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BIOLOGICAL CONTROL IN LATIN AMERICA

Forum

On-farm Production of Microbial Entomopathogens for use in Agriculture: Brazil as a Case Study

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Abstract

In Brazil, the production of beneficial microorganisms by growers exclusively for their own use is a practice known as "*on-farm* production". Regarding *on-farm* bioinsecticides, they were initially deployed for pests of perennial and semi-perennial crops in the 1970s but, since 2013, their use has extended to pests of annual crops such as maize, cotton, and soybean. Millions of hectares are currently being treated with these *on-farm* preparations. Local production reduces costs, meets local needs, and reduces inputs of environmentally damaging chemical pesticides, facilitating establishment of more sustainable agroecosystems. Critics argue that without implementation of stringent quality control measures there is the risk that the *on-farm* preparations: (1) are contaminated with microbes which may include human pathogens or (2) contain very little active ingredient, impacting on field efficacy. The *on-farm* fermentation of bacterial insecticides predominates, especially that of *Bacillus thuringiensis* targeting lepidopteran pests. However, there has been a rapid growth in the past 5 years in the production of entomopathogenic fungi, mostly for the control of sap-sucking insects such as whitefly (*Bemisia tabaci* (Gennadius)) and the corn leafhopper (*Dalbulus maidis* (DeLong and Wolcott)). In contrast, *on-farm* production of insect viruses has seen limited growth. Most of the ca. 5 million rural producers in Brazil own small or medium size properties and, although the vast majority still do not practice *on-farm* production of biopesticides, the topic has aroused interest among them. Many growers who adopt this practice usually use non-sterile containers as fermenters, resulting in poor-quality preparations, and cases of failure have been reported. On the other hand, some informal reports suggest *on-farm* preparations may be efficacious even when contaminated, what could be explained, at least partially, by the insecticidal secondary metabolites secreted by the pool of microorganisms in the liquid culture media. Indeed, there is insufficient information on efficacy and mode of action of these microbial biopesticides. It is usually the large farms, some with > 20,000 ha of continuous cultivated lands, that produce biopesticides with low levels of contamination, as many of them possess advanced production facilities and have access to specialized knowledge and trained staff. Uptake of *on-farm* biopesticides is expected to continue but the rate of adoption will depend on factors such as the selection of safe, virulent microbial strains and implementation of sound quality control measures (compliance with emerging Brazilian regulations and international standards). The challenges and opportunities of *on-farm* bioinsecticides are presented and discussed.

Keywords Biological control · microbial biopesticides · *Bacillus* · entomopathogenic fungi · baculoviruses

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Introduction

Agriculture is eager for sustainable pest management approaches. Pesticides based on microorganisms (bacteria, viruses, fungi), in some countries also known as biopesticides, are recognized as contributing significantly to modern integrated pest management (IPM) programs. In Brazil, several drivers account for increased sales of biopesticides, including growth of the organic farming sector, consumer demand for produce with low concentration of chemical residues, increasing costs of chemical crop protection, and the negative impact of conventional pesticides to human health and biodiversity.

The number of registered biopesticides in Brazil has increased significantly, from 107 in 2013 to over 400 in 2023 (Agrofit 2023). The area treated with commercial biocontrol agents, mostly biopesticides, has increased considerably in the past decade and it was estimated to be 13.3 million hectares in the agricultural year 2019/2020 (IHS Markit 2021). At least 225 microbial products were commercially available for control of crop insects and mites (Agrofit 2023). These products are used on small farms as well as large-area crops such as soybean, corn, cotton, sugarcane, and coffee, among many others. Indeed, soybeans and sugarcane combined are the major crops using biological agents, accounting for over 60% of the biological control market in Brazil (IHS Markit 2021).

There are approximately 1.1 million large/medium-scale farms and 3.9 million family farms in Brazil (IBGE 2017). Not all these farms use biopesticides and, among those that do, some do not use registered commercial products. A number of rural farmers produce biopesticides and other beneficial microorganisms for their own consumption, a practice known as *on-farm* production. The practice is not illegal but Brazilian law stipulates that the products are for the sole use of the producer and not for sale to third parties. Key aspects of commercial and *on-farm* biopesticide production are summarized in Table 1.

Although there are still no robust surveys about the extent of *on-farm* production in Brazil, the area treated with *on-farm* biopesticides was estimated to be 3.1 million hectares in 2019/2020 (IHS Markit 2021). Several large growers produce *on-farm* biopesticides to treat over 20,000 ha of crops each year. An even greater number of small- and medium-size farms use *on-farm* produced biopesticides.

The *On-farm* Production of Entomopathogenic Microorganisms in Brazil

On-farm production of microbial biopesticides is not new in Latin America (Table 2). In Brazil, production of the fungus *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) by individual sugar cane mills and cooperatives for control of sugarcane spittlebugs (Hemiptera: Cercopidae) has been practiced since the 1970s (Li et al. 2010). A few viruses were also used by growers. For example, AgMNPV used since 1982/1983 for the control of *Anticarsia gemmatalis* Hübner in soybean was one of the largest biological control programs in the world (Moscardi 1999). Only recently have farmers initiated *on-farm* production of entomopathogenic bacteria. The uptake and use of commercial products based on *Bacillus thuringiensis* (Bt) increased following outbreaks of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), in soybean and cotton in 2013/2014. According to Bettiol (2022), outbreaks of this pest also boosted the *on-farm* production of Bt since there was a shortage of chemical and commercial biopesticides at the time. The *on-farm* approach continues to be embraced by farmers due to its appeal in reducing costs through replacement of more expensive chemical pesticides. This is particularly relevant for grain producers, usually with lower gains per cultivated area compared to other crops.

The *on-farm* production of bioinsecticides focuses primarily on bacterial and fungal entomopathogens due to the ease of large-scale *in vitro* cultivation on relatively inexpensive substrates. Entomopathogenic bacteria preparations are mostly based on subspecies of Bt (e.g., *kurstaki* and *aizawai*). The most widely used entomopathogenic fungi (EPF) belong to the genera *Metarhizium*, *Beauveria*, and *Cordyceps* (formerly *Isaria*), as shown in Table 3.

Insect-Pathogenic Bacteria

Brazilian Bt-based commercial biopesticides are produced by submerged liquid fermentation (SLF), by well-equipped national and international private companies, and then formulated before sales. In general, the liquid fermentation process conducted under optimal conditions leads to the production of vegetative cells containing spores and protein crystals, the latter being primarily responsible for the insecticidal activity of this microbial insecticide. Bt mass production poses a greater challenge than other bacteria of the same genus because of its complex cultural requirements as outlined by Jallouli et al. (2020). Cultural conditions (e.g., pH, temperature, oxygen supply, and composition of culture medium) have to optimize the development of the crystal toxin responsible for pest death (Ndao et al. 2017). Conditions favoring Bt vegetative growth may not be adequate for spore formation (Anderson and Jayaraman 2003) and optimal conditions for δ-endotoxin yields may also differ, even though sporulation and toxin formation occur simultaneously during fermentation (Jallouli et al. 2020). Rigid control of fermentation parameters is not always considered by *on-farm* producers who, in general, adopt simpler and cheaper cultivation systems (Fig. 1). The preference for SLF by rural producers is driven mainly by the fact that it is less labor intensive and has shorter fermentation times compared to solid-state fermentation (SSF). Most often production is conducted in non-hermetic containers with poor-quality water and no control over temperature, pH, and oxygen. This precarious production process, combined with the lack of specialized technical support, often leads to low-quality preparations. A small proportion of rural producers have invested in modern bioreactors, aseptic cultivation

Table 1 Contrast between commercial and *on-farm* bioinsecticides in Brazil

	Commercial	On farm
Microbial entomopathogens	Bacteria, fungi and viruses. According to labels of commercial products, the main entomopathogenic species are *Bacillus thuringiensis* (Bt), *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*, and a number of viruses. However, some of these scientific names need updating. For instance, a commercial *I. fumosorosea* strain has been shown to be, *Cordyceps javanica* (Faria et al., under submission)	Mainly Bt, but non-registered bacteria such as *Chromabacterium subtsugae* and *Saccharopolyspora spinosa* have also been reported to be used. Production of fungi (same species as in commercial products) was less common in the past few years, but there is recent interest due to their potential for controlling sap-sucking insects and other pests. Production of viruses is quite limited (refer to Table 3 for detailed information on main entomopathogens and targets)
Source of strains	Only strains approved by MAPA (Ministry of Agriculture, Livestock and Food Supply) are allowed as active ingredients of commercial products. Strains are usually those approved for organic agriculture (but also allowed for conventional agriculture) and adopted by majority of companies due to lower registration costs and speed of registration, or exclusive strains which can be directly licensed by companies from R&D institutions or Brazilian culture collections	Usually, small and medium-sized growers use commercial products as inocula. More sophisticated properties either use strains isolated from commercial products or strains directly licensed from R&D institutions or Brazilian culture collections. An increasing number of growers are acquiring inocula from companies who also sell culture media for *on-farm* production. Non-registered strains isolated from soil samples, infected insects, and other substrates are also adopted by a small proportion of farmers. More rarely, non-registered strains from international culture collections are used, which is prohibited by current legislation
Need for registration of formulated products and/or non-formulated preparations	Due to the broad definition of "pesticides" in the Brazilian legislation, both chemicals and biologicals are regulated under law no. 7.802, from 1989. This law was regulated by Decree 4.074 (2002). Specific points in the law/decree were then modified. Both formulated products and non-formulated preparations are commercialized in the Brazilian market, according to formulation definitions adopted by Faria and Wraight (2007)	*On-farm* production is still unregulated, although there are currently at least two projects being discussed in the National Congress (PL 658/2021 and PL 3668/2021, in the Chamber of Deputies and the Federal Senate, respectively). A few large farmers seek to register their microbial preparations, considering that the current legal framework on *on-farm* production is still fragile. In other cases, these farmers have the intention to sell such products legally in the future
Mass production ("fermentation") processes	Liquid fermentation (Bt) performed in industrial reactors. Fungi are mostly produced by solid fermentation (cereal grains, almost always cooked rice), although a few low-concentration non-formulated preparations, based on mycelia and/or blastospores and produced through liquid fermentation, have been recently launched. Viruses are usually multiplied in reared insects	Only liquid fermentation has been adopted for Bt and other bacteria. For fungi, liquid fermentation is also usual, although some properties adopt solid fermentation. Viruses are usually multiplied in reared insects
Shelf-life	Out of 225 commercial microbial biopesticides for control of insects and mites registered at MAPA, shelf-life information is only available for about 40% of them. Quoted shelf-lives are variable, depending on microbial strain, formulation, and storage temperature. According to labels, bacterial products may be stable from 1.5 to 18 months in the 22–30 °C range. For fungal products, usually 1–3 months in the same range. For viral products, refrigeration is commonly recommended, and shelf-lives vary from 1 to 5 years	For bacteria, stability is claimed to be as high as 3 months in cold rooms if commercial preservatives are added to preparations, but for fungal preparations (for which such additives are not available), it is a matter of days. Most properties keep fungal preparations for up to 3 days in cold rooms after the end of production

Table 1 (continued)

	Commercial	On farm
Quality control	Companies are supposed to follow quality standards directed by MAPA, especially the number of infective propagules per kg or L. There is a lack of harmonization among guidelines adopted by different companies. Currently, MAPA is hearing evidence from different bodies, such as Croplife Brazil (representative of a number of biopesticide companies), in order to establish more comprehensive guidelines	This is likely to be the main hurdle faced by the *on-farm* movement since high contamination levels are very likely to be found in most preparations made in open tanks and non-sterile conditions. Quality control is not performed by unsophisticated farmers since it would require considerable investments in facilities, equipment, and trained personnel. Among large farmers, quality control is performed on some properties or by specialized laboratories, but there is still a lack of a harmonized guideline clearly indicating which tests are necessary and stating acceptable contamination levels

conditions and trained staff and, therefore, they produce high-quality preparations. In this sense, the terms “home-made” or “artisanal” used pejoratively by critics of *on-farm* production do not always reflect the reality.

Another concern of *on-farm* preparations is their shelf-life. According to Monnerat et al. (2020), the unformulated Bt broth must be used within 3 days or refrigerated if not used immediately. However, there is no assurance on the quality of the product, especially after long-term storage.

Insect-Pathogenic Fungi

The most widely used infection propagules are aerial conidia produced on cereal grains (e.g., rice) via SSF. The hydrophobic conidia have a longer shelf-life than propagules usually obtained through SLF. Conidia have been applied in the field under tropical conditions for decades with satisfactory results against a number of agricultural pests (Li et al. 2010; Mascarin et al. 2019). Most of the fungus-based products currently registered with MAPA (Ministry of Agriculture, Livestock and Food Supply) use aerial conidia as active ingredients. In contrast, the SLF allows for the production of a more diverse set of propagules, including blastospores, submerged conidia, mycelium, and in some cases, overwintering structures such as chlamydospores and microsclerotia (McCoy et al. 1975; Jackson et al. 1997; Jaronski and Jackson 2008; Ravensberg 2011). Despite extensive research to optimize infective propagule yields and prolonged shelf-life under non-refrigerated storage conditions (Mascarin et al. 2015a, b; Jaronski and Mascarin 2016), most Brazilian growers who practice SLF for fungal species do not adopt this knowledge. In this ready-to-use strategy, downstream processes such as harvesting and drying the fungal propagules are not performed, which reduces costs significantly compared to the traditional SSF approach usually employed by the biopesticide industry. Under certain conditions, mycelium might be the main fungal biomass produced during *on-farm* liquid cultivation, but, although its bioactivity has been shown against soil pests (Krueger et al. 1992), its field efficacy against aerial pests remains uncertain. Therefore, liquid fermentation of commonly used EPF, especially when it aims to control aboveground pests, should seek the production of single-celled infective propagules (submerged conidia or mainly blastospores), which is strongly influenced by the species/strain, medium composition, and cultivation conditions.

The need for immediate use of the fermented broth after the end of production, sometimes when the conditions for field application are not the most appropriate (absence or very low densities of the target pest, unfavorable weather conditions, etc.), often creates difficulties for rural producers. In cases where the wait is long, we have received reports on the formation of a mycelial mat on the surface

Table 2 Examples of *on-farm* production of microbial entomopathogens in Latin American countries reported in the literature

Pathogen	Target	Crop	Country	Reference
Metarhizium anisopliae	Spittlebugs (Hemiptera: Cercopidae)	Sugarcane, pastures	Brazil, Guatemala, Mexico, Nicaragua	Alves and Lopes 2008, Li et al. (2010)
Beauveria bassiana	*Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae)	Coffee	Colombia	Espinel et al. (2018)
Simplicillium lanosoniveum	*Leptopharsa heveae* Drake & Poor (Hemiptera: Tingidae)	Rubber tree	Brazil	Faria et al. (2020)
AgMNPV	*Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae)	Soybean	Brazil, Argentina, Paraguay, Mexico	Moscardi (1999), Williams et al. (2013)
PeluSNPV	*Perigonia lusca* (Fabricius) (Lepidoptera: Sphingidae)	Yerba mate	Argentina, Paraguay	Haase et al. (2015)
ErelGV	*Erinnyis ello* (Linnaeus) (Lepidoptera: Sphingidae)	Cassava	Brazil	Fazolin et al. (2020)
CoveNPV	*Condylorrhiza vestigialis* (Guenée) (Lepidoptera: Crambidae)	Poplar	Brazil	Sosa-Gómez et al. (2020)

Table 3 Entomopathogenic microbes most commonly reported by farmers as being currently produced in Brazil, and their main targets

Microorganism	Main targets (Order)[a]	Main crops	Production process
Bacteria			
Bacillus thuringiensis	*Chrysodeixis includens* (Walker) (Lep.)	Soybean, cotton	Liquid fermentation
	Spodoptera frugiperda (J.E. Smith) (Lep.)	Corn	
Saccharopolyspora spinosa	*Liriomyza trifolii* (Burgees) (Dip.)	Melon	
Chromobacterium subtsugae	*Euschistus heros* (Fabricius) (Hem.)	Soybean	
Fungi			
Beauveria bassiana	*Bemisia tabaci* (Gennadius) (Hem.)	Soybean, cotton	Liquid and/or solid fermentation
	Dalbulus maidis (DeLong & Wolcott) (Hem.)	Corn	
	Scaptocoris castanea (Perty) (Hem.)	Soybean, cotton	
	Hypothenemus hampei (Ferrari) (Col.)	Coffee	
	Sphenophorus levis Vaurie (Col.)	Sugarcane	
	Cosmopolites sordidus (Germar) (Col.)	Banana	
Cordyceps spp.	*Bemisia tabaci* (Hem.)	Soybean, cotton	
	Dalbulus maidis (Hem.)	Corn	
Metarhizium anisopliae	*Mahanarva* spp. (Hem.)	Sugarcane, pastures	
	Scaptocoris castanea (Hem.)	Soybean, cotton	
	Euschistus heros (Hem.)	Soybean	

[a]This is not an exhaustive list. Although the microorganisms and main targets have been individualized, a common approach is the concomitant application of several microorganisms (tank mix). Mention of targets does not mean that the control results obtained under field conditions are necessarily satisfactory

layer of the fermented broth, which can cause nozzle clogging and other inconveniences. In some cases, additional investments in cold chambers, necessary for a slight increase in shelf-life, ends up making liquid fermentation of fungi economically challenging.

Another topic that deserves attention is the dose of infective propagules used for inundative biological control strategies. The use of significant doses of virulent, aerial conidia usually results in control levels of target pests in the range of 40–60% (Alves et al. 2010), although levels above

Fig. 1 On-*farm* liquid fermentation performed with limited (**A**) or extensive process control using a hermetic bioreactor (**B**) for fungal and bacterial microbes. *On-farm* production of fungi—solid-state fermentation in plastic bags filled with cooked rice grains (**C**). Baculovirus-infected caterpillars collected in a soybean field (**D**)

80% have been reported in some cases (Magalhães et al. 2000; Alves et al. 2003; Ausique et al. 2017). According to some researchers, commercial EPF-based products should be applied at the minimal dose of 1×10^{12} infective propagules per hectare and contain no more than 5×10^6 CFU (colony-forming units) contaminants per gram or mL (Alves et al. 2010; Faria et al. 2022a). We believe that the doses to be applied by *on-farm* producers should follow the same order of magnitude of most commercial products ($\geq 10^{12}$), although lower doses would be acceptable in cases in which microbial metabolites present in fermented broths would prove useful. Minimum concentrations of infective propagules and maximum of contaminants in liquid *on-farm* preparations for some entomopathogenic microorganisms are suggested in Table 4.

Many rural producers still use *on-farm* fungus-based preparations without observing other crucial conditions for field effectiveness, including lack of evaluation of the quality of the infective propagules at the time of application, tank mixing of microorganisms with undesirable products

Table 4 Desirable characteristics of *on-farm* liquid preparations for some entomopathogenic microorganisms

Microorganism	Minimum concentration[a]	Maximum contamination[b]	Reference(s)
Bacteria			
Bacillus thuringiensis	2.5×10^9 viable spores/mL	5×10^2 CFU/mL	Monnerat et al. (2020)
Fungi			
Metarhizium anisopliae	1×10^8 blastospores/mL	5×10^2 CFU/mL	Iwanicki et al. (2020)
Beauveria bassiana	1×10^9 blastospores/mL	5×10^2 CFU/mL	Mascarin et al. (2015a,b)
Cordyceps spp.	1×10^9 blastospores/mL	5×10^2 CFU/mL	Mascarin et al. (2015a)

[a]The minimum concentration for Bt refers to the lower limit established for the "reference specification" # 28 (SDA/SMC Joint Normative Ruling n. 01, November 28, 2017), whereas for fungi these concentrations refer to the lower limits experimentally reached in studies by Iwanicki et al. (2020) or Mascarin et al. (2015a,b), who reached concentrations in the range of $1–5 \times 10^8$ blastospores/mL, $1–3 \times 10^9$ blastospores/mL, and $1–3 \times 10^9$ blastospores/mL for *Metarhizium anisopliae*, *Beauveria bassiana*, and *Cordyceps* sp., respectively

[b]For specific contaminants, the number of maximum colony-forming units (CFU) per mL of fermented broth have been proposed by Monnerat et al. (2020), such as for *Escherichia coli* ($\leq$400 CFU/mL), *Enterococcus* spp. ($\leq$50 CFU/mL), *Streptococcus* spp. (none), *Salmonella* spp. (none), and fungi (none). Faria et al. (2022a) suggested higher contamination limits for fungus-based commercial products due to post-production steps that may require additional handling (drying, harvesting, formulation, packaging, and so on), but the limits proposed by Monnerat et al. (2020) seem more applicable to *on-farm* operations, which is why it is also being suggested for liquid preparations with entomopathogenic fungi

(incompatible chemical pesticides, for example), and application at times with low relative humidity and/or intense solar radiation.

The Effect of Microbial Metabolites

As paradoxical as it may sound, there are reports of some microbial biopesticides being efficacious even though they were produced under non-sterile conditions. Presumably, the improved performance can be attributed to products secreted in the culture broth or on the crop, including plant growth hormones (e.g., indoleacetic acid), siderophores, antibiotics, enzymes, and other molecules that may boost plant growth and prime or elicit defense responses in plants against biotic and abiotic stresses (Parnell et al. 2016; Trivedi et al. 2017; Koskey et al. 2021; Akhtar et al. 2022). The mechanisms of action of metabolites on plants, insects, and other living communities are still poorly understood and needs further investigation. *On-farm* products may be dependent on both the microorganism itself but also its secondary metabolites. Progress is slowly being made in unravelling the role of secondary metabolites of microbial biopesticides and biofertilizers and the dual function of these agents i.e. organisms used for control also exhibit plant growth promoting properties and vice versa. Intriguingly, *B. subtilis* and *B. amyloliquefaciens* have recently been shown to be virulent against insects (Torres et al. 2022). These bacteria are produced *on-farm* as inoculants and/or biocontrol agents for the management of plant pathogens, and may also eventually occur in SLF as contaminants (Valicente et al. 2018; Bocatti et al. 2022). In both situations, it is likely that metabolites produced by these bacteria (and probably others not yet reported) could also be contributing to the insecticidal properties of *on-farm* preparations.

Potential Impact of *On-farm* Biopesticides on Human Health

Most strains of entomopathogenic microorganisms commonly used as active ingredients in commercial biopesticides are considered safe for humans and other vertebrates (Steinhaus 1949; Ignoffo 1973; Glare and O'Callaghan 2000). In Brazil, commercial strains and/or those with "reference specifications" (approved by MAPA for use in organic agriculture) have been evaluated for safety to vertebrates and non-target invertebrates, which reinforces the importance of avoiding the adoption of other entomopathogenic strains by *on-farm* producers. Although quite rare, some fungi, such as species within the genus *Metarhizium*, can cause infections in immunocompromised patients (Nourisson et al. 2017). Short-term allergies have been reported in biofactory workers exposed to "clouds" of aerial conidia during the handling of solid substrate colonized by EPFs (Roberts and St. Leger 2004), which could be avoided with some care, especially with the use of personal protective equipment.

The most common contaminants in solid-state fermentation are saprophytic fungi, which can be easily recognized by trained people, but which can go unnoticed by inexperienced eyes. If *on-farm* production is practiced without adequate technical supervision, it can result, for example, in the mistaken production of *Penicillium* spp. (Roberts and St. Leger 2004) or toxicogenic fungi such as *Aspergillus* and *Fusarium*, which would pose a risk to human health.

There have not been reports of negative effects of entomopathogenic fungal metabolites present in commercial products on humans and other non-target organisms (Hu et al. 2016). This may be because they are absent or present in extremely low concentrations when produced on solid substrates. The levels may be higher in liquid media. According to Strasser et al. (2000), the secretion of oosporein by the fungus *B. brongniartii* in liquid medium can reach 300 mg per liter, while in solid substrate it is about one hundred times lower. Metabolites of certain fungal species that are not routinely determined or legislatively regulated, such as beauvericin, are being called "emerging mycotoxins". This particular metabolite is more associated with *Fusarium*-contaminated food and feed (Gruber-Dorninger et al. 2017; Dreassi et al. 2019). In contrast, EPF strains secreting beauvericin produce very little of this compound (Scheepmaker et al., 2019). If these metabolites prove to be a risk to human health in the context of the *on-farm* production, the screening of safe strains and the establishment of cultivation conditions that minimize their secretion in SLF would be important points to be addressed in the near future.

Brazilian legislation requires proof of the absence of β-exotoxin in commercial products based on Bt, which can be secreted into the culture medium and has a broad spectrum of action, including on mammals (Palma et al. 2014). Bonis et al. (2021) also demonstrated the possibility that strains of Bt, especially the *aizawai* and *kurstaki* subspecies, could be related to foodborne infections in humans. De Bock et al. (2021), in turn, stated that there is a low possibility of finding high levels of Bt in fresh foods above the established limit of 1×10^5 CFU per gram, adopted for the pathogen *B. cereus* and, consequently, biopesticides based on Bt present low risk to human health.

The main concern in the multiplication of beneficial microorganisms in liquid media has been the high potential for contamination with bacteria pathogenic to humans and/or their toxic metabolites. As already reported, studies have shown that, at the end of the *on-farm* multiplication process in open containers, the concentration of biocontrol microorganisms is usually extremely low or even null, although there is a pool of contaminants, mostly bacteria, in the fermented broth (Valicente et al. 2018; Santos et al. 2020; Bocatti et al. 2022). Problems with contaminated food could more likely occur in specific situations, such as after application of preparations contaminated by human pathogens in plant structures intended for *in natura* consumption. Some microorganisms potentially pathogenic to humans have already been detected in *on-farm* productions, raising concerns for handlers (personnel involved in production and application of fermented broths, harvest, and distribution of foodstuff), and even consumers of fresh plant parts. In a study conducted by Valicente et al. (2018), Bt samples from three rural properties were poor in spores or crystals typical of this species, but several potentially pathogenic bacteria were found, including *B. cereus*, *Microbacterium* sp., *Enterococcus casseliflavus*, and *E. gallinarum*. Similarly, Santos et al. (2020) showed contamination of all 12 samples of entomopathogenic or nitrogen-fixing bacteria from five rural establishments. These authors not only reported a low yield of *B. thuringiensis* var. *kurstaki* but contamination with high counts of fecal and total coliforms, including *Salmonella*, suggesting unsatisfactory and precarious hygienic conditions in the production process. When analyzing 18 samples of two nitrogen-fixing bacteria, *Bradyrhizobium* sp. and *Azospirillum brasilense*, from rural establishments in five Brazilian states, Bocatti et al. (2022) found that only one of them contained the target bacterium. Furthermore, almost half of the isolated bacteria belonged to genera pathogenic to humans, such as *Enterococcus* (present in more than 60% of the samples), and others which showed resistance to one or more antibiotics. In the three above-mentioned studies, non-hermetic fermentation tanks were mainly adopted, in addition to inappropriate culture media and growth conditions.

Lastly, entomopathogenic viruses are recognized for their high specificity to target insects and low risk to human health. The *on-farm* production of this group of biological control agents, normally conducted through field harvesting and freezing of infected caterpillars, poses a very low risk to human health. Caution should be taken when diseased caterpillars are blackened and have strong odor characteristic of putrefaction stage, indicating bacterial contamination due to septicemia.

Potential Impact of *On-farm* Biopesticides on the Environment

It is still too early to infer the real effect that *on-farm* liquid preparations, consisting of microbial biomass and numerous metabolites, could bring to non-target organisms after release into agroecosystems. Certainly, much of the potential damage could be mitigated with the use of previously screened microbial strains, appropriate production systems, and adoption of reliable quality control protocols. However, strains not authorized by MAPA are being produced *on-farm*, some of which being obtained from international culture collections (Table 1) and, surprisingly, even from public research institutions. To make matters worse, even species without proof of natural occurrence in the country have been commercialized as inocula for *on-farm* production, such as the bacteria *Chromabacterium subtsugae* and *Saccharopolyspora spinosa* (Santos et al. 2020). The bacterium *C. subtsugae* shows activity against some representatives of the orders Diptera, Coleoptera, Hemiptera, and Lepidoptera (Martin et al. 2007; Sial et al. 2019), while the metabolites of *S. spinosa*, known as spinosyns, act on insects of the orders Lepidoptera, Diptera, and Coleoptera (Toews and Subramanvam 2003; Kirst 2010). Additionally, although spinosyns are believed to pose low risk to natural enemies (Kirst 2010), the legally required studies with *S. spinosa* have not been conducted with representatives of the Brazilian fauna.

According to Scheepmaker et al. (2019), "microbial control agents and consequently their secondary metabolites, are not known to accumulate in the environment because they are degraded within complex microbial communities/ degradative food webs". Therefore, these authors suggest that little emphasis should be placed on data requirements on degradation of secondary metabolites in the environment. Nevertheless, the risk of negative effects on the native microbial community from frequent applications of fermented broths (a common practice adopted by *on-farm* practitioners), both in the soil and on the phyllosphere, needs to be better evaluated.

Finally, Santos et al. (2020) also warned of the potential risk of cultivating plant pathogenic microorganisms in *on-farm* production, especially when carried out in non-hermetic systems, which could result in significant economic damage if released into agroecosystems.

Legislation on the *On-farm* Production of Microbial Biological Control Agents

Until 2012/2013, the legality of *on-farm* production of microbial biological control agents in Brazil was not questioned, perhaps because of its confinement to a few crops/ pests, and because liquid fermentation of entomopathogenic bacteria in rural properties was, to our knowledge, nonexistent at the time. As already discussed, production of fungi was performed only on solid substrates (typically with low contamination levels and low production of metabolites) and entomopathogenic viruses were obtained from field-collected diseased caterpillars. However, over the last few years, we have seen a heated debate about the legality of this practice. Critics of the *on-farm* practice referred to

Law No. 7,802 of July 11, 1989, known as the Pesticides Law, which in Art. 3 establishes that pesticides (including products of biological origin) can only be produced if previously registered with a federal agency. On the other hand, defenders of *on-farm* production referred to Decree No. 6,913/2009, which amended Decree 4,074 of January 4, 2002, which in turn regulates the Pesticides Law. The Art. 10D of the aforementioned Decree, in § 8, established that "Phytosanitary products with approved use for organic agriculture produced exclusively for their own use are exempt from registration". Supporters of this view also argued that, as these preparations were allowed to be used in organic areas, they could also be used in conventional crops. Subsequently, to avoid ambiguity, the wording of § 8 was amended by Decree 10,833 of October 7, 2021, which made it clear that the exemption applies to both organic and conventional production systems.

The Brazilian government's support for *on-farm* production was again confirmed through Decree 10,375, of May 26, 2020, which established the National Bioinputs Program, with the purpose of expanding and strengthening the use of such inputs in the agricultural sector, including *on-farm* microbial preparations. However, some points still lack additional regulations. For example, EMBRAPA (2021) has suggested the use only of strains with reference specifications and/or registered at MAPA, the need to identify the rural establishments that practice *on-farm* production, and finally the need to indicate a duly trained technician responsible for production and quality control.

In this long interpretation path on the legality of the *on-farm* production (Table 1), some producers have sought alternatives to overcome the uncertainty imposed by the lack of a sound legal framework. For example, some large rural producers are registering their *on-farm* preparations, what would make this practice unquestionably legal if the current understanding were to be modified and, moreover, would allow them to transport and use their microbial preparations in other farms they may own in different locations. According to current understanding, *on-farm* preparations can only be used on the sites in which they are produced, making their transport to other properties (even if from the same owner) prohibited. Finally, it is important that the Brazilian regulatory framework under construction be in line with international regulations.

Future Prospects

Important actions for an increasing uptake of *on-farm* biopesticides should be taken into consideration, namely (a) public policies to guarantee access of small- and medium-sized farmers to correct *on-farm* approaches (given the high probability of contamination in open fermenters, *on-farm* production by cooperatives, associations or alike appears as an especially relevant alternative); (b) technological developments aiming greater stability of *on-farm* biopesticides, and sound strategies for their employment in the field; (c) quality control of *on-farm* preparations, to ensure acceptable safety standards (yet to be properly discussed/implemented).

The common understanding that commercial bioinsecticides currently available in the Brazilian market necessarily have a high-quality standard is not always true. Most commercial products based on fungi, for example, have short shelf-lives under non-refrigerated conditions, likely < 1–2 months at 30 °C if the harmonized definition proposed by Faria et al. (2022b) were adopted: "the time for the initial concentration of active ingredients to fall by 20% at a given temperature (and also ambient RH if hermetic packaging is not used)". Additionally, these products are sometimes poorly concentrated or, in extreme cases, almost devoid of infective and viable structures (in some cases, surprisingly as low as 1×10^4 CFU/mL). The recent publication of the document "Quality control of commercial products based on fungi for the management of invertebrates (insects, mites, and nematodes) in agricultural systems" (Faria et al. 2022a, in Portuguese), aimed at commercial biofactories, is an important first step in the attempt to harmonize concepts and protocols to provide commercial products with characteristics that lead to increasingly promising and consistent field results. Similar technical reports presenting robust and accurate protocols directed to the *on-farm* users will be invaluable to ensure minimum quality attributes in microbial preparations.

Regarding blastospores produced via SLF, their field effectiveness under tropical field conditions must be shown. Additionally, all (or at least the majority) of fungal strains approved by MAPA were selected based on studies with aerial conidia, and blastospores and other propagules from a given strain would not have necessarily the same effectiveness under field conditions. In this sense, despite favorable results under greenhouse and other low-UV environments, rigorously performed field trials with blastospores in annual crops and other non-protected crops are lacking.

The diversity and novelty in microbial strains have not been wisely explored in Brazil. A simple illustration of this situation is that the majority (> 80%) of commercial EPF based products are comprised of only two strains, one from *M. anisopliae* and the other from *B. bassiana*, usually alone but also together. Furthermore, a number of EPF are still underexplored as commercial or *on-farm* biopesticides, such as *Cordyceps* spp., *Metarhizium rileyi*, *Akanthomyces* spp. (formerly *Lecanicillium*), *Hirsutella thompsonii*, and *Aschersonia aleyrodis*. Besides, some of the strains currently with reference specification at MAPA do not provide satisfactory control against some of their targets, as we have seen in our laboratories (not published).

The availability of additional strains with reference specification and/or registered, considerably more effective than the current ones, would have a positive impact on the effectiveness of *on-farm* preparations.

High-quality inocula for *on-farm* production is critical to the success of this approach. In our opinion, *on-farm* multiplication from samples of registered commercial products is morally reprehensible, as it can compromise the financial health of companies that have invested in the development of commercial products. Furthermore, these products are not 100% pure and therefore their use as an inoculum tends to result in contamination or poor growth. It would be advisable to use pure inocula acquired from specialized companies that have registered products for this purpose or directly from Research & Development institutions.

Final Remarks

In recent years, few issues in the Brazilian agricultural sector have been as controversial as the *on-farm* production of beneficial microorganisms, due to the conflicting interests between the actors involved, including rural producers and commercial biofactories. However, *on-farm* production of bioinputs has been rapidly consolidating. At the same time, sales of commercial biopesticides in Brazil have been growing at a rate above the world average (BIP Spark 2021), showing that there is enough room for both approaches. In fact, a number of rural producers we interviewed use both commercial biological products and *on-farm* preparations. Only rural producers who understand that the preparations obtained via *on-farm* production are made up of living organisms and that they must be treated as such, including care during and after the fermentation process (growing conditions, storage, compatibility with chemical, and application strategies), are more likely to be successful in biological pest control.

The widespread adoption of microbial preparations by farmers will demand additional attention in the use of chemical inputs. In turn, this mind shift may favor other sustainable approaches, including the conservational biological control approach ("Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests," according to Eilenberg et al. 2001). Entomopathogens are valuable and almost always low-risk resources, and their production performed correctly has the potential to guide the Brazilian agriculture toward a more sustainable direction. Adoption of *on-farm* production has been considered by many as an irreversible practice, but its level of adoption will also depend on the strong performance of research institutions, rural outreach, and regulatory bodies.

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Declarations

Conflict of Interest The authors declare no competing interests.

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