it was found that the population was quite stable but was reduced ($3x10^3$ CFU/g of soil) in treatments that received the application of CP, while the treatments with *Trichoderma* sp. without application of CP maintained a higher population of the fungus ($1x10^4$ CFU/g soil). Additionally, the compatibility of *Trichoderma* sp., with CP used in the seedling production system was tested. The *Trichoderma* sp. isolate was compatible with the pesticides: Hymexazol, Propamocarb, Betaciflutrin, Metolachlor, Lambdacyha-lothrin, Lambdacyhalothrin + Thiamethoxam, Radian, Chlorpyrifos, Dimethoate, Emamectin Benzoate and Acetamiprid; while, incompatibility was observed with Thiram, PCNB, Captan, Carboxin + Captan, Carbendazim, Iprodione, Pyraclostrobin, Chlorpyrifos, Chlorothalonil and Ciprodinil + Fludioxonil was observed. The results show that *Trichoderma* sp. can be included in intensive production systems of broccoli seedlings, favoring their growth[75].

Avocado (*Persea americana*) is a fruit tree of economic importance in Ecuador with export potential. The application of *T. harzianum* (0.18 g/plant at a concentration of $4x10^8$ CFU/g of product) in the seedling substrate of the Criollo cultivar was evaluated. It was determined by nutrient absorption analysis that the inoculated plants showed higher level concentrations of macronutrients such as nitrogen, calcium and magnesium and micronutrients such as manganese and copper in the root system compared to the control without application, indicating that this beneficial fungus acts as a plant growth promoter[76].

Andean blackberry (*Rubus glaucus*), is a native fruit of the Andes with great productive potential. Two technological systems were tested for the management of the blackberry crop: a) clean (with moderate use of synthetic products and chemical fertilization) and b) organic (with use of organic fertilizers (OF) and BP), both systems were managed with and without application of *Trichoderma* sp. Clean management, with applications of the fungus produced the best results when evaluating the number of buds per branch, fertilized flowers, fruits per branch and yield, obtaining a yield between 19.04 $t.ha^{-1}$ and 24.97 $t.ha^{-1}$, depending on the production area[77]. The blackberries are badly affected by black foot disease, an important disease in producing areas, which was found to be caused by the fungus *Dactylonectria torresensis*[78]. The antagonistic capacity of isolates of *Trichoderma* spp. against the pathogen was determined. Under *in vitro* conditions, a 68% inhibition of radial growth of the pathogen was observed, while in the greenhouse there was a reduction in the percentage of root neck necrosis of 35%[80]. Studies were also carried out to determine alternatives for the control of this disease. Carbendazim and alternative products such as Myrtaceae extract showed the lowest incidence of the disease at the root and neck level of the plant, with 22% and 36% respectively, compared to 74% in the control[79].

In a further trial, on the productivity (yield and weight of the fruit) of the blackberry crop the application of *T. asperellum* (1.53 x 10^9 CFU/g) produced an increase in yield between 13% and 29% compared to the control. Application of the fungus resulted in larger berries with an increase of 1 g/berry in the treatments where the beneficial fungus was applied[78,50]. During the trials, is was confirmed that, with periodic (monthly) inoculations of *Trichoderma* sp. to the soil, the establishment of the fungus was achieved, reaching high population levels in the rhizosphere of the crop[81].

A survey was made on the use of microorganisms by small farmers in blackberry production. The information was collected in two of the main producing provinces of this fruit in Ecuador (Tungurahua and Bolívar). It was concluded that farmers who have been beneficiaries of the Project have adopted the practice of using BM as part of the integrated management of their crops. It was established that 53% of the bioproducts used have bacteria of the *Bacillus* genus as active ingredients, 31% are based on *Trichoderma* and 16% the remainder correspond to other types of with the addition of combinations of microorganisms. Farmers stated that microorganisms are used for different functions such as soil conditioning (53%), control of downward wilt disease (41%) and to prevent diseases (6%). The cost of microorganisms varies according to the bio-inputs used, finding that 42% of farmers spend less than 30 USD/200-liter tank, 35% spend $50/tank, 10% spend $40/tank, and 13% spend more than $50/tank. Eighty-one percent of blackberry producers apply microorganisms twice a year, 16% three times a year and 3% once a year. Sixty-six percent of the blackberry producers indicated reaching a crop yield of 12 to 18 kg/plant, 25% of 18 to 27 kg/plant and 9% had yields below 12 kg / plant, values that in all the cases are higher than the national average reported by MAG of 4 kg / plant. Of the farmers surveyed, 80% stated that they had received training from INIAP in the use of microorganisms.

In tests carried out in organic banana crops (*Musa acuminata*), the effect of the inoculation of beneficial microorganisms (*Trichoderma asperellum, Purpureocillium lilacinum* and *Arthrobotrys* sp.) on the populations of phytopathogenic nematodes was evaluated. While application of the fungi did not reduce the populations of pathogenic or free-living nematodes, plants treated with *T. asperellum* showed an increase in the number of healthy roots, which favored the increase in productivity of the crop[82].

In addition, beneficial fungi (*Beauveria* and *Paecilomyces*) were evaluated for the control of the thrip *Chaetanaphothrips signipennis* which causes red rust damage that causes severe losses to small and medium-sized organic banana producers and those producing plantains for export[83]. Unfortunately, these beneficial fungi caused a mottling of the fruit when applied directly to the bunch and could not be recommended[84]. As a consequence, products accepted in organic agriculture were evaluated, finding a 90% reduction in damage from red rust banana thrips on fruits through the application of pyrethrin[85], and up to 100% with the application of *Saccharopolyspora spinosa*[83]. In a survey of two associations of small banana producers in Ecuador (Asociación Tierra Fértil and ASOPROLIFLO), 95% of the producers consider that the losses due to red rust banana thrips are important and all are willing to use the technology that INIAP offers for the control of the red rust banana thrips; but they want to continue training through continuous knowledge update workshops.

Discussion

Biological control is the control of pest or plant diseases by applying biological agents suppress a pest or prevent the development of a disease by a pathogen. It is a sustainable approach to pest management, contributing to the reduction in the use of pesticides as part of an IPM strategy[86]. This type control is based on the premise that it is important to counteract habitat loss and environmental disturbance associated with intensive crop production by conserving and managing biological control agents[87]. These organisms provide a valuable ecosystem service through their contribution to BC[88]. Therefore, the conservation of BM is of fundamental importance for the sustainability of crop production[89].

Trichoderma spp., is the main antagonistic fungus that has been formulated and is widely used in agricultural applications due to its well-known BC mechanism. It is also known for its beneficial impact on plant growth, decomposition processes and bioremediation[90]. These benefits bring enormous advantages to the agricultural industry by applying environmentally friendly agricultural practices.

Recent work has pointed out that common plant diseases such as root rot, seedling disease, wilting, fruit rot, among others, can be prevented and/or controlled with *Trichoderma* spp.[90]. Commonly, BCAs do not affect to non-target organisms, however, it has been reported that Trichoderma spp., can also affect other microorganisms[91]. For this reason it is recommended to apply Trichoderma sp. alone and not in combination with other biocontrol agents.

The widespread use of *Trichoderma* spp. in formulated products (wettable powder and granules) has attracted the attention of researchers to discover more about other potential benefits of *Trichoderma* spp., especially as PGP and its impact on plant yield[50,76,82,84,92], for which its application is recommended as part of agricultural crop management programs. The use of formulations allows the generation of products in which the microorganism has a substrate that allows it to have stability, maintaining concentration and viability, and prolonging its shelf life[51]. More research is needed to generate new prototypes that allow the production of quality products that obtain efficient results when applied to agricultural crops.

The regulatory issue is of utmost relevance to regulate the production of bio-inputs in a country[66]. For this reason, the regulations implemented in Latin America allow the establishment of evaluation parameters for the registration of products, guaranteeing their quality. These control processes must be rigorous in order to avoid the commercialization of deficient products that cause the farmer to lose confidence and credibility in bio-inputs.

In Ecuador, there is a trend towards the adoption of biological controls in the production systems of small farmers in which their benefits have been justified through the results of the project "Biocontrol for Sustainable Farming Systems"[93]. This approach leads to the economic growth of poor rural communities and the development of access to local and international markets. Furthermore, by adopting and promoting biological production systems, Ecuador can promote its agricultural sector not only for production, but also as an attractive place for ecological and community tourism. To a large extent, this agricultural development has allowed Ecuador to work from within towards success in achieving the ONU Sustainability Goals[93].

Conclusions

The use of beneficial fungi, mainly *Trichoderma* sp. and *Beauveria* sp., has increased in agriculture due to the positive results that have been confirmed through research generated in different Latin American countries that have justified the efficiency of beneficial fungi in PC as well as their functionality as PGP, affecting plant nutrition. In addition, it has been confirmed that the use of biological control assists the development of a sustainable agriculture that conserves natural resources such as soil and water, as well as reducing pollution of the environment by reducing the use of agrochemicals in agricultural crops. However, regulation of the commercialization of these products is an issue that must continue to be strengthened so that users of bio-inputs obtain high quality, efficient products through quality control procedures that allow formulations and product efficacy to be verified.

In Ecuador, the BC issue has taken on importance and currently there is a favorable attitude among farmers to use bio-inputs and reduce the application of agrochemicals due to the results obtained in the Project "Biocontrol for Sustainable Farming Systems". These results have generated trust and credibility in farmers on the use of microorganisms in agriculture as a component of integrated crop management that protects the environment and offers clean products benefiting consumer health.

References

1. Sarwar M. The killer chemicals as controller of agriculture insect pests: The conventional insecticides. Int J Chem Biomol Sci 2015; 1(3): 141-147.
2. Notarnicola B, Sala S, Anton A, McLaren S J, Saouter E, Sonesson U. The role of life cycle assessment in supporting sustainable agri-food systems: A review of the challenges. J Clean Prod 2017; 140: 399-409.
3. González H, Fuentes N. Mecanismo de acción de cinco microorganismos promotores de crecimiento vegetal. Rev Cienc Agr 2017; 34(1): 17–31. DOI: http://doi.org/10.22267/rcia.173401.61
4. Fenner K, Canonica S, Wackett LP, Elsner M. Evaluating pesticide degradation in the environment: Blind spots and emerging opportunities. Sci 2013; 341(6147): 752-758. DOI: https://doi.org/10.1126/science.1236281
5. Verger P, Boobis A. Reevaluate pesticides for food security and safety. Science 2013; 341(6147): 717-718. DOI: https://doi.org/10.1126/science.1241572
6. Brent K, Hollomon D. Fungicide resistance: the assessment of risk. Bruselas, Bélgica: Global crop protection federation Brussels; 2007.
7. Badii MH, Tejada LO, Flores AE, Lopez CE, Quiróz H. Historia, fundamentos e importancia. En: Badii MH, Flores AE, Galán LJ, editores. Fundamentos y perspectivas de control biológico. Monterrey: Cienc UANL; 2000. p 3-17.
8. Guédez C, Castillo C, Cañizares L, Olivar R. Control biológico#: una herramienta para el desarrollo sustentable y sostenible . Academia 2008; 7(13): 50-74.
9. Celis Á, Mendoza C, Pachón M, Cardona J, Delgado W, Cuca LE. Extractos vegetales utilizados como biocontroladores con énfasis en la familia Piperaceae. Una revision. Agron Colomb 2006; 26(1): 97-106.
10. Trujillo Amaga J. Control biológico por conservación: enfoque relegado. Perspectiva de su desarrollo en Latinoamérica. Ceiba; 1992. 33(1): 17-26.
11. Cristancho Ardila MA. Control biológico de enfermedades. En: Cristancho Ardila MA, editor. Enfermedades del cafeto en Colombia [Internet]. Colombia; 2003. p. 55-63. Recuperado a partir de:https://biblioteca.cenicafe.org/bitstream/10778/993/9/7.%20Control%20biol%C3%B3gico%20de%20enfermedades.pdf
12. Cotes A, Fargetton X, Kohl J, Díaz A, Gómez M, Grijalba M, et al. Control biológico de fitopatógenos, insectos y ácaros [Internet]. Mosquera: Corporación Colombiana de Investigación Agropecuaria; 2018 [citado 22-de mayo de 2020]. 516 p. Recuperado a partir de: file:///C:/Users/usuario/Downloads/33519.pdf
13. Duarte Cueva F. El control biológico como estrategia para apoyar las exportaciones agrícolas no tradicionales en Perú: un análisis empírico. Contabilidad y Negocios 2012; 7(14):81-100.
14. Zin NA, Badaludinn NA. Biological functions of Trichoderma spp. for agriculture applications. Ann Agric Sci 2020; 65(2): 168-178. DOI: https://doi.org/10.1016/j.aoas.2020.09.003
15. Shields M, Johnson AC, Pandey S, Cullen R, González Chang M, Wratten S, et al. History, current situation and challenges for conservation biological control. Biol Control 2019; 131: 25-35. DOI: https://doi.org/10.1016/j.biocontrol.2018.12.010

16. Heimpel GE, Mills NJ. Biological control: ecology and applications. Cambridge: Cambridge University Press; 2017.
17. van Lenteren JC, Bale J, Bigler F, Hokkanen HMT, Loomans AJM. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu Rev Entomol 2006; 51(1): 609-634. DOI: https://doi.org/10.1146/annurev.ento.51.110104.151129
18. Castillo Carrillo CI. Biodiversity in Ecuador and its immense potential for agricultural pest control. En: Chong PA, Newman DJ, Steinmacher DA, editors. Agricultural, forestry and bioindustry, biotechnology and biodiscovery. Switzerland: Springer; 2020. p. 143-161. DOI: https://doi.org/10.1007/978-3-030-51358-0_9
19. Lucero ML, Peña LA, Cultid L, Bolaños MA. Manejo integrado de chisas en fincas de minifundio del departamento de Nariño (Colombia). Cienc Tecnol Agropec 2006; 7(1): 70-72. DOI: https://doi.org/10.21930/rcta.vol7_num1_art:63
20. Solis Soto A, Garcìa Gutierrez C, González Maldonado MB, Medrano Roldán H, Galán Wong LJ. Toxicidad de blastosporas de Beauveria bassiana (VUILL) contra palomilla del manzano Cydia pomonella L. (Lepidoptera: tortricidae) Folia Entomol Mex 2006; 45(2): 195-200.
21. Jaramillo J, Montoya E, Benavides P, Góngora CE. Beauveria bassiana y Metarhizium anisopliae para el control de broca del café en frutos del suelo. Rev Colomb Entomol 2015; 41(1): 95-104.
22. Mascarin MG, Jaronski ST. The production and uses of Beauveria bassiana as a microbial insecticide. World J Microbiol Biotechnol 2016; 32(11):177. DOI: https://doi.org/10.1007/s11274-016-2131-3
23. Ortiz Urquiza A, Keyhani NO. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 2013; 4(3):357-374. DOI: https://doi.org/10.3390/insects4030357
24. Humber RA. Evolution of entomopathogenicity in fungi. J Invertebr Pathol 2008; 98(3):262-266. DOI: https://doi.org/10.1016/j.jip.2008.02.017
25. Espinel Correa C, Torres Torres LA, Villamizar Rivero LF, Bustillo Pardey AE, Zuluaga Mogollón MV, Cotes Prado AE. Hongos entomopatogenos en el control biológico de insectos plaga. En: Espinel Correa C, Torres Torres LA, Villamizar Rivero LF, Bustillo Pardey AE, Zuluaga Mogollón MV, Cotes Prado AE, editores. Control biológico de fitopatógenos, insectos y ácaros [Internet]. Colombia: Corporación Colombiana de Investigación Agropecuaria; 2018. p. 334-367. Recuperado a partir de: http://editorial.agrosavia.co/index.php/publicaciones/catalog/download/21/13/166-1?inline=1
26. McCoy C, Quintela ED, De Faria M. Environmental persistence of entomopathogenic fungi. In: Baur ME, Fuxa JR, editors. Factors affecting the survival of entomopathogens [Internet]. Lousiana: University Agricultural Center, Southern Cooperative Series, Bulletin 400; 2002. p. 20-30.
27. Jaronski S. Ecological factors in the inundative use of fungal entomopathogens. Biocontrol 2010; 55:159-185. DOI: https://doi.org/10.1007/s10526-009-9248-3
28. Fernandes ÉK, Rangel DE, Braga GU, Roberts DW. Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. Curr Genet 2015; 61(3):427-440. DOI: https://doi.org/10.1007/s00294-z
29. Jaronski ST. New paradigms in formulating mycoinsecticides. In: Goss GR, Hopkinson MJ, Collins HM, editors. Pesticide formulations and application systems [Internet]. Filadelfia: ASTM STP 1328; 1997. DOI: https://doi.org/10.13140/RG.2.1.5044.9762
30. Burges HD. Formulation of microbial biopesticides: beneficial organisms, nematodes and seed treatments. Dordrecht: Springer; 1998. 412 p.
31. Wraight S, Ramos ME. Application parameters affecting field efficacy of Beauveria bassiana foliar treatments against Colorado potato beetle Leptinotarsa decemlineata. BioControl 2002; 23(2):164-178. DOI: https://doi.org/10.1006//bcon.2001.1004
32. Lacey LA, Wraight SP, Kirk AA. Entomopathogenic fungi for control of Bemisia tabaci biotype B: foreign exploration, research and implementation. In: Gould J, Hoelmer K, Goolsby J, editors. Classical biological control of Bemisia tabaci in the United States. A review of interagency research and implementation

[Internet]. Dordrech: Springer; 2008. p. 33-69. Recuperado a partir de: file:///C:/Users/usuario/Downloads/Entomopathogenic_Fungi_for_Control_of_Bemisia_taba.pdf

33. Malpartida Zevallos J, Narrea Cango M, Dale Larraburre W. Patogenicidad de Beauveria bassiana (Bals) Vuill., sobre el gusano defoliador del maracuyá Dione juno (Cramer) (Lepidoptera: Nymphalidae) en laboratorio. Ecol Aapl 2013; 12(2):75-81. DOI: https://doi.org/10.21704/rea.v12i1-2.440
34. Charoenrak P, Chamswarng C. Efficacies of wettable pellet and fresh culture of Trichoderma asperellum biocontrol products in growth promoting and reducing dirty panicles of rice. Agric Nat Resour 2016; 50(2):243-249. DOI: https://doi.org/10.1016/j.anres.2016.04.001
35. Díaz García A, Gómez Álvarez MI, Grijalba Bernal EP, Santos Díaz AM, Cruz Barrera FM, León Moreno DM, et al. Desarrollo y escalamiento de bioplaguicidas. En Díaz García A, Gómez Álvarez MI, Grijalba Bernal EP, Santos Díaz AM, Cruz Barrera FM, León Moreno DM, editores. Control biológico de fitopatógenos, insectos y ácaros [Internet]. Colombia: Corporación Colombiana de Investigación Agropecuaria; 2019. p. 628-691. Recuperado a partir de: http://editorial.agrosavia.co/index.php/publicaciones/catalog/download/23/14/304-1?inline=1
36. Medrano M, Ortuño N. Control del Damping off mediante la aplicación de bioinsumos en almácigos de cebolla en el Valle Alto de Cochabamba - Bolivia. Acta Nova 2007; 3(4): 660-679.
37. Arévalo E, Cayotopa J, Olivera D, Gárate M, Trigoso E, Costa B, et al. Optimización de sustratos para la producción de conidias de Trichoderma harzianum. Por fermentación sólida en la región de San Martín. Perú. Rev Investig Altoan 2017; 19(2): 135-144. DOI: https://doi.org/10.18271/ria.2017.272
38. Valdés Ríos EL. Caracteres principales, ventajas y beneficios agrícolas que aporta el uso de Trichoderma como control biológico. Rev Cient Agroeco 2014; 2(1): 254-264.
39. Woo SL, Ruoco M, Vinale F, Vinale F, Nigro M, Marra R, et al. Trichoderma-based products and their widespread use in agriculture. Open Mycol J 2014; (8): 71-126. DOI: https://doi.org710.2174/1874437001408010071
40. Tijjani A, Bashir K, Mohammed I, Muhammad A, Gambo A, Musa H. Biopesticides for pests control: A review. Journal of Biopesticides and Agriculture 2016; 3(1): 6-13.
41. Vallejo Ilijama MT. Caracterización y clasificación de Trichodermas nativos aplicando diferentes medios de cultivo a nivel de laboratorio artesanal [tesis maestría]. [Ambato]: Universidad Técnica de Ambato; 2014 [citado 26 de mayo de 2020]. Recuperado a partir de:https://repositorio.uta.edu.ec/handle/123456789/7691
42. Leon Ttacca B, Ortiz Calcina N, Condori Ticona N, Chura Yupanqui E. Cepas de Trichoderma con capacidad endofitica sobre el control del mildiu (Peronospora variabilis Gäum.) y mejora del rendimiento de quinua. Rev Investig Altoandin 2018; 20(1): 19-30. DOI: https://doi.org/10.18271/ria.2018.327
43. Tirado Gallego PA, Lopera Álvarez A, Ríos Osorio LA. Estrategias de control de Moniliophthora roreri y Moniliophthora perniciosa en Theobroma cacao L.: revisión sistemática. Corpoica Cienc Tecnol Agropecu 2016;17(3): 417-430.DOI: https://doi.org/10.21930/rcta.vol17_num3_art:517
44. Hidalgo K, Suárez C. Uso de Trichoderma spp. para control del complejo Moniliasis – Escoba de Bruja del cacao en Ecuador. INIAP - Estación Experimental Tropical Pichilingue 2016; 1 (4): 13-16.
45. Asero J, Suquilanda M. Evaluación de Trichoderma harzianum y Penicillium sp. en el control de “Oidio” (Sphaeroteca pannosa) en rosas (Rosa sp.) variedad aalsmer gold Ascázubi, Pichincha, Ecuador; 2007. p. 1-13.
46. Sabando Ávila F, Molina Atiencia LM, Garcés Fiallos FR. Trichoderma harzianum en pre-transplante aumenta el potencial agronómico del cultivo de piña. Rev Bras Ciênc Agrár 2017; 12(4): 410-414. DOI: https://doi.org/10.5039/agraria.v12i4a5468
47. Jaimes Suáres YY, Moreno Velandia CA, Cotes Prado AM. Inducción de resistencia sistémica contra Fusarium oxysporum en tomate por Trichoderma koningiopsis Th003. Acta Biol Colomb 2009; 14(3): 111-119.
48. Gerhardson B. Biological substitutes for pesticides. Trends Biotechnol 2002; 20(8): 338-343. DOI: https://doi.org/10.1016/s0167-7799(02)02021-8
49. López Perez M, Rodríguez Gomez D, Loera O. Production of conidia of Beauveria bassiana in solid-state culture: current status and future perspectives. Crit Rev Biotechnol 2015; 35(3): 334-341. DOI: https://doi.org/10.3109/07388551.2013.857293

50. Viera W, Noboa M, Martínez A, Báez F, Jácome R, Medina L, et al. Trichoderma asperellum increases crop yield and fruit weight of blackberry (Rubus glaucus) under subtropical Andean conditions. Vegetos 2019; 32:209-215. DOI: https://doi.org/10.1007/s42535-019-00024-5

51. Viera W, Noboa M, Bermeo J, Báez F, Jackson T. Parámetros de calidad de cuatro tipos de formulaciones a base de Trichoderma asperellum y Purpuricillium lilacinum. Enfoque UTE 2018; 9(4): 145-153. DOI: https://doi.org/10.29019/enfoqueute.v9n4.348

52. Perdomo Quishpe CE. Desarrollo de cuatro prototipos de bioformulaciones en base a conidias de Trichoderma asperellum [tesis licenciatura]. [Quito]: Universidad Central del Ecuador; 2018 [citado 26 de julio de 2020]. Recuperado a partir de: http://www.dspace.uce.edu.ec/handle/25000/16686

53. Mamani Rojas P, Limachi Villava J, Ortuño Castro N. Uso de microorganismos nativos como promotores de crecimiento y supresores de patógenos en el cultivo de la papa en Bolivia. Rev Latinoam Papa 2012; 17(1): 74-96.

54. Cruzat R, Ionannidis D. Resultados y lecciones en biocontrol de enfermedades fungosas con Trichoderma spp [Internet]. Santiago de Chile: Fundación para la Innovación Agraria; 2008 [citado 12 de junio de 2020]. 30 p. Recuperado a partir de: http://bibliotecadigital.fia.cl/bitstream/handle/20.500.11944/2075/62_Libro_Trichoderma.pdf?sequence=1&isAllowed=y

55. Borrelli NP. Selección in vitro de cepas nativas del género Tricoderma a partir de aislamientos de suelo para el control de Sclerotinia sclerotiorum [tesis liceniatura]. [Buenos Aires]: Universidad de Buenos Aires; 2017 [citado 26 de agosto de 2020]. Recuperado a partir de: http://ri.agro.uba.ar/cgi-bin/library.cgi?a=d&c=ti&d=2017borrellinicolaspablo

56. Agamez Ramos E, Barrera Violeth J, Oviedo Zumaqué L. Evaluación del antagonismo y multiplicación de Trichoderma sp. en sustrato de plátano en medio líquido estático. Acta Bioló Colomb 2009; 14(3): 61-70.

57. González Caneloríguez M, Puertas Arias A, Jiménez Arteaga MC, Danger Hechevavarría L, López Álvarez S. Uso de Trichoderma harzianum Rifaii para el control biológico de Sclerotium rolfsii Sacc. en el cultivo de zábila (Aloe vera L.). Rev Arbitr Interdiscip Koinonía 2017: 11(3): 213-225.

58. Borges Chagas LF, Chagas Junior AF, Rodrigues de Carvalho M, de Oliveira Miller L, Orozco Colonia BS. Evaluación del potencial de solubilización de fosfato de cepas de Trichoderma (Trichoplus JCO) y efectos sobre la biomasa del arroz. J Soil Sci Plant Nutr 2015; 15(3): 794-804. DOI: https://doi.org/10.4067/S718-95162015005000054

59. Lagler JC. Biological inputs: different perceptions making focus in the biological fertilization. Agron Ambiente 2017; 37(1): 73-89.

60. Instituto Colombiano Agropecuario (ICA). Productos registrados bioinsumos. Colombia; 2018. Recuperado a partir de: https://www.ica.gov.co/getdoc/2ad9e987-8f69-4358-b8a9-e6ee6dcc8132/productos-bioinsumos-mayo-13-de-2008.aspx

61. Servicio Nacional de Sanidad y Calidad Agroalimentaria. SENASA. Registro nacional de terapéutica vegetal. Argentina; 2018. Recuperado a partir de: https://www.argentina.gob.ar/inscribir-reinscribir-en-el-registro-nacional-sanitario-de-productores-agropecuarios-renspa

62. Servicio Nacional de Sanidad Agropecuaria e Inocuidad Alimentaria - Unidad Nacional de Sanidad Vegetal (SENASAG). Estado del registro de productos agroquímicos. Bolivia; 2011. http://www.senasag.gob.bo/egp/productossv10.html

63. Servicio Nacional de Sanidad Agropecuaria e Inocuidad Alimentaria Unidad Nacional De Sanidad Vegetal (SENASAG). Registro de Insumos Agrícolas. Bolivia; 2013. ecuperado a partir de: http://www.senasag.gob.bo/registros-e-insumos-agricolas

64. Servicio Agrícola y ganadero (SAG). Inocuidad y biotecnología Lista de Plaguicidas Autorizados. Chile; 2019. Recuperado a partir de: http://www.sag.cl/ambitos-de-accion/inocuidad-y-biotecnologia/76/registros?field_tema_registros_y_listas_tid=All&field_tipo_de_registro_tid=All&title=&items_per_page=15.

65. Ministerio de Agricultura Servicio Nacional de Sanidad Agraria (SENASA). Reporte de Productos Plaguicidas Registrados. Perú; 2019. Recuperado a partir de: https://servicios.senasa.gob.pe/SIGIAWeb/sigia_consulta_producto.html

66. Agencia de Regulación y Control Fito y Zoosanitario (AGROCALIDAD). Dirección de Registro de Insumos Agrícolas. Registro de Fertilizantes. Ecuador; 2019. Recuperado a partir de: http://www.agrocalidad.gob.ec/direccion-de-registro-de-insumos-agricolas/
67. Hidalgo Dávila JL. La situación actual de la sustitución de insumos agropecuarios por productos biológicos como estrategia en la producción agrícola: El sector florícola ecuatoriano [tesis maestría]. [Quito]: Universidad Andina Simón Bolívar; 2017 [citado 16 de marzo de 2020]. Recuperado a partir de: http://repositorio.uasb.edu.ec/bitstream/10644/6095/1/T2562-MRI-Hidalgo-La%20situacion.pdf
68. Jackson TA, Báez F, Villamizar LF. Formulación para mejorar la actividad de bioplaguicidas microbianos. En: León-Reyes A, Barriga N, Aycart JJ, Báez F, Cevallos JM, Erazo N, et al., editores. En: Memorias del 3er Simposio en Fitopatología, Control Biológico e Interacciones Planta-Patógeno: octubre 2017. Archivos Académicos Universidad San Francisco de Quito [Internet]. Quito: Universidad San Francisco de Quito; Editorial USFQ Universidad San Francisco de Quito; 2017 [citado 3 de mayo de 2020]. p. 24. Recuperado a partir de: https://revistas.usfq.edu.ec/index.php/archivosacademicos/article/view/1475/1574
69. Falconí C. Manual de Taxonomía de Trichoderma y Paecilomyces spp. Ecuador; 2011. p. 3-40.
70. Noboa M, Díaz A, Vásquez C, Viera W. Parasitoids of Neoleucinodes elegantalis Gueneé (Lepidoptera: Crambidae) in Ecuador. Idesia 2019; 35(2): 49-54. DOI: https://doi.org/10.4067/S0718-34292017005000015
71. Instituto Nacional de Investigaciones Agropecuarias (INIAP). Informe anual del Proyecto Biocontrol for Sustainable Farming Systems, Ecuador. Quito: INIAP; 2017.
72. Villamizar L, Zeddam JL, Espinel C, Cotes AM. Implementación de técnicas de control de calidad para la producción de un bioplaguicida a base del granulovirus de Phthorimaea operculella PhopGV. Rev Colomb Entomol 2005; 31(2): 127-132.
73. Pumisacho M, Sherwood. El cultivo de la papa en el Ecuador. INIAP – CIP. Quito, Ecuador; 2012. p. 132 – 136.
74. Guapi Auquilla AP. Evaluación de la eficacia del bioformulado de Beauveria bassiana, y tipos de aplicación para el control del gusano blanco de la papa (Premnotrypes vorax), en dos localidades de la provincia de Chimborazo [tesis licenciatura]. [Riobamba]: Escuela Superior Politécnica de Chimborazo; 2012 [citado 26 de mayo de 2020]. Recuperado a partir de: http://dspace.espoch.edu.ec/bitstream/123456789/2201/1/13T0746%20.pdf
75. Báez F, Viera W, Noboa M, Jackson T, Espinosa G. Experiencias de aplicación del hongo Trichoderma sp. en plántulas de brócoli. En: León-Reyes A, Barriga N, Aycart JJ, Báez F, Cevallos JM, Erazo N, et al., editores. Memorias del 3er Simposio en Fitopatología, Control Biológico e Interacciones Planta-Patógeno: octubre 2017. Archivos Académicos Universidad San Francisco de Quito [Internet]. Quito: Universidad San Francisco de Quito; Editorial USFQ Universidad San Francisco de Quito; 2017 [citado 3 de mayo de 2020]. p. 18. Recuperado a partir de: https://revistas.usfq.edu.ec/index.php/archivosacademicos/article/view/1475/1574
76. Sotomayor A, Gonzáles A, Cho K, Villavicencio A, Jackson T, Viera W. Effect of the application of microorganisms on the nutrient absorption in avocado (Persea americana Mill.) Seedlings. J Korean Soc Int Agric 2019; 31(1): 17-24. DOI: https://doi.org/10.12719/KSIA.2019.31.1.17
77. Espín Chico MC. Validación de los componentes tecnológicos limpio y orgánico, con y sin Trichoderma para el manejo del cultivo de Mora de Castilla (Rubus glaucus Benth) en el cantón Cevallos, provincia de Tungurahua [tesis licenciatura]. [Riobamba]:Escuela Superior Politécnica de Chimborazo; 2013 [citado 26 de agosto de 2020]. Recuperado a partir de: http://dspace.espoch.edu.ec/handle/123456789/2370
78. Sánchez J, Iturralde P, Koch A, Tello C, Martínez D, Proaño N, et al. Dactylonectria and Ilyonectria species causing black foot disease of Andean Blackberry (Rubus Glaucus Benth) in Ecuador. Diversity 2019; 11(11): 218. DOI: https://doi.org/10.3390/d11110218
79. Oña Malataxi CI. Evaluación de la eficacia de productos convencionales y alternativos para el control de marchitez descendente (Ilyonectria torresensis) en mora de Castilla [tesis licenciatura]. [Quito]: Universidad Central del Ecuador; 2018 [citado 26 de julio de 2020]. Recuperado a partir de: http://www.dspace.uce.edu.ec:8080/handle/25000/17068

80. Vásquez W, Jackson T, Viera W, Viteri P, Villares M. Integrated Andean blackberry (Rubus glaucus) crop management using beneficial microorganisms by small farmers in the Ecuadorian Andes. In: Feldmann F, Short Heinrichs EA, editors. Proceedings of International Plant Protection Congress, 2015 [Internet]. Berlin; 2015. p. 498. Recuperado a partir de: file:///C:/Users/usuario/Downloads/2015_IPPC2015_Prog_Abstractbook.pdf

81. Torrens G, Báez F, Martínez A, Jácome R, Jackson T. Fluctuación poblacional de Trichoderma spp. en suelo de plantación de mora (Rubus glaucus) en la Provincia de Tungurahua. En: Memorias del 3er Simposio en Fitopatología, Control Biológico e Interacciones Planta-Patógeno: octubre 2017. Archivos Académicos Universidad San Francisco de Quito [Internet]. Quito: Universidad San Francisco de Quito; Editorial USFQ Universidad San Francisco de Quito; 2017 [citado 3 de mayo de 2020]. p. 61. Recuperado a partir de: https://revistas.usfq.edu.ec/index.php/archivosacademicos/article/view/1475/1574

82. Navia D, Delgado A, Viera W, Báez F, Jackson T. Application of bioproducts in Ecuadorian agriculture: case banana. En: Conference Proceedings of Bioproducts for Sustainable Agriculture. Quito: INIAP; 2017. p. 57.

83. Arias de López M, Corozo Ayovi RE, Delgado Arce R, Osorio Villegas B, Rojas JC, Rengifo D, et al. Cómo reducir la mancha roja causada por thrips en banano [Internet]. Quito: El Triunfo, Ecuador: Instituto Nacional de Investigaciones Agropecuarias; 2019 [citado 26 de mayo de 2020]. Boletín Divulgativo No. 443. Recuperado a partir de: file:///C:/Users/usuario/Downloads/BoletinDivulgativo443.pdf

84. Delgado A, Hall R, Navia D, Viera W, Jackson T. Efecto de hongos benéficos sobre las poblaciones de Chaetanaphothrips signipennis en el cultivo de plátano. En: Memorias del LIX Convención Nacional de Entomología. Trujillo: Sociedad Entomológica del Perú; 2017a. s.p.

85. Delgado A, Hall R, Navia D, Viera W, Báez F, Arias M, et al. Evaluation of pyrethrum, extract of Saccharopolyspora spinosa, Beauveria bassiana and Metarhizium anisopliae for the control of Chaetanaphothrips signipennis, a pest of banana. En: Proceedings of the 50th Annual Meeting of the Society for Invertebrate Pathology. San Diego: Society for Invertebrate Pathology; 2017b. p. 78-79.

86. O´Brien PA. Biological control of plant diseases. Australas Plant Pathol 2017; 46(4): 293-304. DOI: https://doi.org/10.1007/s13313-017-0481-4

87. Begg G, Cook S, Dye R, Ferrante M, Frankc P, Lavigne C, et al. A functional overview of conservation biological control. Crop Prot 2017; 97: 145-158. DOI: http://doi.org/10.1016/j.cropro.2016.11.008

88. Pimentel D. Environmental and economic costs of the application of pesticides primarily in the United States. En: Peshin R, Dhawan AK, editors. Integrated pest management: Innovation-Development Process. Dordrecht: Springer; 2009. p. 47-71. DOI: https://doi.org/10.1007/978-1-4020-8992-3

89. Barzman M, Bàrberi P, Birch ANE, Boonekamp P, Dachbrodt Saaydeh S, Graf B, et al. Eight principles of integrated pest management. Agron Sustain Dev 2015; 35: 1199-1215. DOI: http://doi.org/10.1007/s13593-015-0327-9

90. Zin NA, Badaluddin NA. Biological functions of Trichoderma spp. for agriculture applications. Ann Agric Sci 2020; 65(2): 168-178. DOI: https://doi.org/10.1016/j.aoas.2020.09.003

91. Ros M, Raut I, Santisima Trinidad AB, Pascual JA. Relationship of microbial communities and suppressiveness of Trichoderma fortified composts for pepper seedlings infected by Phytophthora nicotianae. PloS One 2017; 12(3): e0174069. DOI: https://doi.org/10.1371/journal.pone.0174069

92. Viera W, Noboa M, Martínez A, Jácome R, Medina L, Jackson T. Trichoderma application increases yield and individual fruit weight of blackberries grown by small farmers in Ecuador. Acta Hortic 2020; 1277: 287-292. DOI: https://doi.org/10.17660/ActaHortic.2020.1277.42

93. Viera W, Jackson T. Ecuador demonstrates a sustainable way forward for small farmer producers. Chron Horticult 2020; 60(3): 19-21.

Notes

Funding source: Ministry of Foreign Affairs and Trade New Zealand

Interest conflicts These investigations did not present conflicts of interest.

Acknowledgment: The authors thank the "Biocontrol for Sustainable Farming Systems, Ecuador" Project for its contribution to generate information that has allowed the promotion of the use of bio-inputs and has produced results that benefit the Ecuadorian agricultural sector. In addition, we thank the Ministry of Foreign Affairs and Trade New Zealand for funding the Project. Finally, thanks to all the farmers and technicians who were part of the activities developed in the Project.

Ethical aspects: All the investigations were carried out following the experimental procedures according to the INIAP guidelines and the code of ethics, in order to generate reliable results that contribute to a sustainable agriculture in Ecuador.

ID of article:091/JSAB/2020

Editor's Note:*Journal of the Selva Andina Biophere (JSAB)* **remains neutral with respect to jurisdictional claims published on maps and institutional affiliations.**

Alternative link

http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S2308-38592020000200006&lng=es&nrm=iso&tlng=en (html)