# Responsible use of biological products in coffee growing

Special Edition - Year 2022

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# Responsible use of biological products in coffee growing

Monte Carmelo - MG
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# SUMMARY

# FOREWORD

Always strengthening the responsible production of differentiated coffees, monteCCer created the Responsible Use of biological products in coffee growing Program, whose objective is to encourage the study and rational use of chemical fertilizers combined with the technology of biological inputs in the control of pests and diseases and in the improvement of soil fertility, without risks to the environment. In the development of this Program, monteCCer relied on the valuable partnership of the Federal University of Uberlândia (UFU) - Campus Monte Carmelo; and from this partnership, this booklet was created.

All the content presented here was prepared by professors from the Institute of Agrarian Sciences and the UFU Institute of Biotechnology, experts in the subject, who were concerned took a didactic approach, with the initial dissemination of information in the format of posts on monteCCer's social networks, in the interest of reaching a wide audience. Now assembled, this content makes up this booklet; bringing the latest research of biologicals carried out in the field, in accessible and clear language.

We leave our thanks and recognition for this unprecedented work to the UFU professors – Ana Carolina Silva Siquieroli, André Luiz Firmino, Bruno Sérgio Vieira, Cleyton Batista de Alvarena, Edson Aparecido dos Santos, Gilberto de Oliveira Mendes, Gleice Aparecida de Assis, Paula Cristina Natalino Rinaldi, Vanessa Andaló, Wedisson Oliveira Santos; to the supporters - Volcafé and Tim Hortons; and monteCCer's Board of Directors.

We hope wherever this rich material arrives - on farms or in academic chairs - it will be put to good use.

***New times demand new paradigms.***

Francisco Sérgio de Assis
President of monteCCer

Regis Damasio Salles
Director of monteCCer

# 1. What are biological inputs?

- **Products, processes, and technologies of microbial, vegetable, or animal origin** intended for use in agricultural production (Decree 10375/2020);

- **used to control pests and diseases,** improve soil fertility, and promote plant growth.

**Also known as biological products, bio-inputs, or simply biological** they include inoculants, bio-pesticides, and soil conditioners.

**They present low risk to the environment and humans,** besides being completely biodegradable;

**provide sustainable and environmentally friendly agricultural practices,** generating healthy products for society.

# 2. What organisms can be used as biological inputs?

Considered as a high potential alternative for agriculture due to its efficiency and sustainability.

| MACROBIOLOGICAL | MICROBIOLOGICAL |
|---|---|
| - Parasitoid insects<br><br>- Predatory insects and mites | - Fungi, viruses, bacteria, and nematodes for pest and disease control |

## Parasitoids

Parasitoids, which need a host to complete their life cycle. They can parasitize different stages of pest development

Examples: Families of Hymenoptera and Diptera.

## Predators

Predators exhibit predatory behavior at different stages of their life cycle. They need a certain amount of prey to develop into adulthood.

## Entomopathogenic fungi, viruses, bacteria, and nematodes

Are microbiological control agents (AMCs) and have been shown to be safe in relation to their possible effects on humans and other non-target organisms in the environment.

## In 2020, MAPA granted the registration of 95 biological control pesticides:

- 15 registrations are composed of microorganisms such as *Beauveria bassiana*, *Trichoderma asperellum*, *Clonostachys rosea*, and *Metarhizium anisopliae*;

- 4 are products containing small parasitoid pest wasps (*Habrobracon hebetor*, *Telenomus podisi*, *Trichogramma galloi*).

# 3. Where do the organisms in biological products come from?

**The organisms present in biological products are found naturally in the rhizosphere of plants,** in the soil, and also from insects colonized by microorganisms.

*Hypothenemus hampei* infected with *Beauveria bassiana* (A) and fruit with the coffee berry borer infected by the fungus.
Source: Jéssyca Gonçalves Duarte (2018).

After isolation and identification of the organism, research is carried out in compliance with current legislation for the establishment of protocol and ***in vitro*** evaluations, product formulation, agronomic tests, and commercial product registration.

For a biological product to be registered, it must meet the standards of the National Health Surveillance Agency (ANVISA); the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA); and the Ministry of Agriculture, Livestock and Supply (MAPA).

At the Federal University of Uberlândia - Campus Monte Carmelo, isolates of *Bacillus spp*. found in Cerrado soils are being tested in research related to the management of nematodes in coffee and against halo spot (*Pseudomonas springae pv. garcae*), with great potential of these isolates for the future production of a commercial biological product.


*Bacillus spp*. isolates from the collection of bacteria at the Laboratory of Microbiology and Phytopathology (LAMIF) at UFU/Monte Carmelo.
Source: Bruno Sérgio Vieira (2021).

# 4. Does biological input work?

**yes!** This is confirmed by national and international surveys.

A 2019[1] study conducted at the **Federal University of Uberlândia (UFU), Campus Monte Carmelo**, showed that *Bacillus spp.* isolates reduced by more than 80% the severity of halo spot in seedlings kept in a greenhouse.

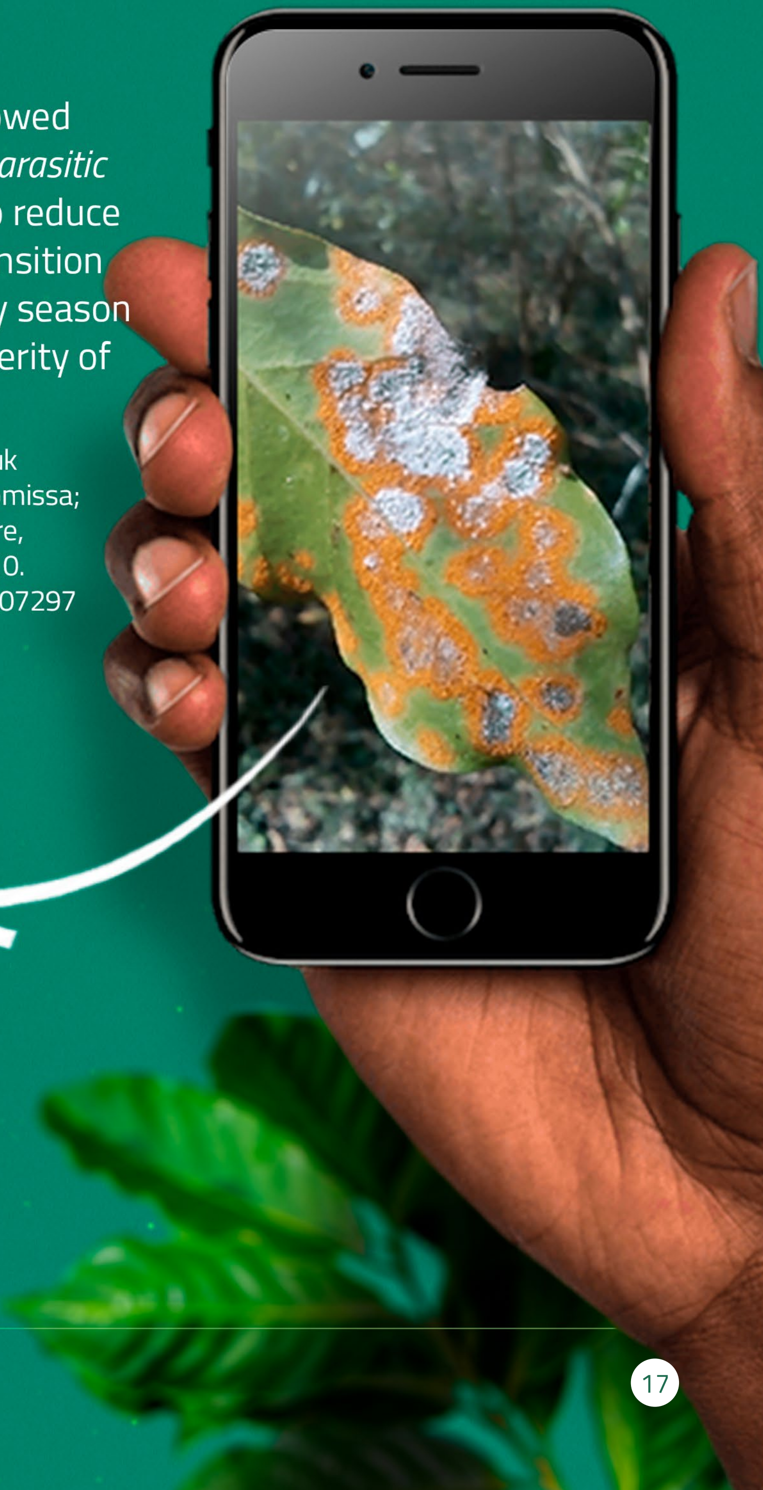
[1] Fabricio Júnio e Silva; Bruno Sérgio Vieira; Ana Carolina Silva Siquieroli (2019). Scientific, 47(4), 364-370. http://dx.doi.org/10.15361/1984-5529.2019v47n4p364-370

Another study by UFU, Campus Monte Carmelo, carried out in 2020[2], showed an interaction between the fungus Beauveria bassiana and the entomopathogenic nematode *Heterorhabditis amazonensis* in the control of the coffee borer, causing mortality in larvae and adults of insects.

[2] Igor Bitencourt Lima Moreira; Vanessa Andaló; Gleice Aparecida of Assisi; Lucas Silva de Faria; Fabio Janoni Carvalho; Renan Zampiroli (2020). Coffee Science, 15, 1-9, https://doi.org/10.25186/v15i.1779.

And a survey carried out in Ethiopia (center of origin of Arabica coffee) in 2021[3] showed the potential of the *hyper-parasitic fungus Lecanicillium lecanii* to reduce the spread of rust in the transition from the rainy season to dry season when the incidence and severity of rust would be at its peak.

[3] Bevene Zewdie; Avco J.M. Tack; Biruk Ayalew; Girma Adugna; Sileshi Nemomissa; Kristoffer Hylander (2027). Agriculture, Ecosystems & Environment, 311, 1-10. https://doi.org/10.1016/jagee.2021.107297



Coffee rust, caused by the fungus *Hemileia vastatrix* (orange spores) and the *hyperparasite* fungus *Lecanicillium lecanii* (white spores) (Zewdie et al., 2021).

# 5. Is it possible to use biological input in coffee growing?

The search by coffee growers for an increasingly responsible production has driven the use of biological inputs in the production process. Several advantages are attributed to the use of biological products. Such as:

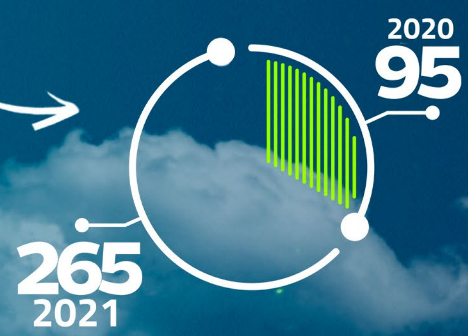
- preservation of natural enemies;
- does not induce resistance;
- coffee valuation, especially in the foreign market with increasing demand for products with low chemical application.

# Precautions with the use of biologicals:

- application dependent on environmental conditions;
- the storage of biological inputs requires special care;
- coffee valuation, especially in the foreign market with increasing demand for products with low chemical application.

BRAZIL. Ministry of Agriculture, Livestock and Supply – MAPA. Federal Government. National Bioinputs Program is launched and will boost the use of biological resources in agriculture. 2020. https://www.gov.br/agricultura/ps-br/assuntos/noticias/program@-nacional-de-bioinsumos-@-lancado-e-ker-boost-use-of-resources-biological-in-agriculture-brasieira Accessed: Mar 17, 2021.

In 2020, 95 biological phytosanitary products were registered in Brazil. Currently there are 265 registered biological products, including bio-insecticides, bio-fungicides, bio-acarides and bio-formicides.




In coffee growing, there are successful cases in the application of biological inputs to control pests and diseases, such as the action of *Beauveria bassiana* on the coffee borer and rosette cochineal and *Trichoderma harzianum* in the control of *Rhizoctonia solani* and *Fusarium solani*.

BRAZIL. Ministry of Agriculture Livestock and Supply - MAPA. Federal Government. MAPA registers a record of 95 biological control pesticides in 2020.2021. https//www.gou.br/agricultura/pt-br/assuntos/notícias/Mapa-registra-recorde-de-95-defensivos-biologicos-em-2020. Accessed on: Mar. 17, 2021.

# 6. Is it possible to manufacture biological inputs on the farm?

**› Yes!** "On-farm production" is possible as long as the property has adequate infrastructure and skilled labor.

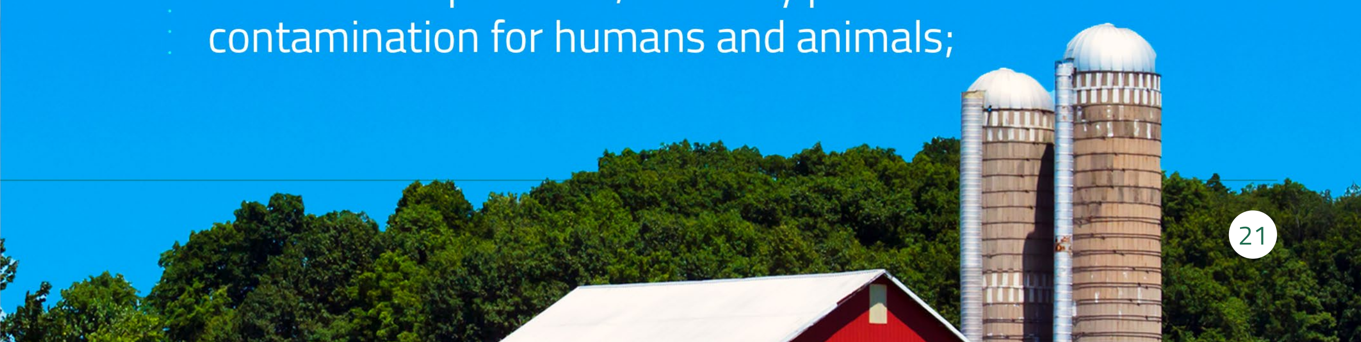
The production of microorganisms used to control pests and diseases on the farm can reduce cost and facilitate access to products.

## Examples of microorganisms produced within farms:

- **Bacteria of the genus *Bacillus*** for the control of fungal diseases, bacteriosis, and nematodes.
  Source: Prof. Bruno Sérgio Vieira (2021)
- ***Trichoderma spp.* fungus species** are used as biological control agents for root pathogens. They also function as plant growth promoters.
  Source: https://estoesagricultura.com/

## Risks and possible problems observed in the production of organic products on the farm:

- Contaminations make the product unsuitable for use as a bio-pesticide, and may present a risk of contamination for humans and animals;



- inadequate concentration of propagules interferes with control results, reducing the effectiveness of biological agents, in addition to interfering with logistical and application issues;

- inadequate production of propagules.
  Ex.: there are specific fermentation conditions for the production of resistant spores and metabolites of *Bacillus subtilis*.

## *Trichoderma* production: an on-farm experience in the Laboratory of Biologicals - Condominium in Mineiro - Goiás

It's possible!

Santos, J.C.S, Aguilera, J.G, Vian, R., Santos, C. S. O., Barbosa, A. 2018


**Palavras-chave**: *controle biológico, produtividade, fungos*

## Introdução

O uso de fungos entomopatogênicos tem proporcionado grandes ganhos no controle de doenças do solo e foliares para a cultura da soja [*Glycine max* (L.) Merrill], em várias regiões do Brasil. Dentre estes, as espécies pertencentes ao gênero *Trichoderma* são amplamente empregadas por sua maior plasticidade de adaptação, pelo maior espectro e estímulo no desenvolvimento das plantas. A produção "*on farm*" é um desafio que os produtores tentam superar, principalmente pela necessidade de mão-de-obra qualificada e infraestrutura adequada, que permita a obtenção de uma produção com o máximo de qualidade e controle em todas as fases do processo. Neste trabalho, é apresentada uma experiência de sucesso na produção de *Trichoderma asperellum* em uma fazenda no município de Mineiros, Goiás.

## Relato da Experiência de Sucesso

A produção de *Trichoderma* na Fazenda Selia teve início após o Curso de Produção "*on farm*" ministrado nos anos de 2016 e 2018, o qual forneceu as primeiras noções do que seria necessário para que se conseguisse realizar a produção na fazenda, melhorando o processo a partir das informações obtidas no curso, as quais foram complementadas pelas experiências de outros produtores, em especial os relatos da Paraíba-PA e de Rio Verde-GO. O

**Figura 1.** Processo de produção do Fungo *Trichoderma asperellum*. A: inoculação do fungo em arroz; B: arroz inoculado com o fungo; C: sala de crescimento; D: arroz com *T. asperellum*.

## Conclusões - Recomendações

A produção "*on farm*" de *Trichoderma* é possível, mas necessita da preparação de pessoal especializado e uma constante adaptação no

# 7. What are the precautions for using a biological product?

Biological products are regulated by the Pesticides Law, decrees, and reference specifications for this class of pesticides. In 2020, the **National Bio-inputs Program** (PNB) was created by decree Nº10,375. The PNB aims to improve the regularization of products of biological origin; as well as expand the supply, access, and encourage adoption and correct usage.

The regulation seeks to guarantee the standardization of the products developed, and consequently their quality and efficiency. Regulatory acts made it possible to register bio-input products by biological target, and once registered, they can be used on any crop in which the pest is present.

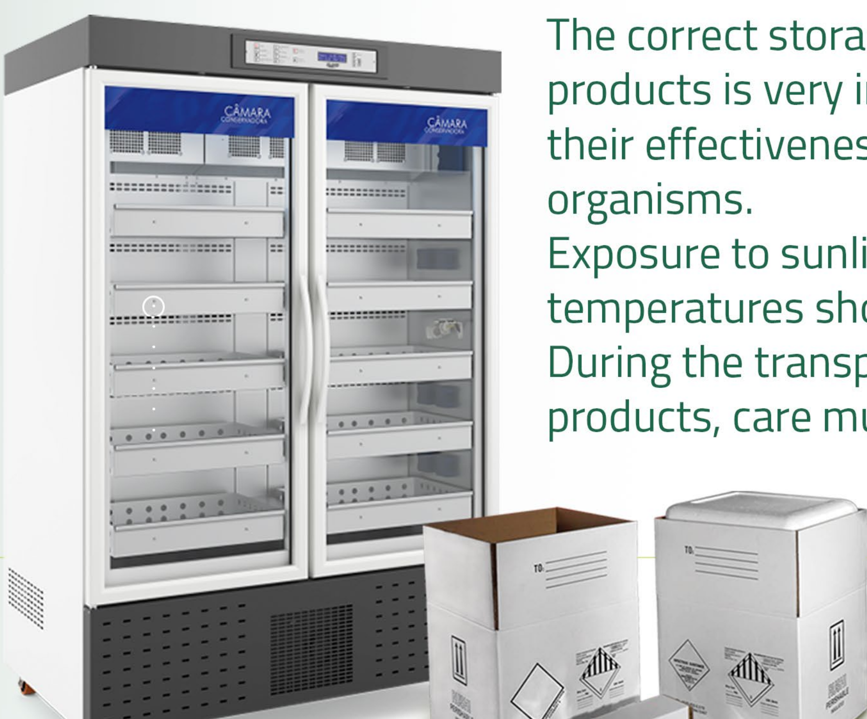
Bio insumos

## ACQUISITION:

MAPA
ANVISA
IBAMA

- evaluates the agronomic efficiency and practicality for the control of a given pest;
- its toxicity to humans;
- and the danger to natural resources.

## STORAGE:



The correct storage of biological products is very important to guarantee their effectiveness since they are living organisms.
Exposure to sunlight and high temperatures should be avoided.
During the transport of biological products, care must be taken.

## MONITORING:

Before applying biological products, it is necessary to monitor the pest or disease in order to know the correct time to apply the product. Application is recommended when reaching the control level, before causing economic damage.

## APPLICATION:

In the application of a biological product:

- **a high quality product should be used.**
  In those based upon fungi or bacteria the recommended viability should generally be above 80%. This index needs to be guaranteed by the manufacturer and verified in laboratories accredited by MAPA.
- **the dosage indicated by the manufacturer.**
  By using smaller doses than recommended in order to save money, the product will not function properly nor bring about desired results.
- **the application technology must be used in the correct manner.**
  Applications are recommended during the mildest temperatures and least sunny times.

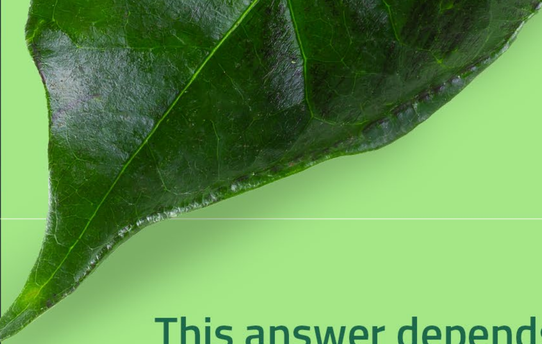
  It is important to observe the correct application times, considering the circadian rhythms of the biological agent, and also the compatibility with other products applied in the area, such as chemical fungicides, insecticides, while respecting the application intervals.

# 8. Does biological work in the same way as chemical?

The use of biological control in coffee crops has increased considerably in recent years; aiming to reduce the application of chemical phytosanitary products and provide a more sustainable activity that meets the requirements of the internal and external market.

However, the question remains:

**Do biologicals work in the same way as chemical controls?**





**This answer depends on several factors, and other relevant questions.**

In the case of microorganisms used to control pests and diseases, adequate climatic conditions are necessary for the biological agent to preserve its viability and promote effective control of the insect/pathogen. **An example is the application of *Beauveria bassiana* in the control of the coffee borer.** Despite the proven effectiveness of this fungus, mild temperatures and higher humidity are necessary to guarantee the fungus' survival, germination, and sporulation. In addition, the application of fungicides to the crop at times close to the application of *B. bassiana* can harm the microorganism and, consequently, reduce pest control (SANTINATO et al. 2017).

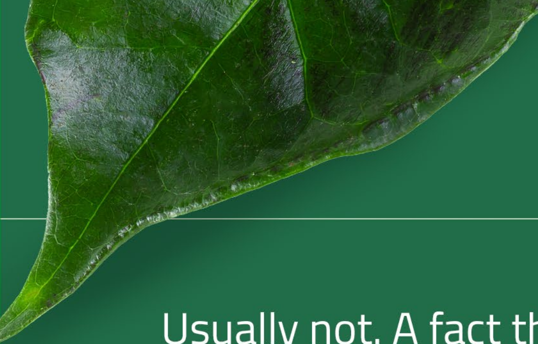
Regarding the use of chemical controls, it is necessary to promote the alternation of molecules presenting different mechanisms of action in the pest/pathogen, avoiding resistance, and consequently, higher control costs.

## Do biologicals promote the same pest/disease control time compared to chemicals?

If the application and handling conditions are suitable for the biological control agent microorganism, the time of action may be relatively short, and even similar to the chemical one.

**An example is the fungus *B. bassiana***, which infects the coffee borer via integument, germinating in a period of 12 to 18 hours. After 72 hours the insect is completely colonized and dies due to the toxic substances released by the fungus (ALVES, 1998).

## Do biological control agents and chemical phytosanitary products have the same mode of action?



Usually not. A fact that becomes very important in the use of different control strategies in the integrated management of pests and diseases. *Bacillus subtilis*, for example, acts in an antagonistic way, releasing substances that act and compete with pathogens. Fungicides from the chemical group of strobilurines, widely used to control coffee plant diseases, act directly on the mitochondria. Thus, the integrated use of biological and chemical inputs is a promising alternative for avoiding pest and disease resistance, in addition to contributing to the increase of natural enemies in the crop, promoting greater sustainability of the production process.

# 9. Can fungi and bacteria help in plant growth?

Beneficial microorganisms can inhabit internal tissues and the surface of leaves and roots of plants, helping their development through the acquisition of nutrients such as nitrogen and phosphorus, production of plant hormones, and biological control of pests and diseases.

## Nitrogen-fixing bacteria

Biological fixation is carried out by converting nitrogen from the atmosphere into chemical forms usable by plants. The best known bacteria are the rhizobia, which form nodules in legumes such as soybeans. But there are several other bacteria in the soil capable of fixing nitrogen, including in coffee plantations.

1 Marliane C. S. Silva et al. (2020) IfesCiência Magazine, 6, 12-21 DIVERSITY OF NITROGEN-FIXING BACTERIA IN COFFEE CROPS (*Coffea arabica L.*) | Ifes Ciência Magazine]


Resumo

**Resumo:** Os microrganismos são considerados importantes fatores biológicos para o crescimento e desenvolvimento das plantas, sendo as bactérias fixadoras de nitrogênio (BFN) auxiliando diretamente a nutrição nitrogenada. Entretanto, poucos estudos têm avalido esta importante comunidade bacteriana na cultura do café. Assim, o objetivo do presente estudo foi avaliar a diversidade de BFN nos solos e nos frutos de cafeeiros em oito propriedades. Após a extração de DNA, a subregião hipervariável V4, do rDNA da região 16S foi amplificada e sequenciada na plataforma Ilumina Miseq. A predição funcional de batérias com potencial de fixação de nitrogênio molecular foi realizada com o programa PICRUSt2. Cento e dezoito Amplicons de Sequencias Únicas (ASVs) foram preditas como potencias fixadoras. Destas, apenas quatro foram detectadas em frutos. O filo mais abundante foi Proteobacteria (71 %) seguido de Firmicutes (23 %). A abundância relativa das BFN em relação ao total de bactérias do solo variou entre os três e cinco por cento. Nos grãos, essa abundância variou desde menos de um por cento a quase seis por cento. Apenas uma ASV da família Rhizobiaceae foi encontrada simultaneamente nos frutos e no solo. O gênero *Bradyrhizobium* foi detectado em todas as amostras de solo. Concluimos que importantes BFN fazem parte da microbiota presente no solo e fruto de café e que existe um compartilhamento entre eles. As evidências mostram que o gênero *Bradyrhizobium* pode ser considerado um importante componente da microbiota *core* do solo do cafeeiro

Silva, M. de C. S., Gomes Reis Veloso, T. ., Lorenzoni Entringer, T. ., Borchardt Bullergahn, V. B. B., Louzada Pereira, L. ., Márcia Anastácio, L. ., & Megumi Kasuya, M. C. . (2020). DIVERSITY OF NITROGEN-FIXING BACTERIA IN COFFEE CROPS (Coffea arabica L.). *Revista Ifes Ciência* , *6*(3), 12-21. https://doi.org/10.36524/ric.v6i3.852

Fomatos de Citação

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Seção

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## Availability of phosphorus

Microorganisms are able to solubilize soil minerals that contain phosphorous and decompose organic material, making phosphorus available to plants. Some examples of microorganisms with this capacity are:

- **bacteria:** *Agrobacterium, Bacillus, Pseudomonas, Rhodococcus, Serratia* and *Xanthomonas;*
- **fungi:** *Aspergillus* and *Penicillium.*

Fungus *Aspergillus niger.*
Photo: Gilberto Mendes

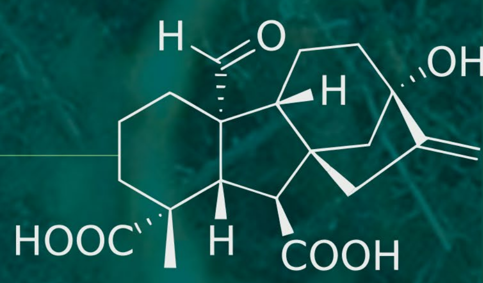
# Mycorrhiza

Fungi that are associated with plant roots in a symbiosis providing benefits for plant growth, such as:

- they enlarge the soil exploration area, enhancing the absorption of water and nutrients;
- increase the availability of nutrients;
- and provide a greater tolerance to water stress, pathogens, and toxic compounds such as heavy metals;
- thus improving soil quality.

# Phytomon Production

Microorganisms can produce plant hormones that stimulate plant development, promoting root and shoot growth. **Auxins** and **gibberellins** are examples of phytomonones produced by fungi and bacteria.



A study carried out at the Federal University of Uberlândia, Campus Monte Carmelo, demonstrated that coffee seedlings inoculated with the soil fungus *Aspergillus niger* showed **higher height, stem diameter, number of leaves, root dry mass, and root volume.**[1]

1 Vithória C Araujo, Kamila F. Rossati, Laura V. Xavier, Vinícius A. Oliveira, Glecia J. S. Carmo, Gleice A. Assis, Gilberto O. Mendes (2020). Rhizosphere, 15, 100236. https://-doi.org/10.1016/j.rhi-sph.2020.100236

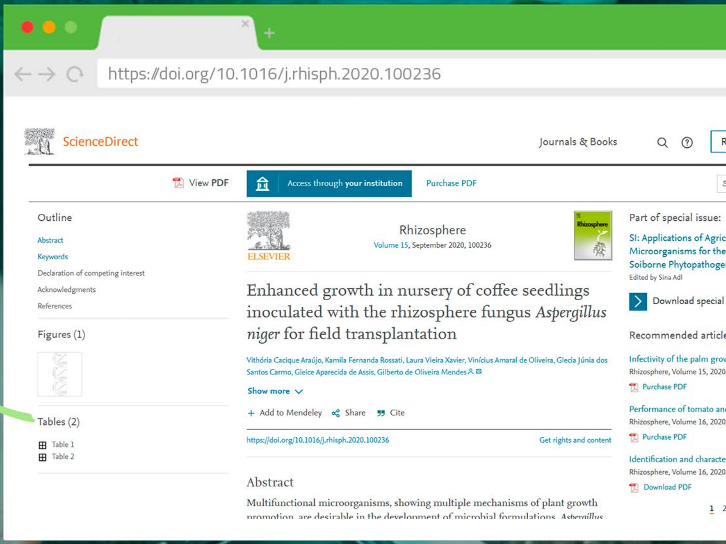



Photo: Vithória Cacique Araújo

# 10. How do fungi control nematodes?

Among the various natural enemies of nematodes commonly found in soils, those with the greatest potential as biological control agents, are bacteria and fungi (FERRAZ & SANTOS, 1995).

## Nematophagous fungi

Fungi known as nematophagous are the most studied organisms and have sophisticated strategies to infect or capture nematodes; and can be divided into: predators, endo-parasites, opportunistic (parasites of eggs and sedentary females), and those producing toxic metabolites to nematodes.

(STIRLING, 1991).

# Predatory fungi

A group of nematophagous fungi having great potential in the biological control of nematodes are the opportunistic fungi or parasites of eggs and females, with emphasis on the species *Purpureocillium lilacinum* and *Pochonia chlamydosporia*.

**Examples of predatory fungal genera:** *Arthrobotrys, Monacrosporium, Dactylaria, Dactylella* and *Duddingtonia.* (Figure 1)

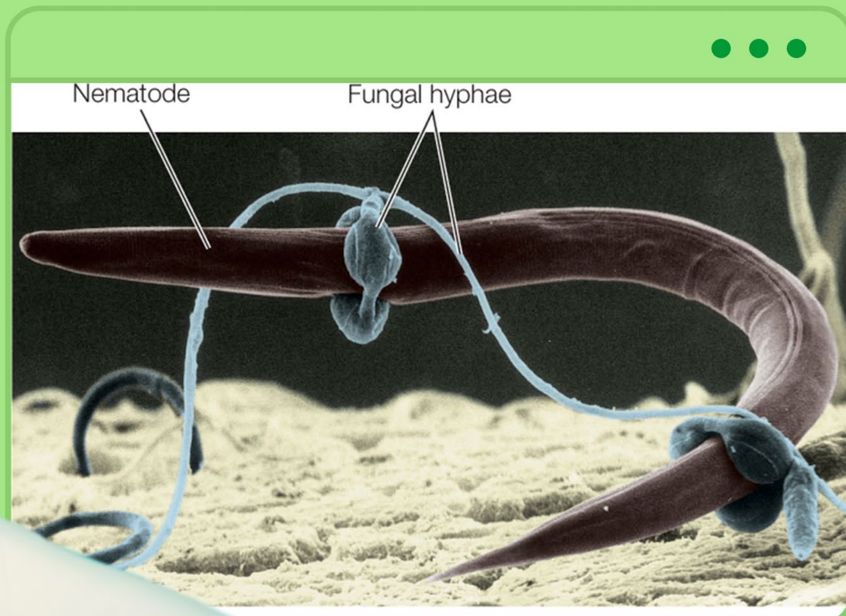


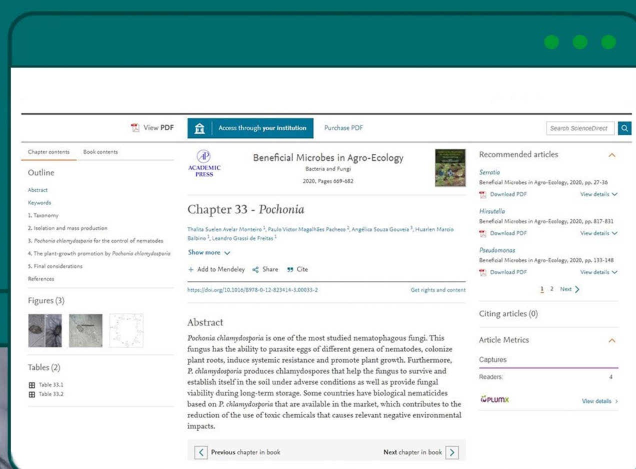

**Figure 1**
N. Allin & G.L. Barron/Biological Photo Service.

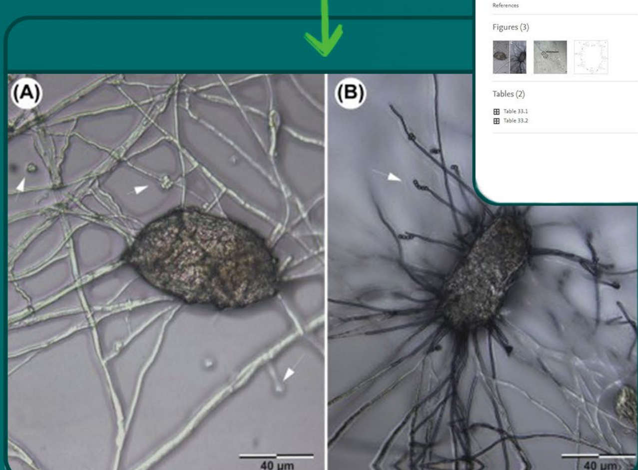
# Egg parasitic fungi

A group of nematophagous fungi having great potential in the biological control of nematodes are the opportunistic fungi or parasites of eggs and females, with emphasis on the species *Purpureocillium lilacinum* and *Pochonia chlamydosporia*.

## Figure 2

*Pochonia chlamydosporia var. chiamydosporia* (A) and *Pochonia chiamydosporia var, catenulata* (B) parasitizing eggs of *Meloidogyne javanica*, Monteiro et al. 2020. Chapter 33 Pochonia, https://oi.org/10.1016/B978-0-12-823414-3.00033-2.



# Fungi producing toxic metabolites

Fungi producing toxic metabolites, represented by the genera *Aspergillus, Pleurotus, Penicillium, Trichoderma* and *Murothecium*, require further studies on the effect of possible toxic substances to nematodes that can be produced by such fungi. Although the main mode of nematode control by *Trichoderma spp.* is the production of toxic compounds, there are reports of parasitism of phytonematode eggs by this fungus genus (EAPEN et al., 2005).

# 11. How do bacteria control nematodes?

The use of bacteria is widely available to promote the reduction of nematode population levels in many crops. Rhizobacteria, as biological control agents, act to promote plant growth and protect against nematodes through different action mechanisms. For example, producing substances that inhibit eggs and juveniles (FERRAZ; BROWN, 2016).

## *Bacillus*

*Bacillus amyloliquefaciens* is capable of forming a biofilm on roots as a protective mechanism against nematodes (ABDEL-SALAM et al., 2018). In addition, it is a plant growth-promoting rhizobacterium. That is, it is able to colonize the soil and roots of plants, resulting in increased plant growth. *Bacillus subtilis* produces toxins that interfere with the reproductive cycle of nematodes (ARAUJO et al, 2002). These bacteria are capable of producing enzymes that break down chitin-based compounds and proteins that make up the nematode body. It hinders the orientation of nematodes and impairs the recognition of the root of the host plant by the nematodes.

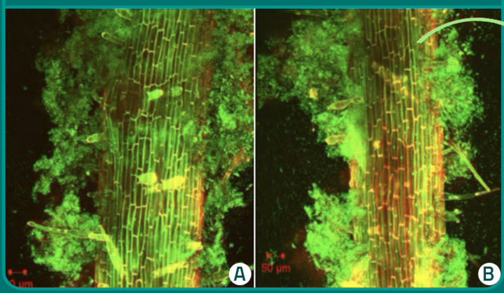



Figure 1
*Bacillus subtilis* (A) and *B. licheniformis* (B) colonizing the outer surface of active roots and forming a bacterial biofilm (green). Source: FMC/Chr Hansen.

## *Pausteuria*

Their endospores come into contact with moving juveniles on the ground and attach externally to their cuticle; then sprouting germ tubes that pierce and propagate inside the bodies, thus beginning intense formation of new spores, which causes the death of the nematode.

(FERRAZ; BROWN, 2016).

Figure 2 Spores of the endoparasite bacterium *Pausteuria penetrans*, a biocontrol agent for Meloidogyne species, adhered to juveniles in Fertantes (S. Ferraz and University of California/Davis).

# 12. Does a biological product control any disease?

## A biological product DOES NOT control any disease!

A first step in the development of a potential biological control agent is the definition of the target.

**Example:** Coffee rust, caused by the fungus *Hemileia vastatrix* or coffee root-knot nematode (*Meloidogyne exigua*).

## Survey and collection of natural antagonists in the environment.

Surveys of natural enemies may begin at the center of origin of the target species or in the regions of occurrence of this pathogen. These natural enemies may be specific to control a given disease(s).

## Identification and characterization of biological control agents

After the work of field collections of the natural enemies (antagonists) of the pathogens, the researchers should clarify the identity of the antagonists selected as the most promising to act on the target species.

## Studies involving antagonistic mechanisms of action

Evaluations of the potential of antagonist isolates obtained as biocontrol agents should be investigated in the laboratory and in a greenhouse, in order to verify their efficiency in handling the target species in question.

## Specificity

The specificity of the antagonist in relation to the target species should also be determined and some characteristics are desired as a biological control agent, such as safety for non-target species, effectiveness, and virulence.

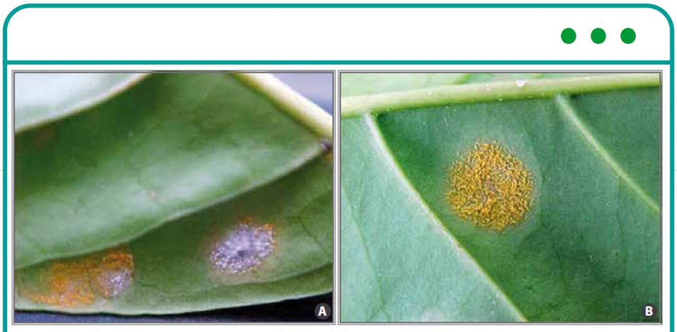
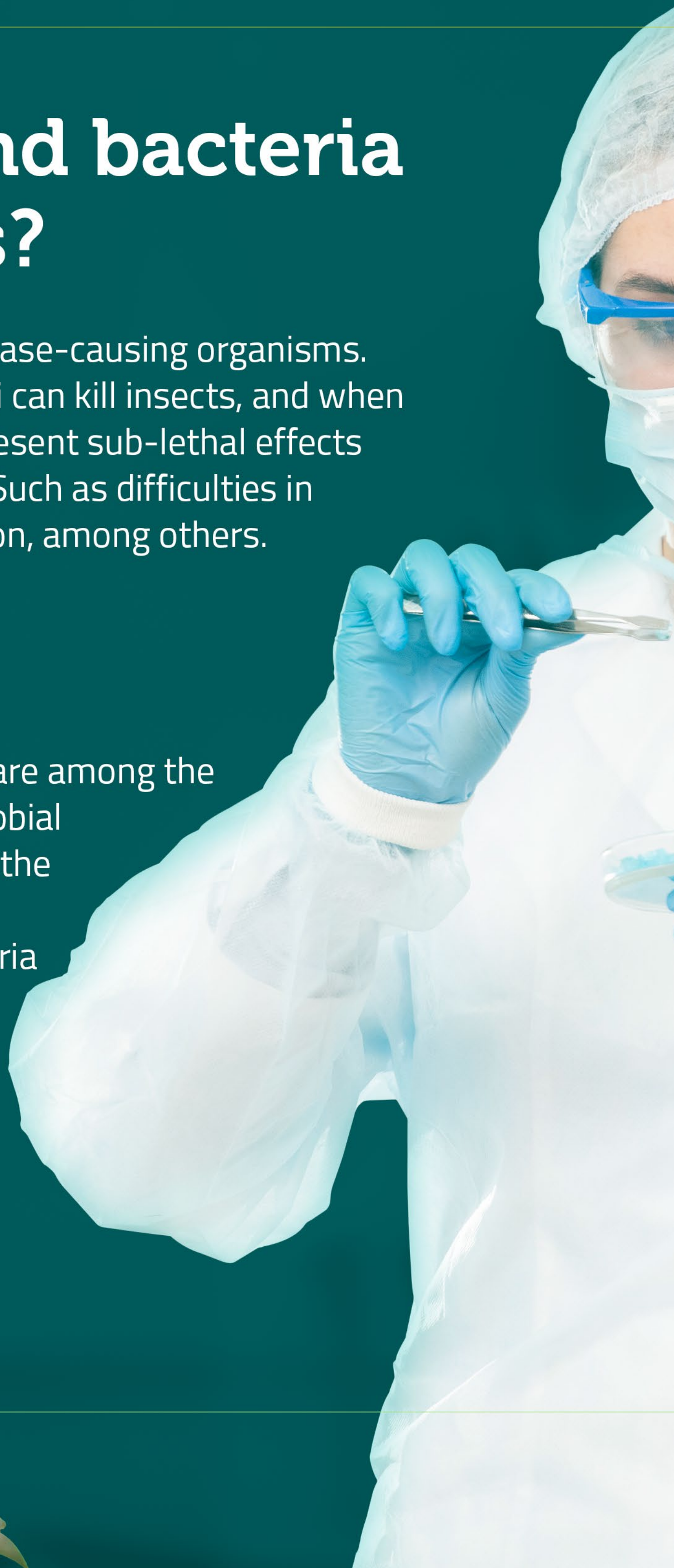



**Figure 1**

Rust pustules with the presence of the hyperparasitic fungus *Verticillium hemileae,* with a white center (A); and rust pustule on the lower leaf surface (B).

# 13. Do fungi and bacteria control insects?

Insects are susceptible to many disease-causing organisms. Several species of bacteria and fungi can kill insects, and when death does not occur, insects can present sub-lethal effects by contact with entomopathogens. Such as difficulties in locomotion, feeding, and reproduction, among others.



## BACTERIA

Pathogenic bacteria of insect pests are among the most commercially successful microbial insecticides. These organisms enter the host only after insects ingest them. Inside the insect's body, these bacteria produce toxins that break through the intestinal wall where they proliferate, causing widespread infection and the insect’s death.

Some naturally occurring insect pathogenic bacteria have been isolated and mass-produced for commercial use. One of them, *Bacillus thuringiensis* (Bt), is the most applied biological control agent in the world. The Bt toxin first paralyzes the intestine and then the entire body of the insect, leading to death. Like most other bacterial pathogens, Bt is specific and lethal to certain pest insects; especially for *Lepidoptera, Diptera* and *Coleoptera*.

## FUNGI

Unlike bacteria that need to be ingested, the process of infection by entomopathogenic fungi occurs mainly by contact. These pathogens also have the ability to infect all stages of host development - eggs, larvae, pupae, nymphs or adults.

Fungi penetrate the insect mainly through the cuticle. Once inside the insects, fungi rapidly multiply in the internal body cavity. Death is caused by tissue destruction and occasionally by toxic metabolites produced by the fungi.

After the death of the host, entomopathogenic fungi often emerge from the body of insects and produce spores. Such spores will continue the cycle in another host (Figure 1).

**Figure 1**
Banana borer killed by
*Beauveria bassiana.*

*Beauveria bassiana* and *Metarhizium anisopliae* are the most studied and applied fungi in pest control. The coffee borer (*Hypothenemus hampen*), for example, is a pest widely distributed in the producing regions, and can be controlled using the fungus *B. bassiana.*

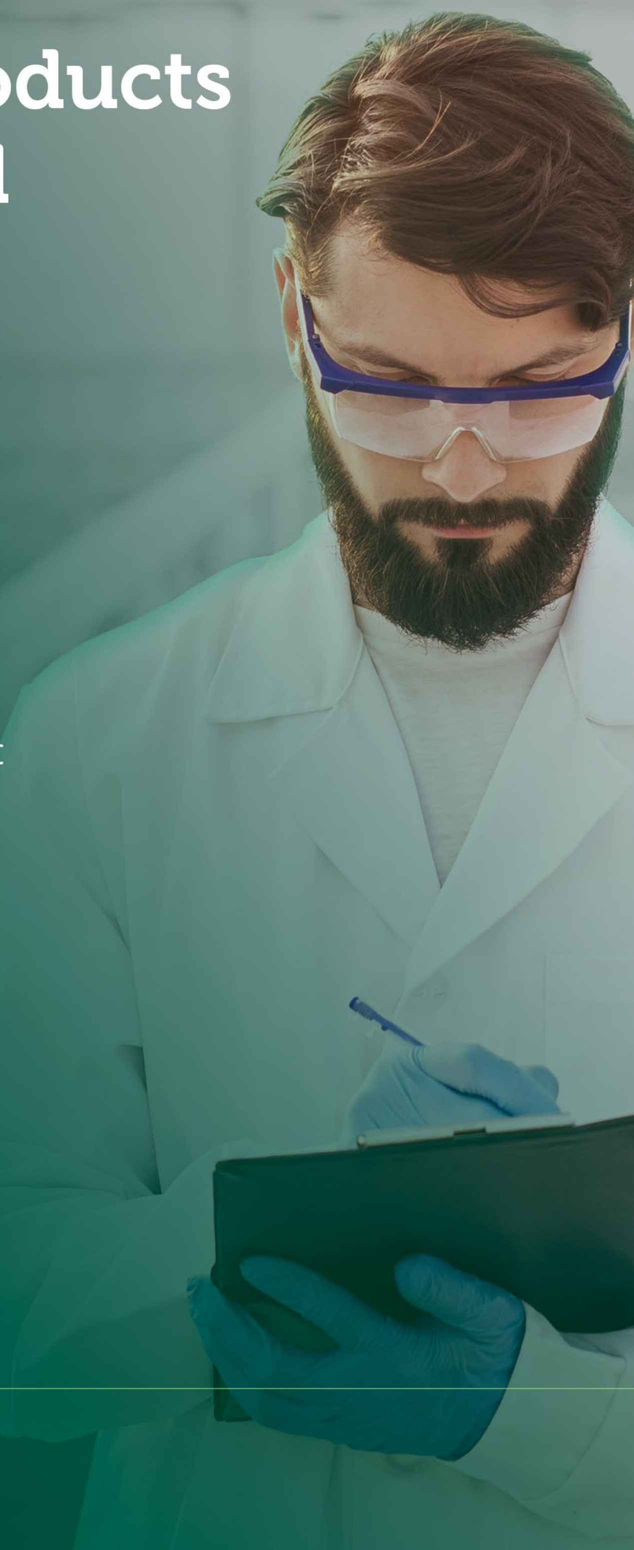
Due to their inherent insect-killing abilities, their distribution in the environment, and by not causing disease to humans; entomopathogenic fungi and bacteria are successfully applied worldwide to control agricultural pests.

# 14. Are there products with viruses and nematodes for insect control?

**Yes!**

They are biological products formulated based on viruses and entomopathogenic nematodes that contribute to making agricultural activities more sustainable.

Viruses interfere with host metabolism, causing physiological dysfunction. They penetrate the digestive system of insects through contaminated food reaching the larva midgut.



The alkaline environment dissolves food and releases viral particles that initiate the process of infecting midgut epithelial cells.

Symptoms are variable; however, most viruses cause loss of appetite, mobility, and epidermis coloration; liquefying the viscera contained in the insect's body. There are several cases of diseases of viral etiology, and the *Baculoviridae* family concentrates the most important viruses used in the biological control of insect pests.

Commercial virus-containing products are used to control important agricultural pests such as the soybean caterpillar (Figure 1), fall army worm, soybean looper caterpillar, and *Helicoverpa armigera*.

Figure 1 - Soybean caterpillar infected with *Baculovirus*.

**Entomopathogenic nematodes** (EPNs) are those capable of killing insects mainly due to a symbiosis established between the nematode and a bacterium, which is responsible for causing the degradation of insect tissues and death due to generalized infection.

These nematodes present, during their life cycle, a larval form of resistance known as infective juvenile (I), found free in the soil. JIs infect adult insects, larvae, or nymphs; penetrating holes such as mouth, anus, or spiracles; or even perforating less resistant regions of the exoskeleton. Some species of EPNs have a corneal tooth in the anterior region of the body that facilitates penetration.

Once in the insect's body cavity, the JIs release the bacteria contained therein, initiating an infection. These bacteria quickly kill the insect - usually between 24 and 48 hours (Figure 2).

**Figure 2**
Entomopathogenic nematodes coming out of the dead insect.

The entomopathogenic nematode *Heterorhabditis bacteriophora* is registered for the control of beetles: brasileirinho, sugarcane weevil, and cupuaçu borer.

# 15. Viability of stored syrup from biological products

## To prepare the phytosanitary syrup it is necessary to:

**a.** Identify the target.

**b.** To know the severity or infestation of the harmful agent to the crop.

**c.** Define the product.

**d.** Define the application method.

**e.** Define the dose - which varies with the concentration of the biological agent.

**f.** Set the application rate.

**g.** Set droplet size - there are few studies for droplet size with biologicals.

**h.** Select the spray tip.

**i.** Prepare the syrup.

**j.** Carry out the application.

## Syrup preparation

**a.** Recommended preparation:
Add the products to the tank and immediately start the application.

**b.** What can go wrong and increase the time the mixture remains in the tank:

I. Tractor or sprayer mechanical problems.
II. Inadequate weather conditions.
III. Work with concentrated ready-to-use syrup.
IV. Reduction of the application rate.

## Problem.

**a.** The syrup stays in the tank longer than planned.
There is no guidance on this in the product inserts and labels.

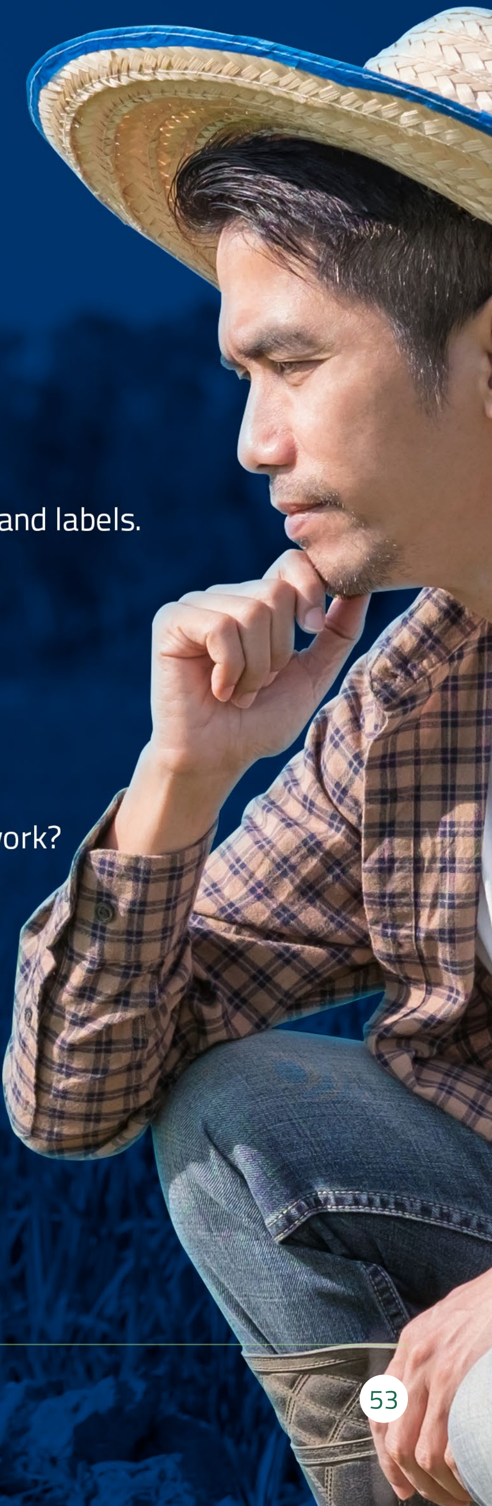
## Decision-making:

**a.** What to do with the product stored in the tank?
I. Discard the syrup! Where?
II. Apply to the crop! At what cost? Is it going to work?

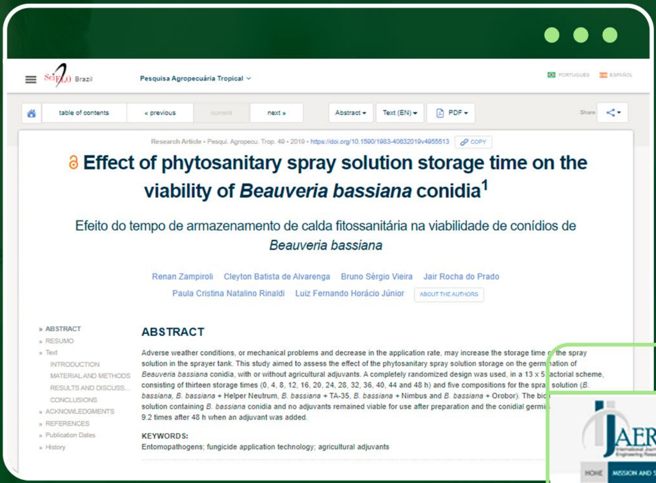
**b.** At night are weather conditions favorable for the application?
Perhaps not, if winter is cold and dry.

**c.** What does the manufacturer recommend in this condition?

**d.** What has the research sought to clarify for this condition?
There is little conclusive research on this matter.



I. In one study, the syrup stored for 48 hours maintained the conidial germination at 84% by adding some adjuvants, and the viability remained above 70% for 4 hours.




https://doi.org/10.1590/1983-40632019v4955513

II. In another study the addition of an emulsifier did not contribute to raising the coffee berry borer mortality.


https://doi.org/10.1590/1983-40632019v4955513

## How long can the syrup be stored?

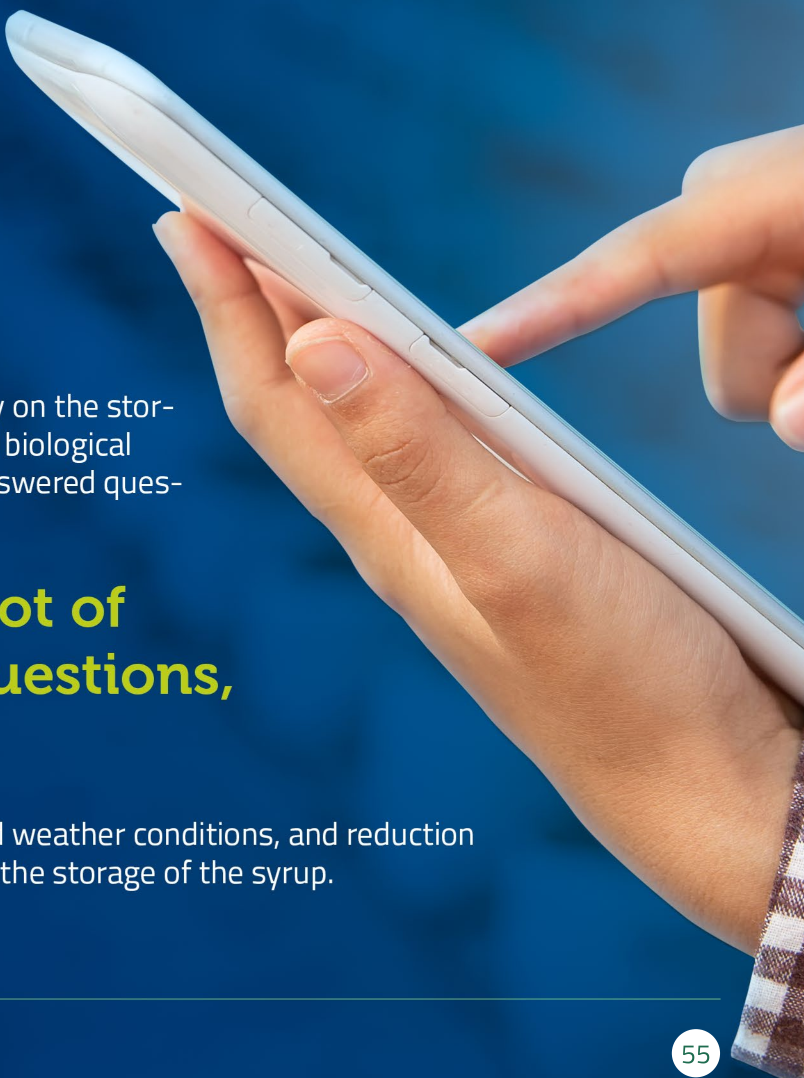
**a.** Considering a tank of 2,000 liters, a rate of 500 L ha$^{1}$, at 6 km h$^{1}$, in spacing of 4x0.8m and operational efficiency of 70%, it takes 36 minutes ha$^{1}$; or 2.4 hours to apply the tank. That's the average syrup time in the tank.

**b.** Are there reactions between chemicals and biologicals? It is likely, but will reactions affect the biological microorganism? The answer directly impacts tank mixing decisions.

**c.** If the syrup remains in the tank for 24 hours what should you do?

I. The manufacturer's recommendation is to discard the commercial product. Where? Do you know the inert materials that make up the biological formulation?

II. In *On-Farm* production, follow the same rule recommended by the industry chemistry.



## Does the syrup remain viable after being stored?

Perhaps. It will depend mainly on the storage time, its composition and biological agent. There are a lot of unanswered questions, aren't there?

## There are a lot of unanswered questions, aren't there?

But mechanical problems, bad weather conditions, and reduction in application rate can extend the storage of the syrup.

# 16. What are predatory insects?

Predators are those organisms that capture, kill, and feed on various prey throughout their life cycle. They play an important role as a mortality factor of pest arthropods in agroecosystems and can promote natural balance by controlling potential pest populations.

Predators have been widely reported in coffee plantations in several Latin American and **African** countries since the 1970s as natural pest mortality factors.

Predatory thrips and the ant *Crematogaster curvispinosus* were found feeding on the coffee borer (*Hypothenemus hampei*).

Several works indicate predatory wasps as efficient natural enemies in controlling the miner (*Leucoptera coffeella*) as well as lacewings, such as *Chrysoperla externa* and *Ceraeochrysa cincta*, which mainly consume caterpillars and pest pupae.

Several species of predatory mites belonging to the *Phytoseiidae* family can also be found in the coffee agroecosystem, especially the genera *Euseius*, *Amblyseius* and *Iphiseiodes*, acting on the main phytophagous mites and reducing damage to the crop and the number of applications of phytosanitary products.

Knowing the main predatory species present in the agroecosystem is extremely important to select environmental management techniques that favor the conservation and increase of those desirable species.

# 17. How do parasitoids act in pest control?

Parasitoid insects are those whose immature forms live as parasites on other insects; consuming their tissues externally or internally, leading the host to death. Parasitoids can attack the different life stages of the host, such as eggs, larvae, nymphs, pupae, and adults.

**Figure 1**
Parasitoide *Cotesia congregata* in Manduca sexta, Lyle J. Buss, University of Florida

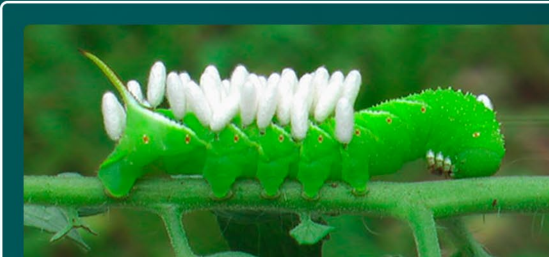


**Figure 2**
*Pupários de Cotesia* sp. in Manduca sexta, Elijah J. Talamas, ARS USDA

They occur naturally in the environment and are also mass produced in the laboratory. They are considered important tools in the natural and applied biological control of pests, mainly against caterpillars and bedbugs in various agricultural crops.

Among the most successful cases of biological control programs applied with parasitoids in Brazil, we highlight the use of *Cotesia flavipes* in the control of sugarcane borer caterpillars (*Diatraea saccharalis*); species of *Trichogramma sp*. (Figure 3) in the control of moth eggs such as *Spodoptera frugiperda, Helicoverpa armigera, Tuta absoluta*, among others; the use of *Telenomus podisi* (Figure 4) and *Trissolcus basalis* to control bedbugs eggs from the *Pentatomidae* family, such as the brown bedbug, *Euschistus heros*; in addition to *Citricola Ageniaspis* and *Tamarixia radiata* against citrus pests.

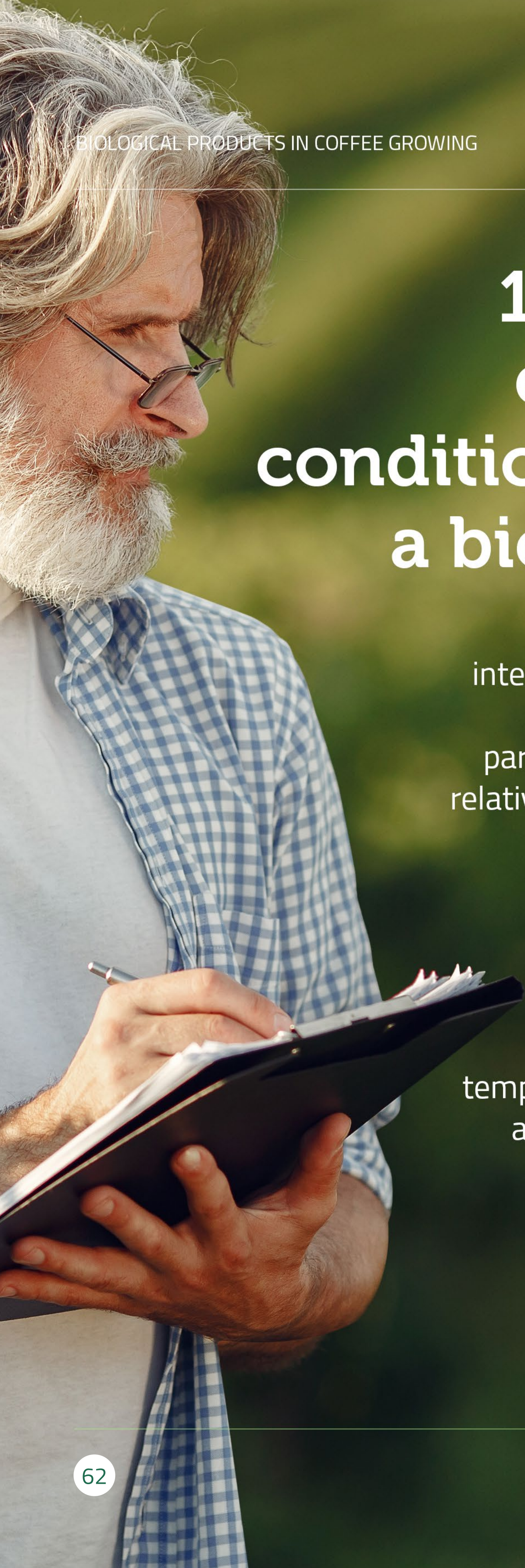
**Figure 3**
Adult of *Trichogramma sp.*, Bornand, C. C. BY-NC 2.0

**Figure 4**
*Telenomus podisi* in brown bedbug eggs (*Euschistus heros*), R. R. Rufino, EMBRAPA

In Brazil there are more than 50 registered commercial products based on parasitoid insects. Commercial products can be released into the field manually or by means of drones. They are widely used in soybean, corn, cotton, citrus, and tomato crops.

Although commercial products with parasitoids are not yet used in coffee growing, there is a great diversity of naturally occurring species that parasitize the main coffee pests; especially the leaf miner (*Leucoptera coffeella*), mainly concentrated in the families *Eulophidae* and *Braconidae*.

The preservation of natural areas, environmental enrichment and rational management with phytosanitary products favor the natural occurrence of parasitoids in the agroecosystem.



# 18. Under what environmental conditions can I apply a biological agent?

Environmental conditions may interfere with the viability and efficiency of the applied biological. The main parameters are related to temperature, relative humidity, and ultraviolet radiation.

## Temperature

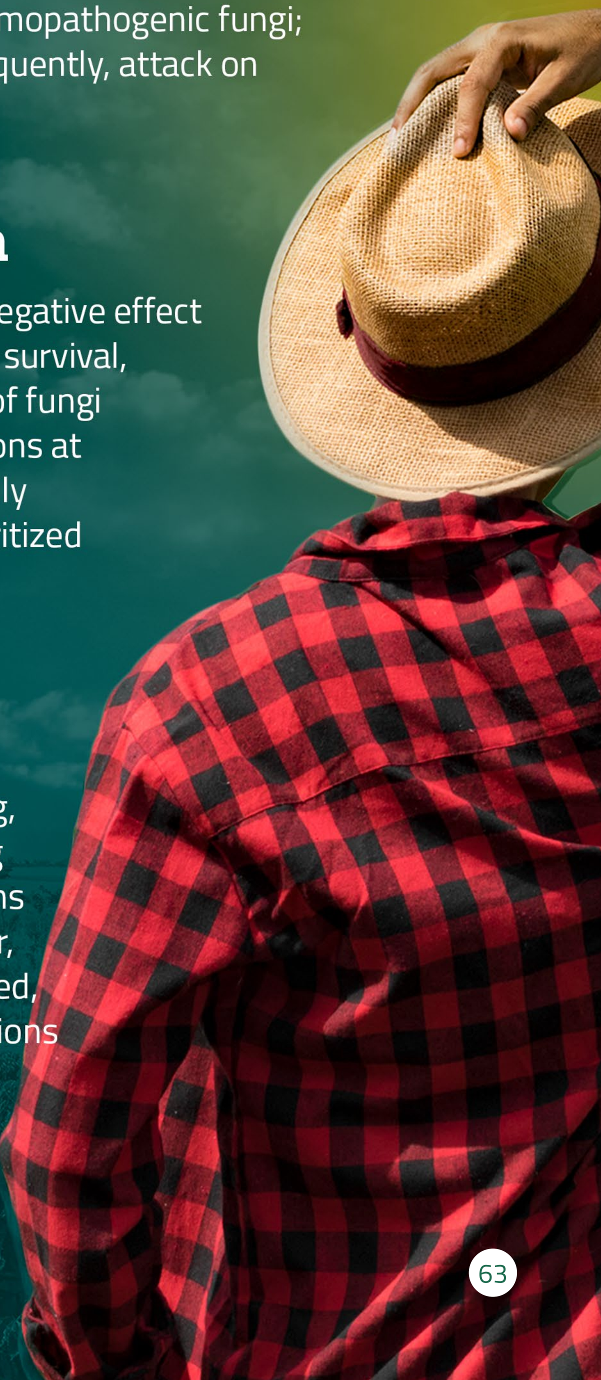
It is important to know the biological response to different temperatures and to know the favorable and unfavorable temperatures for its development. In general, biologicals are better adapted to conditions of temperatures close to 25°C.

## Relative humidity

Biologicals require environmental conditions of high relative humidity, especially microorganisms such as entomopathogenic fungi; in order to favor germination and consequently, attack on susceptible hosts.

## Ultraviolet radiation

Exposure to ultraviolet radiation has a negative effect on spore germination, as well as on the survival, reproduction, dispersion, and virulence of fungi and bacteria. In this context, the conditions at the time of application should be carefully monitored, and applications can be prioritized at the end of the day and at night.



## Formulations

The formulations of biological agents contribute to their stabilization, handling, and application; in addition to protecting and enhancing their effects. Formulations can be solid— powder, wettable powder, granule, or pellets; or liquid - water-based, oil-based or emulsions. These formulations may contain additives to mitigate the effects caused by temperature, relative humidity, and ultraviolet radiation, ensuring greater biological efficiency.

# 19. Mixing in tank between biological and chemical product

## Is there a recommendation to mix between the companies that make the products?

### YES

**Based on the conditions and property.**

**a)** According to Adeney Bueno, EMBRAPA researcher, the joint use of biological and chemical products can be done as long as the chemical pesticide is physiologically or ecologically selective to the control agent present in the biological product.

**b)** In these works, it can be observed that the combination between chemical and biological products is possible and may be viable to the producer.

**https://repositorio.unesp.br/bitstream/handle/11449/180368/brenha_jam_dr_jabo.pdf?sequence=3&isAllowed=y. https://repositorio.ufu.br/bitstream/123456789/31043/3/AtividadeInseticidasBiol%C3%B3gicos.pdf.**

### NO

**Am I able to do it on my own?**

**a)** Not without a responsible professional.

**b) It will be necessary to know:**

I. The formulation
II. The dose
III. The concentration
IV. The other products

**In many leaflets, the manufacturer says to avoid it, and I have respected this recommendation.**

## Is it desirable that biological and chemical products are compatible?

**a) Yes, in this paper the authors concluded that this combination:**

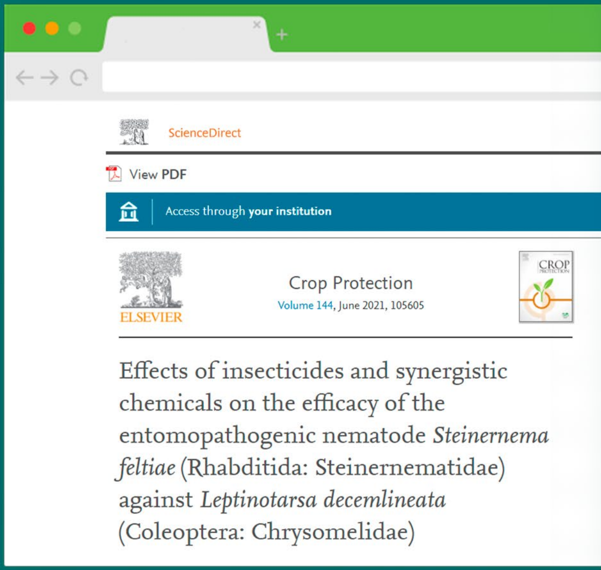
I. Would reduce operating costs.

II. Would facilitate the applications operational.

III. Encourage possible additive action on chemical and biological products, thus increasing the control effectiveness.

IV. Would reduce cases of chemical resistance.

Further studies still need to be conducted.




https://doi.org/10.1016/j.cropro.2021.105605.

## How would this combination be useful to the producer?

**a) A chemical with an immediate shock effect.**

**b) A biological with a more lasting and prolonged effect.**

## To avoid chemical and biological mixing; is it best just not to mix them in the tank?

I. Was the spray properly decontaminated?

There are several products for tank decontamination.

II. Is the triple washing carried out in the tank and hydraulic system?

Pure water is able to greatly reduce chemical residues, but it may not be enough.

## The mixture's success is dependent on the products dose and concentration when added to the syrup.

a) In commercial production, the concentration is known.

b) On-Farm Production is possible too! Can the production be trusted?

I. Without knowing the concentration, it is not possible to recommend a mixture.

## Does mixing between products only occur in the tank?

**a) No, depending on the absorption time, formulation, time on the leaf surface, and two successive applications in a short period of time; mixing can occur within the plant.**

I. You know the process of product interaction.

**Example:** think of a cubicer followed by a biological or vice versa.

**a.** What is the optimal interval between these applications?

## Before mixing the biological and chemical product in a tank, pay attention to these questions, so you will be better prepared to carry out the application.

# 20. Do I need a license to use a biological product?

Agronomists, Forestry Engineers, and Agricultural Technicians are the competent and qualified professionals to fill in the AGRONOMIC RECIPE, a mandatory document for the recommendation of PHYTOSANITARY OR RELATED PRODUCTS.

The Agronomic License guarantees the PHYTOSANITARY PRODUCT is being recommended based on the particularities related to the biological target. Due to the large number of factors related to the occurrence of a phytosanitary problem, only qualified professionals can issue such a document.


As the recommendations for controlling phytosanitary problems are intrinsic to that problem, and very dynamic for the recommendation of BIOLOGICAL INPUTS, it is necessary to issue the AGRONOMIC RECIPE when the product is intended for PLANT PROTECTION.

However, LOW HAZARDOUS PRODUCTS can be dispensed from the AGRONOMIC RECIPE, which will appear on the LABEL and in the PRODUCT PACKAGE LEAFLET. The products registered for ORGANIC AGRICULTURE are exempt from AGRONOMIC RECIPE.

# 21. Must the biological product be registered for the culture and the control target?

Biological products in Brazil can be microbiological, biological, semiochemical, or biochemical agents. The regulation of all stages that allow the use of products in the field is carried out based on analyses by IBAMA, MAPA and ANVISA.

MAPA Ministério da Agricultura e do Abastecimento

ANVISA Agência Nacional de Vigilância Sanitária

IBAMA MMA

When purchasing a biological product, the farmer has technical and scientific support regarding the efficiency and quality of the product and its lower risk to human health and the environment.

Conventional phytosanitary products are registered for the biological target and for the crop, according to all research in the registration process; however, **BIOLOGICAL PRODUCTS**, due to their safer characteristics to human health and the environment, can be used in **ANY CROP**, provided that the **BIOLOGICAL TARGET** is present (ACT Nº 6, JANUARY 23, 2014).

The packaging also does not need to include the term "caution poison" and the skull with crossed shinbones. The indication of the culture on the label or package leaflet of the products by the registering company is optional.

Biological products are regulated by Law Nº 7,802 of July 11, 1989. Due to the various studies and the best characteristics that these products have, there have been many advances in regulation. The possibility of using the product, regardless of the crop, stimulated the development and greater supply of products for the various agricultural crops in Brazil.

# 22. Application technology for liquid biological products

## The liquid application uses water or oil as a vehicle for the dispersion of phytosanitary products. For biologicals, the main vehicle is water.

a) For biologicals, the main vehicle is water.

## In coffee growing, the main vehicle is water, in terrestrial and aerial application.

a) Water is a good vehicle to disperse the control agent over the crop.

b) Water helps in the development of structures of pathogenic agents.

## Application technology for liquid biological products?

a) Fungi
b) Bacteria
c) Viruses
d) Entomopathogenic nematodes

## Possibilities for application of biologicals in coffee growing.

| a) Pulverization | b) Irrigation |
|---|---|
| **I.** Turbo atomizers. | **I.** Pivot center. |
| **II.** Remotely piloted aircraft. | **II.** Drip. |
| **III.** Agricultural aircraft. | |

## Advantages of liquid application.

a) Producer/operator experience with the machine.

b) Distribution of the organism directly on the target.

c) Non-corrosive to sprayer or irrigation hydraulics system.

d) Reduced operator contamination.

## Points of attention for the spraying of biological products.

a) Concentration of commercial product must be known regardless of production method

- **I.** Direct impact on the dose.
- **II.** Direct impact on control effectiveness.
- **III.** Direct impact on technology.
  - **1.** If it doesn't work, you lose credit and it's hard to recover.

b) Application fee

c) Number of applications

d) Droplet size

e) Tip type.

f) Turbine airflow effect.

g) Water evaporation prevents the prolifera-tion of the biological agent.

h) Possible clogging of tips due to granulometry of the structures of biological agents.

i) Should water quality represent the same concerns as chemical syrup?

**I.** Hardness.

**II.** pH.

**III.** Electrical conductivity.

**IV.** Surface tension.

## Attention points for the spraying of biological products.

a) The formulation.

b) Inert.

c) Possible use of adjuvants to improve the biological agent arrival at the control target.

# 23. Biological control of weeds

## What is the importance of weeds?

Weeds are plants that interfere with crops by competing for water, light, and nutrients.

## Does biological control work for weeds?

Biological control of weeds is feasible and used in many places. Indirectly, this control is practiced by keeping a straw protecting the soil, plant material harbors natural weeds enemies. There are also insects, fungi, bacteria, and mites with the potential to control plants.

## Are there commercial products for biological weed control?

Plant or microorganism metabolites, or the microorganisms themselves, are marketed as BIOLOGICAL PRODUCTS for weed control. Biological herbicides are effective and produced from microorganisms such as *Alternaria, Colletotrichum, Streptomyces*, and *Xanthomonas*, among others. Some examples of commercial products marketed outside Brazil are: Opportune™, Stumpout™, Velgo® and Smolder WP.

## Biological products for weed control in Brazil.

Biological control of weeds is the result of large investments in Brazil. Institutions such as the Federal University of Uberlândia, in partnership with public or private entities, work on the development of biological products for weed control and the results indicate great potential for the production and commercialization of these products in the near future.

# 24. Remineralizers in coffee growing

## Is the use of rock powders interesting in Coffee Growing?

Limestone rocks (limestone), phosphates (reactive natural phosphates) or gypsum (mineral gypsum) powders are efficient as soil conditioners or nutrient sources in acidic soils with low fertility for Ca, Mg and P, respectively for coffee growing. Soil remineralizers based on silicate rocks have highly variable efficiency.

## What are soil remineralizers?

Legally, soil remineralizers are rock powders submitted only to physical processing (grinding), generally silicate, with minimum levels of $K_2O$ of 1%, $\Sigma$CaO, MgO and $K_2O$ ≥ 9.0 % and levels of As, Cd, Hg and Pb below 15, 10, 0.1 and 200 mg/kg, respectively, capable of promoting benefits to the soil — source of nutrients or conditioner— or to the plants— growth, productivity.

## What is the remineralizers composition?

They are materials rich in various minerals, such as feldspars, micas, and quartz, with highly variable reactivity and solubility.
In general, they stand out with high levels of Si and Al and medium to low levels of K, Fe, Ca, Mg, P, and micronutrients.

## Some remineralizers have more than 10% of K2O. Should this content be considered as available to plants?

No! Most of the K is strongly attached to the silicate structures in these materials, so it is practically insoluble in water. A small portion is soluble in citric acid, usually less than 3%, and can be released into the soil within a few months after application, under conditions that favor dissolution.

## Are soil remineralizers efficient?

The efficiency of remineralizers is very dependent on the characteristics of materials, soil, and management.

## Can soil remineralizers replace limestone in soil acidity correction?

Although silicate materials have the potential to correct soil acidity, their low reactivity makes them unfeasible as correctives.

## What do I need to know to decide on the use of remineralizers in coffee growing?

- Price per nutrient point considering the soluble content

- Solubility of materials in water, citric acid, or neutral ammonium citrate

- Soil reactivity

- Local experimental demonstration attesting efficiency

# 25. Soil microbiology

Microbial biomass is the living and active part of soil organic matter, composed mainly of fungi, bacteria, and archaea.

## DID YOU KNOW THAT?

A teaspoon of soil contains 100 million to 1 billion bacteria.

**These microorganisms play a fundamental role in the sustainable functioning of natural processes in the soil, and can act in:**

- Nutrient cycling, affecting ecosystem productivity and stability;
- Storage of carbon and mineral nutrients;
- Biodegradation of pollutants and pesticides;
- Soil structuring;
- Promoting plant growth;
- Protection against pests and diseases.

Changes in the community and microbial activity can lead to changes in the chemical and physical properties of the soil – which could mean a sign of soil improvement or degradation. The soil microbial community is tied to agricultural sustainability, so it can be used as a potential indicator for assessing soil quality.

## Can microbial biomass reflect changes in organic matter and soil development?

Yes. As soil microorganisms participate in processes that affect the ecosystem and are linked to soil fertility, they can be used as indicators of soil quality.

A study conducted by researchers at UFU*, Monte Carmelo campus, showed that the diversity of bacteria can be an indicator for changes in soil functioning.

* https://doi.org/10.14393/SN-v34-2022-62940

# 26. Care and risks in the production and handling of Bio-inputs

*On-farm* production, also known as "production on farms" of biological inputs, consists of the multiplication of bacteria and/or fungi within the property for its own use.

However, there is great concern regarding the multiplication of microorganisms. Inadequate technique and the absence of quality control steps during the on-farm production of microorganisms can lead to the multiplication of low quality, unsafe, and ineffective products for biological control, putting the crop at risk. In addition, contamination by human disease-causing agents or the proliferation of species that produce toxins harmful to human health may occur.

The production of biological inputs requires scientific knowledge and the adoption of strict quality control processes at all stages. What is expected is a product free of contaminants and effective in controlling pests and diseases.

In order not to compromise the integrated management of pests and diseases, a strategy for the conservation of these microorganisms in the area is necessary, which can be done through the application of selective pesticides when the use of chemical control is indispensable.

The effect of pesticides on biological inputs may vary depending on the species and strain of the microorganism, the chemical nature of the products, and the doses used. These products can act by inhibiting the vegetative growth, reproduction, sporulation of microorganisms, and cause genetic mutations, which can lead to a decrease in the virulence of a particular pest or pathogen.

Thus, it is important to know the action of phytosanitary products in determining their selectivity and compatibility with the biological product, in order to minimize the impacts both on the environment and on the resident microbiota.

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