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“ON FARM” PRODUCTION OF MICROORGANISMS IN BRAZIL

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ABSTRACT - The use of microorganisms is an excellent strategy for controlling agricultural pests and diseases. In recent years, rural producers have manufactured microorganisms on their properties for direct application in crops, a practice known as on farm production. In this work of literature review, aspects related to the home production of microorganisms, advantages, disadvantages and future perspectives are addressed. The practice is not illegal. Producers are supported by the Organic Law, which allows the production of agricultural inputs for their own use. One of the main advantages of homemade multiplication is the reduction in the cost of purchasing the products and the inexistence of transport and storage costs. Quality control is essential for using the technique, as the main risk is contamination with human and animal pathogens. The main multiplied microorganisms are: *Trichoderma* sp., *Metarhizium anisopliae*, *Bacillus subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. thuringiensis*, *Bauveria bassiana*, *Nomuraea riley* and *Azospirillum brasilense*. These microorganisms act in the induction of resistance, parasitism, antagonism and the production of enzymes that degrade the cell wall, as well as the production of toxic substances, essential characteristics for the alternative control of pests and diseases. On farm production is driven by the demand for healthier foods, the occurrence of chemical resistance problems and the lack of new products, traditionally used in conventional agriculture.
Keywords: sustainability, economics, *Trichoderma* sp., *Bacillus* sp.

PRODUÇÃO “ON FARM” DE MICRORGANISMOS NO BRASIL

RESUMO - O uso de microrganismos constitui uma excelente estratégia de controle de pragas e doenças agrícolas. Nos últimos anos, produtores rurais têm fabricado em suas propriedades microrganismos para aplicação direta nas lavouras, prática conhecida como produção “*on farm*”. No presente trabalho de revisão de literatura, são abordados aspectos relacionados à produção caseira de microrganismos, vantagens, desvantagens e perspectivas futuras. A prática não é ilegal. Os produtores se amparam na Lei dos Orgânicos, que permite a produção de insumos agrícolas para uso próprio. A multiplicação caseira tem como uma das principais vantagens a redução do custo da aquisição dos produtos e à inexistência dos custos de transporte e armazenagem. O controle de qualidade é essencial para a utilização da técnica, pois o principal risco é a contaminação com patógenos humanos e animais. Os principais microrganismos multiplicados são: *Trichoderma* sp., *Metarhizium anisopliae*, *Bacillus subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. thuringiensis*, *Bauveria bassiana*, *Nomuraea riley* e *Azospirillum brasilense*. Esses microrganismos atuam na indução de resistência, parasitismo, antagonismo e a produção de enzimas que degradam a parede celular, assim como a produção de sustâncias tóxicas, características essenciais para o controle alternativo de pragas e doenças. A produção “*on farm”* é impulsionada pela demanda de alimentos mais saudáveis, pela ocorrência de problemas de resistência a produtos químicos e a falta de lançamento de novos produtos, tradicionalmente utilizados na agricultura convencional.
Palavras-chave: sustentabilidade, economia, *Trichoderma* sp., *Bacillus* sp.

INTRODUCTION

The increase in the world population, climate change, cases of resistance to chemical products and the decrease in the launch of new products used in the conventional control of pests and diseases, represent challenges for global agricultural production (BOON et al., 2014; MURILLO-CUEVAS et al., 2019; GABARDO et al., 2022).

There is a need to intensify agricultural production in a sustainable way. Microorganisms have the potential to promote the biocontrol of diseases and pests, in addition to promoting plant growth, representing a sustainable and promising solution, which can be used in organic and conventional agriculture (GABARDO et al., 2020a; CANIATO et al., 2020).

Other advantages of biological control are the specificity of agents that act only on the target, the sustainability of the method as it has a lower environmental and living impact, in addition to being non-polluting, having a low probability of selection of resistant pests and the demand of the consumer market, concerned about the adverse effects of chemical products and their residues (BETTIOL; MORANDI, 2009; ALEKSEEVA et al., 2019).

The cost of developing a biological product is also much lower, estimated at 2 to 10 thousand dollars, compared to a chemical with an estimated cost of 250 thousand dollars (MONNERAT et al., 2020). Another

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factor that has driven the use of biological products is the drop in the efficiency of chemical products, and the increasing cost, in addition to the world population.

There is a need for more research related to biological inputs. The research of new methodologies and production systems are important to make the microbial control of agricultural diseases and pests economically viable, both in small and large areas (CANIATO et al., 2020). According to De Ávila et al. (2021), new research results are always needed and have helped to improve large-scale production, formulation, storage and field application techniques.

The “on farm” production has been increasing in recent years, having as one of the main advantages the reduction in the cost of pesticide producers, which can reach 90% (SANTOS et al., 2020). The practice is not illegal. Producers are supported by the Organic Law, which allows the production of agricultural inputs for their own use. In July 2009, decree nº 6.913 was sanctioned, where in its articles 1 and §8 it is determined that “phytosanitary products with approved use for organic agriculture produced exclusively for own use are exempt from registration”. As of this decree, there was an exponential growth in the production of microbiological products on the farms themselves, which was called on farm multiplication.

Currently, even allowed, there is no regulation for this practice on rural properties, but the agriculture sector is already moving to encourage the biosafety of this type of manufacturing, present in small, medium and large agricultural establishments (MONNERAT et al., 2020; EMBRAPA, 2021).

The practice is intense in grain-producing properties in the Center-West region and in Minas Gerais. In the South, this practice is more frequent in Paraná, expanding more recently to Santa Catarina and Rio Grande do Sul. Maranhão, Tocantins, Piauí and Bahia (EMBRAPA, 2021).

In this context, the aim of the work was to gather information about the multiplication of microorganisms “on farm”, contributing to the rational and sustainable control of pests and diseases for farmers. In addition to contributing to the dissemination of knowledge about the use of microorganisms, contributing to the sustainability of agricultural systems.

DEVELOPMENT

Benefits and risks of the on farm process

Vital for a more sustainable protection of different crops, bio-inputs have been gaining ground in crop protection in combating agricultural pests and diseases. The premise of using “on farm” multiplication is to enable the producer to produce their own biological input with action to stimulate plant growth and nutrition or those with a biopesticide function, aiming to reduce production costs. Cost reductions can reach 80 to 90% due to in-house manufacturing and the inexistence of transportation and storage costs (SANTOS et al., 2020). With the reduction in cost, there is greater use of microorganisms.

There are differences between commercial biological products and those produced on the farm. In commercial production, there is higher manufacturing cost, lower recommended dose for application, less contamination, higher quality, longer shelf life, latent active ingredient, formulation, greater tolerance to stress (UR, UV, etc.) and greater control of the multiplication process. In-farm production, on the other hand, there is lower cost, higher recommended dose for application, high risk of contamination, lower quality, shorter shelf life (immediate use), active ingredient without formulation, lower stress tolerance and less control of the process. multiplication (BERNARDO; BETTIOL, 2010; EMERSON; MIKUNTHAN, 2015).

The main concern of home production is quality control. Without this control, contaminating microorganisms that are harmful to the environment can multiply. With the release for home production, research institutions like Embrapa (Brazilian Agricultural Research Corporation) began to worry about quality control to assess the concentration, purity and identity of microorganisms.

There are differences between installations. Some properties invest in infrastructure and specialized personnel, with success stories being reported. Multiplying biological products with high quality control, providing greater productivity without causing health risks for the handlers of these products or the environment (OLIVEIRA et al., 2020; EMBRAPA, 2021). Although producers seek to reduce costs by manufacturing their own bio-inputs, they are still having to make high investments in structures, in order to obtain products with greater quality control.

In other properties, water tanks are used. The producer adds the culture medium, water, defoamer, crystal sugar and the microbial inoculum for multiplication, coming, in most cases, from a registered commercial product and in other cases, even more worrying, the use of products that do not they have a record, they do not even show the identification of the likely microbial agent on the label. For multiplication, air is injected into the solution through a piping system to ensure system aeration (SANTOS et al., 2020). Multiplied is ready for use within 24 to 48 h for bacteria and 96 h of system operation for fungi or bacteria with metabolites.

Poor production systems are inefficient and result in poor quality products. Often, target microorganisms for multiplication do not reach adequate concentrations. In addition, there is a proliferation of contaminants, which results in products with low or no efficiency, including a risk of pathogenicity to humans, animals and plants (SANTOS et al., 2020; EMBRAPA, 2021).

Regardless of the conditions of the installations, it is essential to consider that all systems used are susceptible to contamination, such as air, water, the culture medium, defoamers, sugar and the process itself. When considering adequate facilities for microorganism fermentation processes, one of the most important aspects is the reduction of the possibility of contaminants in the final product. What is achieved through the use of sealed reactors, with air, water and sterilized nutrients inlet, being possible to control, in addition to these parameters, pH, temperature, dissolved oxygen rate, among others (MONNERAT, et al., 2020). In this way, ensuring the multiplication of the microorganisms of interest without the presence of unwanted contaminants and/or metabolites.

The presence of a single cell of a contaminating microorganism may be enough to guarantee its multiplication at rates, sometimes higher than that of the target microorganism, causing an entire batch of the product to be lost. The high number of total coliforms is an indication of unsatisfactory hygienic conditions during the multiplication process, or it may even reveal the possibility of poor quality raw material (EMBRAPA, 2021).

When multiplying microorganisms that are not the targets of the process, the producer may be applying microorganisms that have no desired action or multiplying some microorganism that may be phytopathogenic (OLIVEIRA et al., 2019). With that, turning what would be a cost reduction into a problem that can cause serious losses. This fact can lead to questions regarding the quality, already proven, of biological products registered and released for use, negatively impacting the segment and affecting the credibility of the technology.

According to Santos et al. (2020), some results of samples collected on farms point to the absence of microorganisms that should be present, and the presence of contaminating agents, which makes the producer's initial idea of economy and optimization unfeasible. Furthermore, it can increase the risks arising from the lack of quality control and damage to the environment in general.

It is noteworthy that in the industrial process for the production of products based on microorganisms, strict procedures are followed to ensure the final quality of the product. For the registration of biological products, several protocols are carried out at MAPA, IBAMA and ANVISA. Bureaucratic procedures must be followed to guarantee a product with 100% reliability.

As in any initial movement, in most cases the home production of microorganisms is precarious. The producer first makes a test to, if successful, then think about investing in infrastructure and qualification of the workforce. It is essential that careful protocols are applied for the entire multiplication production system, being essential to carry out quality control of the final product, as well as routine analyzes of the plants that receive applications, in addition to product efficiency tests. It is also necessary to carry out microbiological quality analyzes of the water, the culture media used and the source product of the acquired inoculum. The latter to ensure the adequate source of target inoculum (EMBRAPA, 2021).

A homemade product may not have the level of quality and technology that an industrially produced commercial product. It is also necessary to consider whether it is worthwhile for the producer to adequately incorporate the production of microorganisms into the productive activities of the property (MONNERAT et al., 2020). If so, they must have qualified technical assistance for the development of the entire process, such as the appropriate choice of the system, the culture medium, the multiplication protocols, considering the specificities of each target microbial species, in addition to having strict quality control. If good production practices are followed, coupled with good technical monitoring, it is possible to develop production within the farm in a safe and efficient manner.

Main microorganisms produced

Among the main microorganisms used in the biological control of pests and diseases, fungi of the genus Trichoderma, *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi* and *Bacillus* spp. (GABARDO et al., 2020ab; EMBRAPA, 2021). Bacteria and fungi comprise the largest percentage of biological products for agriculture (SANTOS et al., 2020).

Fungi of the Trichoderma genus are recognized as the most studied and the ones that have been most used as active principle of biofungicides (BETTIOL; MORANDI, 2009; ALEKSEEVA et al., 2019). They are widely distributed due to their cosmopolitan habit, and have been used as excellent biopesticides for phytopathogens in major commercial crops.

These fungi are relevant due to the characteristics that favor them in terms of survival in the environment, such as: being saprophytic and rapidly colonizing substrates, with minimal nutritional requirements; besides producing resistance structures to survive the adversities of the environment. In general, the use of Trichoderma favors the control of soil pathogens, such as: *Rhizoctonia* sp., *Pythium* sp., *Sclerotinia* sp. and nematodes (LOUZADA et al., 2009).

They are used as biopesticides, biofertilizers, growth promoters and natural resistance stimulants. Trichoderma spp. they can be applied separately or in combination with other antagonists or even with fungicides commonly used to control soil phytopathogens (AVILA et al., 2021).

Another example of fungi that have great importance in the natural regulation of insect and mite pest populations are the entomopathogenic fungi of the species *Beauveria bassiana* and *Metarhizium anisopliae,* acting as causais agents of diseases of numerous arthropods (MASCARIN; PAULI, 2010).

These fungi act in contact through the surface of their hosts, requiring direct application to the target and/or the host coming into contact with the inoculum, in order to start the infection process. They are known worldwide and used as biocontrol agents for agricultural pests of various species in different orders, including Hemiptera, such as aphids (LORENCTI et al., 2018). Furthermore, they are of great agronomic interest in almost every country in the world, especially in tropical ones (CANIATO et al., 2020).

B. bassiana, is pathogenic to several insect species, is easily produced *in vitro*. It has a biological cycle that allows its characterization as a facultative parasite. Its conidia can penetrate any part of the insect's cuticle, and this microorganism is used on a commercial scale in several countries, including Brazil, the United States and Mexico (LORENCTI et al., 2018).

In Brazil, the most studied and produced fungus for pest control on a commercial scale is *M. anisopliae* sensu lato, whose use has already reached more than one million hectares in applied and treated area in the field (LI et al., 2010). In view of its importance in the integrated pest management program, this beneficial entomopathogenic fungus is believed to occur naturally in more than 300 species of insects of different orders, including important pests, being successfully used to control leafhopper in sugarcane, *Mahanarva posticata*, in the Northeast and in the control of leafhoppers in the Southeast and Midwest of Brazil (ALVES; CARVALHO, 2014).

The most produced and commercialized structures of *M. anisopliae* are the conidia, produced on the surface of solid culture medium, inside different containers according to the objective and production scale, with rice being the most used substrate for conidial production. In addition, conidia and spores have a high capacity for horizontal dispersion, being transported by various agents over great distances, composing dissemination foci (CASTRILLO et al., 2005). Attacked insects become mummified and covered with a green powdery layer, formed by agglomeration of conidia.

The fungus *Nomuraea rileyi* is also one of the main biological control agents and occurs naturally infecting pest insects of the Coleoptera, Lepidoptera and Orthoptera orders of several cultures. Despite being isolated from several hosts, a preference for hosts of the order Lepidoptera is described (SUWANNAKUT et al., 2005).

The fungus produces epizootics in populations of the soybean caterpillar, *Anticarsia gemmatalis* (Huebner), naturally controlling the pest under certain conditions, and its presence prevents the population of *A. gemmatalis* from reaching the level of economic damage, thus avoiding the application of insecticides, acting as a natural control agent for insect populations in different soy-producing regions in the country (SUJII et al., 2002; ANDRADE et al., 2021).

There are different methodologies for multiplication of fungi using different substrates. Generally cereal grains such as rice, sorghum, millet, wheat straw and rice husk are the substrates most used in production for multiplication, due to their practicality, availability, cost and yield (BERNARDO; BETTIOL, 2010; EMERSON; MIKUNTHAN, 2015). Furthermore, as they are readily biodegradable, they facilitate field application (CARVALHO FILHO et al., 2008).

In the production of fungi, some parameters must be carefully monitored. As the pH (usually requires more acidic pH ≈ 3.5), the culture medium (need for different nutrients according to the microorganism to be multiplied), be aware that the use of antibiotics or contaminant inhibitors can hinder the formation of conidia, as there is longer multiplication time (72 to 96 h for conidia formation), shorter shelf life (immediate use) and lower tolerance to desiccation (due to the use of glycerol in the medium) (MONNERAT et al., 2020).

Several phytosanitary products for the biological control of bacterial-based diseases are also being used. In particular, *Bacillus* spp. stands out for its potential to form endospores resistant to adverse conditions and present a multiplicity of antagonistic mechanisms, thus enabling its long maintenance and survival in specific ecological niches, with great versatility in the mechanisms of action to inhibit the defenses of phytopathogens (GABARDO et al., 2020).

There are products on the national and international market with a record of use in grape, apple, pear, peanut, cucurbit, leafy, cruciferous, pepper, tomato, onion, carrot, herbaceous, ornamental and other crops (COPING, 2004). These products act by inhibiting the germination of spores, the growth of the pathogens' germ tube and mycelium, blocking the pathogen's attack on the leaf surface by forming an inhibition zone and also by inducing resistance in the host.

In Brazil, there is efficiency of *Bacillus subtilis* and *Bacillus pumilus*, due to multiple modes of action against several pathogens, especially fungi. Acting through antagonism, production of various lipopeptides, competition for nutrients and space (GABARDO et al., 2020). Strains of these species are also plant growth promoters. In the control of phytopathogenic bacteria, the main reports are against black rot in crucifers, caused by *Xanthomonas campestris* pv. *campestris* (MONTEIRO et al., 2005). It presents itself as an excellent biocontrol agent and can be found as growth-promoting rhizobacteria in plants, epiphytic and endophytic bacteria (ONGENA et al., 2005).

Coutrim et al. (2018), with bacteria of the genus *Bacillus* spp. it was observed that *B. subtilis* and *Bacillus amyloliquefaciens* inhibit the growth of *Fusarium equiseti* and *F. solani*, showing an inhibition percentage of 38.11% and 40.33%, respectively. Batista et al. (2016), studied the different strains of *Bacillus* spp. and found that they were able to inhibit the mycelial growth in vitro of pathogens: *Fusarium equiseti* and *F. solani*, isolated in *Capsium chinese*.

B. thuringiensis is a gram-positive and entomopathogenic, aerobic or facultatively anaerobic bacterium, naturally found in soil. Like other bacteria, this species can remain dormant as endospores under adverse conditions. During the sporulation phase, bacteria synthesize proteins that accumulate in the periphery of the spores in the form of crystals in one of the cell's poles (GABARDO et al., 2020). These crystals are composed of one or more Cry proteins, also called d-endotoxins or Insecticidal Crystal Proteins (ICPs).

Such proteins are highly toxic and specific, therefore innocuous to most other organisms, including beneficial insects (HERRERO et al., 2001). Protein solubilization depends on the alkaline pH of Lepidoptera and Diptera; a lower effectiveness of these proteins in coleoptera may be due to neutral or low acid pH (LOURENCETTI et al., 2018). Gabardo et al. (2020), also demonstrated that from the bacterium *B. thuringiensis*, great efficacy as an antagonist, significantly decreasing the growth of *Sclerotinia rolfsii* and *S. sclerotiorum* colonies.

Bacteria belonging to the genus Azospirillum have been intensively studied for their possible application in the cultivation of corn, wheat and other crops (EPSTEIN;

BLOOM, 2006). It helps the development of cultures in different ways; among the main benefits generated by bacteria, the production of hormones stands out, which interfere with plant growth, mainly benefiting greater root development, the increase in the process of assimilatory reduction of nitrate available in the soil and biological nitrogen fixation (REIS, 2007; BERTOLDO; MAZARO, 2018).

Bacterial production occurs mainly by agitated liquid cultivation, also known as batch or batch fermentation, or by stationary cultivation or solid fermentation. It is important to monitor the pH, the ideal range being between 6.8 and 7.2 initial (neutral), the concentration of dissolved oxygen (the fermentation of aerobic bacteria (such as Bt) should not be below 20%), the temperature ideal depends on the microorganism to be multiplied, usually between 28 and 32°C, the growth time: varies according to the microorganism (between 24 and 48 h for bacteria and 96 h for fungi or bacteria with metabolites).

Bacterial multiplication usually occurs in a medium containing water, sugarcane molasses, sugar, milk, maize, and monobasic phosphate. The culture medium must contain carbon sources (sucrose, glucose, starch, etc.), organic sources (yeast extract, soy flour, peptones, aa, proteins, etc.) and inorganic sources (salts, minerals such as phosphorus, calcium, potassium, iron, magnesium, etc.), C:N ratio (0.66 to 1) (BERNARDO; BETTIOL, 2010; MONNERAT et al., 2020).

Regarding the sources of inocula: obtained on the farm/area, inocula for multiplication (companies), inocula purchased from competent institutions (EMBRAPA, IB, etc) and commercial products (not indicated). The use of commercial products as inoculum sources presents a high risk of contamination. Contaminants can grow faster than the desired strain, affecting the quality of the final product. In addition, commercial products contain stabilizers and preservatives that can interfere with the fermentation process.

Future

We are in a new era in agriculture, with the use of microbiological agents as growth promoters and agents in the biological control of pests in crops. The use of products of microbial origin, bacteria and fungi, for agriculture has grown exponentially in the world, and in Brazil this growth is significant.

According to ABCBio (Brazilian Association of Biological Control Companies), the biopesticides market in Brazil is estimated at US$ 95.6 million (1% of the pesticide market) and the expected annual growth rate is 20%. In 2021, the market is expected to be US$ 237.8 million. Compared to the conventional pesticide industry, the biocontrol industry is growing 5.3 times faster.

The collection and use of microorganism isolates found naturally in the soil in commercial crops can be an efficient biological control strategy, as these populations are naturally adapted to the local environment and specifically aggressive towards pathogen communities present in that agricultural area.

Problems with lack of quality and contaminants must be solved. One possibility will be the association of farmers with regional research centers. Also allowing the quantification of the level of diversity of these biological agents in the field for the development of specific strategies for managing diseases and pests in different agroecosystems.

As they constitute potential substitutes for chemical products, when exercising actions of biocontrol and/or promotion of plant growth, favoring the preservation of the environment, they have been identified as a viable alternative for ecologically and economically sustainable agricultural production systems, meeting the current demand of the world population.

Embrapa is a protagonist in the development of biological inputs and recognizes that production for its own use has the potential to contribute to the sustainability and competitiveness of Brazilian agriculture. Its researchers have been warning that this activity should be regulated, as there is a risk that poor quality products may harm the good image of bio-inputs, built over the years, with loss of producer confidence, in addition to causing damage to the health and the environment.

CONCLUSIONS

Brazil has a vast potential for the production and use of biopesticides, however, among the aspects that justify the low exploitation of this sector, there is the high cost of commercial products and their limited availability. There is a need to develop and promote social technologies that can expand the access and use of biological agents in Brazilian crops.

Theoretical and practical qualification is required on basic aspects related to the physiology of the target microorganisms of multiplication, as well as good production practices, for the handlers, for the technical responsible for the properties, as well as for the representatives that commercialize some sources of inoculum and the culture media used.

There are conflicts of interest between companies and rural owners. With homemade multiplication, companies can lose their market niche. There are consulting companies that sell kits of fungi and bacterial cultures to farmers, accompanied by an instruction manual.

This work is expected to disseminate information about the production of bioinputs and the potential of biological control to be practiced by farmers and the real possibility of multiplying fungi and bacteria in a simple way to control diseases and pests, contributing to the viability of this system production for organic, conventional and sustainable agriculture.

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