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Original Article

Trichoderma: biological control efficiency and perspectives for the Brazilian Midwest states and Tocantins

Trichoderma: eficiência no controle biológico e perspectivas para os estados do Centro-Oeste brasileiro e Tocantins

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Abstract

Brazil is one of the world leaders in the agribusiness sector tending to directly influence a growing dependence on imported inputs, specifically synthetic agrochemicals. At the state level, in 2013, Tocantins stood out in first place in the ranking of agrochemical consumers, however, these products can cause several problems, such as poisoning to humans, environmental contamination, and increased resistance to phytopathogens. Biological control is an alternative to the use of agrochemicals towards eliminating pests naturally by using living organisms called Biological Control Agents (BCA). Currently, fungi of the *Trichoderma* genus are some of the most used organisms in biological pest control for their relevant characteristics that favor them in terms of survival in the environment, such as high capacity to adapt to ecological conditions, potential to colonize the rhizosphere of plants, mycoparasitism, production of volatile and non-volatile metabolites. In addition, it works on plant growth and productivity. In general, the use of *Trichoderma* favors the control of soil pathogens, such as *Rhizoctonia, Pythium, Sclerotinia*, and nematodes. Thus, this review aims to demonstrate the importance of using *Trichoderma* in biological control, as well as to present an overview and perspectives of research developed by respondents in the Brazilian Midwest region and Tocantins state.

Keywords: *Trichoderma*, biocontrol, growth promotion, research overview.

Resumo

O Brasil é um dos líderes mundiais no setor do agronegócio e essa liderança tende a impactar diretamente numa dependência crescente de insumos importados, especificamente, agroquímicos sintéticos. A nível de estado, em 2013, o Tocantins se destacava em primeiro lugar no ranking de consumidores de agroquímicos, contudo, esses produtos podem causar diversos problemas, como intoxicação ao homem, contaminação do ambiente e aumento da resistência de fitopatógenos. Um método alternativo ao uso de agroquímicos é o controle biológico, o qual busca a eliminação de pragas de forma natural, utilizando-se de organismos vivos chamados de Agentes de Controle Biológico (ACB). Atualmente, entre os organismos mais usados no controle biológico de pragas estão os fungos do gênero *Trichoderma*, isto, por possuir algumas características relevantes que os favorecem em termos de sobrevivência no ambiente, tais como: a alta capacidade de adaptação às condições ecológicas, potencial em colonizar a rizosfera das plantas, micoparasitismo, produção de metabólitos voláteis e não voláteis. Além disso, atua no crescimento e produtividade das plantas. Geralmente, o uso de *Trichoderma* favorece o controle de patógenos do solo, como: *Rhizoctonia, Pythium, Sclerotinia* e nematoides. Assim, a presente revisão visa demostrar a importância da utilização do *Trichoderma* no controle biológico, assim como apresentar um panorama e perspectivas das pesquisas desenvolvidas por pesquisados da região Centro-Oeste brasileiro e no estado do Tocantins.

Palavras-chave: *Trichoderma*, biocontrole, promoção de crescimento, panorama das pesquisas.

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1. Introduction

The Brazilian scenario is marked by agribusiness moving the economy through domestic consumption and production export, contributing to generate employment in various stages of the production chain and technology implementations. According to Pignati et al. (2017), to keep this sector booming, the agricultural scenario has been consuming too many different agricultural inputs, including transgenic and non-transgenic seeds, fertilizers, and agrochemicals. Brazil is known as a world leader in the agribusiness sector; however, this leadership can directly influence a growing dependence on imported inputs, with an emphasis on synthetic agrochemicals, consequently making it one of the leading countries in the consumption of these products.

The Brazilian Midwest and the state of Tocantins have been linked to agricultural activities since the 70s due to the agricultural expansion in the Cerrado and the consolidation as regional agricultural hub resulting from creating soy seeds adapted to soils. It represents one of the main crops for supplying both the domestic and international markets in the region. Along with soy, other crops have a great impact on the economy of the Midwest region and Tocantins state, such as the cultivation of corn, sorghum, beans, cotton, among others (Flores et al., 2012; Menke et al., 2009). In 2013, the state of Tocantins occupied the first place in the ranking of agrochemical consumption among the northern states, with the application of these chemicals being associated with monocultures of soybean, corn, cotton, and sugarcane (Brasil, 2018).

According to art. 2 of Brazilian Federal Law 7802, of July 11, 1989, agrochemicals are defined as products or agents of physical, chemical, or biological processes used in the production, storage, and processing of agricultural products, pasture, and protection of forests, native or implanted, and other ecosystems, as well as urban, water and industrial environments. The goal is to change the composition of flora or fauna seeking to preserve them from the harmful action of living beings considered harmful (Brasil, 1989).

Agrochemicals are substances that act against any agent that may compromise either the cultivation or storage of agricultural production. Although target organisms absorb most of these substances, a significant proportion of agrochemicals are transported to the environment through air or water and they are consistently found in soil, surface water, and groundwater (Oliveira et al., 2021). As a result, through the residual effect of these products, non-target organisms are constantly affected. Another aggravating factor is the use of proven toxic pesticides in Brazil that are banned in other parts of the world. Among the 50 most sold commercial products in Brazil, 22 are banned in Europe, such as trichlorophenol, 2,4-D, paraquat and some triazine herbicides (Oliveira et al., 2021).

The large use of agrochemicals is linked to their impact on crop productivity, but their side effects make their advantages unremarkable (Guarda et al., 2016). When applied inappropriately or exaggeratedly, agrochemicals can cause severe damage, such as harming the soil and organisms present in the environment, increasing the resistance of pests in the current crop and others to come, and even of crops in nearby areas, in addition to polluting rivers, interfering with living organisms, whether terrestrial or aquatic, changing their morphology and function within the ecosystem (Lopes and Albuquerque, 2018). Several studies emphasize the inefficiency of fungicides in combating fungal resistance structures (sclerotia) present in the soil of contaminated crops (Brewer and Larkin, 2005; Silveira et al., 2003; Tsror and Peretz-Alon, 2005). In addition, agrochemicals are harmful to human health, both for those who live nearby and those who will consume such products in the future.

Around the 1950s, a new, much less harmful alternative for agricultural pest control, known as biological control, emerged. Foster was the first researcher to publish on biological control based on the use of the *Trichoderma* fungus against the tobacco mosaic virus, successfully controlling a certain pest (Morandi and Bettiol, 2009).

Therefore, biological control seeks to eliminate these pests in a natural way by using living organisms called Biological Control Agents (BCA), which can be fungi, bacteria, insects, or others. These BCAs may have a positive influence on crops, production, and economy. The growth in the biochemicals market follows the global trend of less use of agrochemicals (Rodrigues et al., 1998). Thus, farmers have adopted new mechanisms to control these pests, and biological control has achieved great prominence in plantations. BCAs can be handled directly (lively inserted) or indirectly (introducing only metabolites produced by the BCA) (Di Piero and Garda, 2008; Grigoletti Júnior et al., 2000; Lazzaretti and Bettiol, 1997; Morandi and Bettiol, 2009). Thus, the use of BCAs can lead to the reduction or elimination of some agrochemicals, consequently reducing the exposure of producers and technicians to agrochemicals, in addition to the absence of residues in food and lower risk of environmental pollution (Lucon et al., 2014).

According to the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA) (2020), the production of biological inputs for controlling agricultural pests and diseases grew by more than 70%, resulting in sales of R$ 464,5 million. In Brazil, most biological control products available in the market have one or more biological agents as active. There are at least 194 products registered with MAPA whose formulation has some beneficial organisms.

BCAs are harmless to human health and the environment (they are natural to the soil microbiota), therefore, such characteristics have caused to decrease the use of agrochemicals and provided a balanced crop in ecosystems. Currently, fungi of the *Trichoderma* genus are among the most used organisms in biological pest control.

2. *Trichoderma:* an Effective BCA

Fungi of the genus *Trichoderma* belong to the phylum Ascomycota, class Sordariomycetes and family Hypocreaceae with easily perceived phenotypic characteristics, ranging from macroscopic fungal parasites to the rhizosphere (De Abreu and Pfenning, 2019). Despite being mesophilic, these fungi are distributed throughout the globe, mostly in tropical and temperate climate regions (Machado et al., 2012).

Currently, it is estimated that the diversity of fungi varies from 2.2 to 3.8 million species (Calaça et al., 2022), and just over 375 species of *Trichoderma* are accepted and can be identified through molecular phylogeny (Cai and Druzhinina, 2021). The genus *Trichoderma* has fungi that are present in the soil, acting as macroscopic parasites and rotting organic matter, considered environmental opportunists (Druzhinina et al., 2011). *Trichoderma* fungi are defined by some common phenotypic characteristics (*eg.* green colonies) (Figure 1), such as wide geographic distribution, rapid growth, and highly capable of parasitizing or preying on other fungi and, in the case of some species, they can establish beneficial interactions with plants, resulting in the promotion of growth and induction of resistance to diseases and abiotic stresses (Harman et al., 2004).

As aforementioned, the *Trichoderma* genus is effective against different phytopathogens for its high capacity to be found in different soils and substrates. In addition, *Trichoderma* shows an accelerated growth that favors habitat colonization. These fungi can grow in soils without agricultural crops and inactivate structures of infection and resistance to existing pathogens (Lucon et al., 2014). Thus, *Trichoderma* isolates can act preventively and not only curatively and can be applied before diseases emerge.

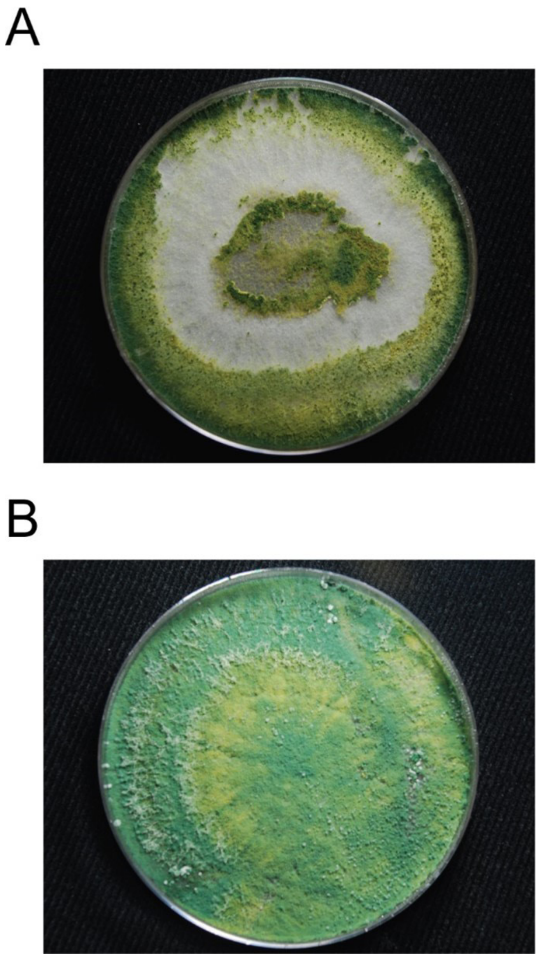

Figure 1. *Trichoderma* isolates collected in Brazil. (A) Isolate *T. harzianum* ALL-42 grown on PDA (Potato Dextrose Agar) medium; (B) Isolate *T. asperellum* T-00 grown on PDA (Potato Dextrose Agar) medium.

Several studies have proved the effectiveness of *Trichoderma* isolates against phytopathogens found in the soil, responsible for root infection and incidence of rot in plants, such as fungi of the genera *Fusarium*, *Rhizoctonia*, and *Sclerotinia* (Lucon et al., 2014). Such an effectiveness of *Trichoderma* in controlling these phytopathogens occurs due to its antagonist actions, which can be classified as i) mycoparasitism – secretion of hydrolytic enzymes acting on the host's cell wall, ii) competition for space or nutrients, and iii) production of metabolites interfering with pest development (Bettiol, 2001) (Figure 2).

Mycoparasitism allows the organism of the genus *Trichoderma* to have a biotrophic and saprophytic nutrition, through which the enzymes secreted by these fungi prevent the germination of spores, growth of hyphae, and the development of sclerotia and chlamydospores (resistance structures) of other species, including pathogens (Druzhinina et al., 2018; Monte et al., 2019). Expanding its antagonistic role, *Trichoderma* species rely on the production of a vast diversity of secondary metabolites that present antibiotic properties to many species, enhancing the effectiveness of population control of other species, as target pests (Hermosa et al., 2014; Monte et al., 2019).

Fungi of the *Trichoderma* genus feed through their hyphae, which secrete hydrolytic enzymes, thus allowing to break the host's cell wall (Monte et al., 2019). A large number of genes can encode enzymes, such as β-1-3-glycanases, N-acetyl-glucosaminidase (NAGase), chitanase, and proteases; these enzymes identify mycoparasitism as an ancestral character of genus (Kubicek et al., 2011; Lopes et al., 2012). Thus, the enzyme arsenal of the genus *Trichoderma* can degrade several compounds in superficial cell from different hosts, allowing these fungi to parasitize other fungi.

Another mechanism used by fungi of the *Trichoderma* is competition for space and nutrients. Competition is a process related to the interaction between two or more organisms engaged in the same resource, such as nutrients, water, light, space, growth factors, oxygen, among others (Machado et al., 2012). Thus, fungi of the *Trichoderma* genus are known to be excellent competitors, preventing access to space and nutrients, resulting in inhibited growth of phytopathogenic populations; in addition to influencing plant growth and productivity.

3. Phytopathogens Fought by the BCA *Trichoderma*

Using BCAs like fungi of the *Trichoderma* genus against phytopathogens allows to control pest population throughout the plant life cycle (Moraes, 1992). *Trichoderma* isolates have been used to control phytopathogens such as *Rhizoctonia solani*, *Fusarium* spp., *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, among others (Figure 3), influencing the productivity of different agricultural crops.

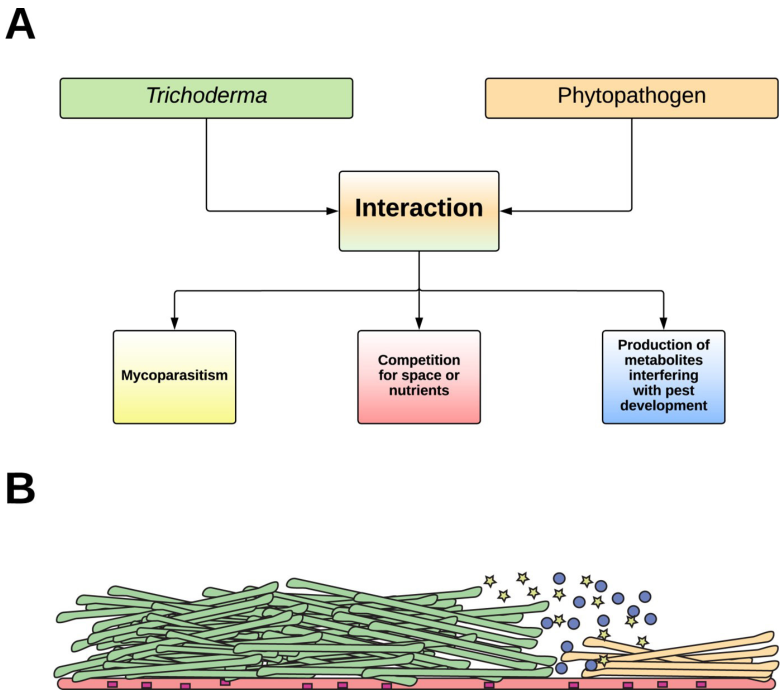

Figure 2. Interaction mechanisms of *Trichoderma* spp. and phytopathogens. (A) Yellow, red, and blue correspond to the antagonistic actions of *Trichoderma* from contact with phytopathogens; (B) Contact of fungal hyphae (*Trichoderma* spp. in green and phytopathogens in orange). Elements in pink correspond to the process of competition for space (long base) and nutrients (rectangles). Blue circles correspond to the metabolites produced by *Trichoderma*. Yellow stars represent the enzymes produced by *Trichoderma* in the mycoparasitic process.

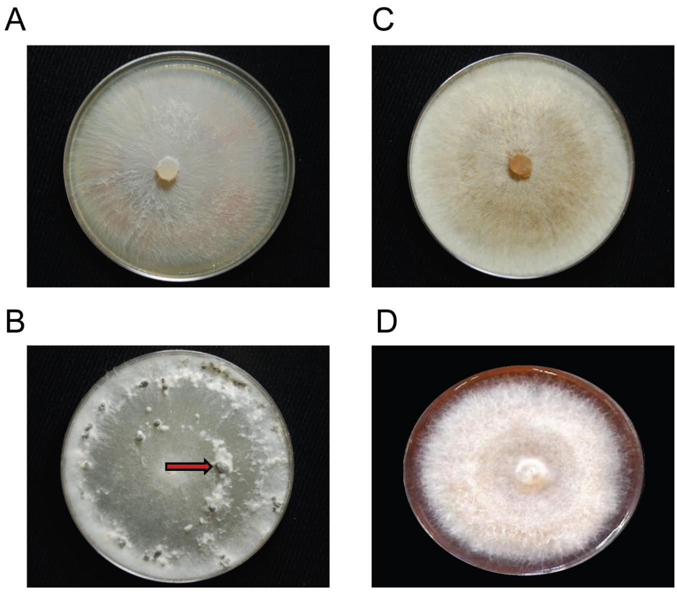

Figure 3. Prominent phytopathogens in the agricultural scenario of the Brazilian Midwest and Tocantins state. (A) *Fusarium oxysporum*; (B) *Sclerotinia sclerotiorum*; (C) *Rhizoctonia solani*; (D) *Colletotrichum gloeosporioides*. The red arrow indicates the sclerodium resistance structure produced by *S. sclerotiorum*.

In 1815, De Candolle described the genus *Rhizoctonia*, and in 1858, Kühn reported the *R. solani* as its prominent species (Ogoshi, 1996). *R. solani* occurs worldwide in several economically important crops such as potatoes, beans, tobacco, corn, and soybeans. Attack by this phytopathogen cause delay in plant development, generating deformation and discoloration and producing toxins that inhibit growth. Each new plant grown implies fruther resistance structures and greater propagation of the fungus in the soil (Dias et al., 2013). Isolates of *R. solani* can perform hyphal fusion, plasmogamy and have been used as a criterion for morphological identification (David et al., 2018).

Fusarium is a group of filamentous fungi widely distributed in soil and plants. Some of its species produce toxins that affect both humans and animals. Among over 100 *Fusarium* reported species, only 12 can be considered pathogenic for humans, including *F. solani*, *F. oxysporum*, and *F. verticilloides*, in decreasing order of frequency (Tapia and Amaro, 2014).

The *Fusarium solani* species is capable of infecting several crops (*eg.* soy, corn, beans, and wheat) at different developmental stages. The soil complexity and the *F. solani* genetic variability make its resulting disease difficult to control, thus continuing on the ground for several seasons (Milanesi et al., 2013). The species cause plant to display yellowish leaves, vascular system discoloration, and plant wilt (Rocha et al., 2016). According to Desjardins (2006), one of its strategies is producing a wide variety of toxic and bioactive secondary metabolites for host colonization.

The *Fusarium oxysporum* species is well represented in the rhizosphere microflora. Although all strains exist saprophytically, some are well known to induce wilt or root rot in plants, while others are considered non-pathogenic. This species is able to compete for nutrients in the soil, affecting the pathogen's germination rate by chlamydospores, in addition to being able to compete for infection sites in the root, thus triggering plant defense reactions and inducing systemic plant resistance (Fravel et al., 2003).

The fungus *Colletotrichum gloeosporioides* causes the disease known as anthracnose, which occurs in tropical and subtropical regions of the world. This fungus affects economically important crops such as cereals, legumes, vegetables, fruits, and ornamental species (Carneiro et al., 2012). *C. gloeosporioides* attack plants by generating lesions and dark, rounded spots, in addition to large and deep necrosis. The pathogen has been reported as increasingly resistant to control by agrochemicalsand lacking any fully effective control method, thus hindering the development of soybean pods and causing impact on crop production (Ribeiro et al., 2016).

The species *Sclerotinia sclerotiorum* causes the disease popularly known as white mold, affecting several cultures all over the world, especially soybean. Its pathological behavior can cause soybean crop yield to reduce by 70% (Meyer et al., 2016, 2017, 2018). The high pathogenicity degree of the *S. sclerotiorum* species may be associated with its ability to form sclerotia that act as a mechanism for resistance and survival and may remain viable for up to 10 years, and when the environment becomes favorable, the sclerotia germinate giving rise to new colonies of the species and contaminating the plants around them (Henson et al., 1999; Leite, 2005; Bolton et al., 2006). In the state of Goiás, white mold, a disease caused by *S. sclerotiorum*, was responsible for a loss of up to 20% in the yield of soybeans in some crops (Pomella and Ribeiro, 2009).

4. Use of *Trichoderma* in Agriculture

The potential of *Trichoderma* species as biological control agents for plant diseases was discovered in the 1930s (Weindling, 1932), followed by the control of many diseases in the subsequent years. These discoveries have led to the emergence of several commercial products made from different species on almost every continent (Howell, 2003).

In Brazil, *Trichoderma*-based products are registered for the control of plant diseases (Bettiol et al., 2019b), including a large portion with conidia of the species *T. harzianum* and *T. asperelum* as active ingredients. Most of these products are recommended for most crops affected by phytopathogens, such as root pathogens like root nematode *Meloidogine javanica* (Sharon et al., 2001), root fungi as *Pythium* spp. (Naseby et al., 2000; Thrane et al., 2000), *Rhizoctonia* spp. (Cúndom et al., 2003), *Phytophthora* spp. (Etebarian et al., 2000; Ezziyyani et al., 2007), and shoot pathogens such as *Venturia* spp., *Botrytis* spp. (Hjeljord et al., 2001; Lisboa et al., 2007), *Crinipellis perniciosa*, causal agent of the cocoa witch's broom (Sanogo et al., 2002). Thus, species of the genus *Trichoderma* are relevant for their favorable characteristics to survival in the environment, such as saprophytic nature and rapid colonization of substrates with minimal nutritional requirements, and resistance structures to survive environmental adversities.

In addition to this antagonistic capacity, these BCAs promote resistance induction and plant growth (Machado et al., 2012), the latter through the production of auxin and auxin-related substances, which lead to the development of roots and shoots (Abdelmoaty et al., 2022). A large part of *Trichoderma* species can form mutualistic interactions with plants, helping them to develop (Abreu and Pfenning, 2019), by stimulating the production of various hormones for different purposes, promoting plant growth, increasing the availability and absorption of nutrients, and inducing increase in root surface and resistance to abiotic stresses (Lucon, 2009). Thus, after the stimulus caused by colonization by fungi of the genus *Trichoderma*, plants will fight against other fungal invasion through the synthesis and accumulation of phytoantitoxins, flavonoids and terpenoids, phenolic derivatives, aglycones, among other antimicrobial compounds (Franken et al., 2002).

Trichoderma can act on plant growth and increase productivity (Harman et al., 2004). The process of adhesion to the root surface can be regulated by small hydrophobic proteins on the outer surface of the cell wall (hydrophobins) and by expansin-like proteins capable of recognizing cellulose and altering the structure of plant roots, making them deeper and more vigorous, providing shoot growth and resistance to biotic and abiotic factors and it can increase plant growth by up to 300% (Brotman et al., 2010). Due to its ability to colonize roots, *Trichoderma* is a biostimulator of root growth and mass, improving a better nutrients and water absorbing (Harman, 2000; Harman et al., 2004).

Therefore, *Trichoderma* has the potential to promote plant growth and tolerance of the plant organism to the parasitism of phytopathogens and may represent a promising sustainable solution to improve agricultural production.

5. *Trichoderma* Research in Brazilian Midwest and Tocantins

In the last five years, research lines aimed at the use of *Trichoderma* as BCA have been widely studied in different research institutions, such as Universidade Federal do Tocantins (UFT), Universidade Federal de Goiás (UFG), Empresa Brasileira de Pesquisa e Agropecuária (EMBRAPA), among others. In the Goiás state, Dr. Cirano José Ulhoa leads a research line consisting of the analysis of hydrolytic enzymes (molecular and biochemical observation) produced by *Trichoderma* spp. by means of biological control and mycoparasitism actions. In the Federal District, Dr. Sueli Corrêa Marques de Mello seeks to demonstrate the benefits of using *Trichoderma* as BCA in different types of plantations in the Federal District by investigating its effectiveness as antagonist to several phytopathogens, reconciling the characterization and identification of actions.

In Tocantins state, Dr. Aloísio Freitas Chagas Júnior and Dr. Gil Rodrigues dos Santos seek to deepen their knowledge on the effects of inoculating *Trichoderma* isolates that promote plant growth, collected in Tocantins soils in different cultures of leguminous plants or non-legume. Their goal is to verify the effectiveness of these isolates as agents for biocontrol of phytopathogens. In line with these studies, in the Federal District, Dr. Eliane Ferreira Noronha has publications demonstrating the advantages of using *Trichoderma* as plant growth inducers.

Despite the efforts of the abovementioned researchers, there are still gaps related to the genetic and physiological understanding of the *Trichoderma* genus. Thus, further studies may elucidate certain functional responses not yet understood, enabling a more comprehensive and effective use of this BCA. Still in the scope of these researchers' studies, another major deficiency is the small number of *Trichoderma* isolates for commercial use. According to Bettiol et al. (2019a), there are approximately 246 *Trichoderma*-based products in Brazil used as BCAs and growth promoters as only 21 of these products are produced in Brazil. Thus, there is still a large field to be explored since organisms isolated in our habitat tend to have a more successful field application.

6. Perspectives

The emergency to isolate native strains of *Trichoderma* from the Midwest region and Tocantins is closely linked to the success of adaptation to this specific environment. Research on the antagonistic potential of the genus *Trichoderma,* in addition to the search for new organisms of this genus, can contribute to the socioeconomic scenario of the Brazilian Midwest and Tocantins, since agriculture is one of the main economic sources in several states. For example, farmers, in general, took two measures in order to increase the amount of soy produced, eliminating the factors that limit greater production (*eg*. soil nutrition, water and, mainly, pests), or upon not achieving the expected outcome, increasing the planting area (expanding, and consequently, deforesting native Cerrado areas). In this new scenario, the use of the BCA *Trichoderma* together with numerous studies, allows greater productivity of different cultures, softening the impact caused by agricultural activity.

It is fundamental to develop better-quality crops and less harmfulness, both to human and the environment. Therefore, the application of BCAs, specifically fungi of the *Trichoderma* genus, directly contributes to build a more sustainable scenario, resulting in less use of agrochemicals and a more rational use of natural resources, in addition to being antagonists to the main phytopathogens of great importance.

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