Chapter

Biological Control in *Capsicum* with Microbial Agents

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Abstract

Capsicum annuum L. has great importance worldwide for its nutritional characteristics and its antioxidant content. It is cultivated in different geographical areas, under field and greenhouse conditions, and its production can be used for fresh consumption or processing. During its growth, it can be affected by biotic factors, such as pests and diseases that negatively affect the production and quality of its fruits, thus making adequate control measures necessary to avoid relevant economic losses. The environmental conditions that occur in its production promote the development of pests and diseases that can progress rapidly, making it increasingly difficult to manage populations of *Capsicum*. Traditionally, chemical pesticides have been used to deal with these problems, but their indiscriminate use has had negative consequences on the environment and human health. Biological control, based on the use of microorganisms, is thus presented as an efficient and sustainable alternative for *Capsicum* cultivation and offers a series of additional benefits. This chapter reviews the control alternatives available with microbial agents and their applications in the protection of *Capsicum* plants.

Keywords: beneficial microorganisms, entomopathogenic fungi, *Beauveria bassiana*, *Trichoderma*, endophytes

1. Introduction

The genus *Capsicum* belonging to the family *Solanaceae* consists of approximately 31 species, of which only five have been domesticated: *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens* [1]. *Capsicum* is known by various names including pepper, chile, chili, chilli, aji, and paprika. Throughout the world, *Capsicum annuum L.* is the most commercially important and widely grown species within this genus. The abundant varieties of *C. annuum*, including sweet peppers and chilli peppers, are important horticultural crops produced worldwide, especially in countries such as Spain and Mexico. Their fruits have remarkable sensory attributes in terms of colour, acidity and aroma, as well as an ample diversity of antioxidants, such as phenolic compounds and flavonoids [2]. Moreover, some types present high levels of capsaicin (8-methyl-*n*-vanillyl-6-nonenamide), which provide them with their spicy flavour in addition to therapeutic applications due to their anti-cancer properties [3].

The growth and development of *Capsicum* can be limited by various abiotic and biotic factors that negatively affect its fruit production and quality. It is estimated that losses caused by biotic factors such as invertebrates, pathogens and weeds can vary from 27 to 42%, which would increase to between 48 and 83% if the crops

were not protected [4]. Among these, pests and plant diseases seriously affect *Capsicum* crops, in addition to the fact that their production is carried out under limited environmental conditions, which translates into a decrease in yield and quality [5]. For the control of these biotic agents, chemically synthesised pesticides have been traditionally used, which have generated several controversies due to their toxicity in humans and animals, and their damaging effects to the environment. Additionally, they can generate resistance in pathogenic microorganisms [6] and insects [7].

Regarding phytosanitary problems, the high temperatures and high levels of humidity generated in intensive production systems promote the development of fungal diseases that can progress rapidly [8]. Among the diseases that affect *Capsicum*, soil-borne diseases caused by pathogens of the genus *Phytophthora*, *Fusarium*, *Pythium* and *Rhizoctonia* are especially significant. At the fruit level, this crop is affected by pathogens such as *Botrytis cinerea* and *Anthracnose*, the latter caused by a complex of *Colletotrichum* species that is considered a serious problem with heavy losses in fruit yield, exceeding 80% [9]. Another important problem in the cultivation of *Capsicum* is viruses, since there are approximately 70 types that can affect this crop [10], especially the cucumber mosaic virus, pepper mild mottle virus and potato virus Y, among others [11–13]. In general, viruses can interfere with the chlorophyll synthesis of the plant, causing chlorosis and mottling of the foliage (mosaic).

On the other hand, several insect pests affect this crop during the entire growth and production cycle, causing significant yield losses ranging from 50 to 90% [14]. This has resulted in an intensive use of insecticides, mainly chemical. Among the plagues that affect *Capsicum* are: whiteflies, like *Trialeurodes vaporariorum* Westwood (Hemiptera: *Aleyrodidae*) and *Bemisia tabaci* Gennadius (Hemiptera: *Aleyrodidae*); flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: *Thripidae*); aphids *Myzus persicae* Sulzer (Hemiptera: *Aphididae*) and *Aphis gossypii* Glover (Hemiptera: *Aphididae*); worms *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*), *Spodoptera litura* Fabricius and *S. exigua* Hübner (Lepidoptera: *Noctuidae*); and mites *Polyphagotarsonemus latus* Banks (Acari: *Tarsonemidae*), *Tetranychus urticae* Koch, *T. ludeni* Zacher and *T. evansi* Baker and Pritchard (Acari: *Tetranychidae*) [15–17]. Within this group, whiteflies, aphids and thrips are considered pests of economic importance worldwide [18]. They have a wide range of hosts, from agricultural species to ornamental plants and are difficult to control due to their high reproductive rate, short life cycle and cryptic behaviour [19–21].

Finally, phytoparasitic nematodes are also organisms present in the cultivation of *Capsicum* and can cause serious problems affecting its performance [22]. Among the nematodes that affect *Capsicum*, those of the genus *Meloidogyne* are especially significant, such as *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. enterolobii* (Yang and Eisenback) and *M. javanica* (Treub) Chitwood, which are responsible for root-knot disease [23–25]. These nematodes are found throughout the world, especially in warm areas and in greenhouses. Other nematodes that also cause economic damage in *Capsicum* include the false root-knot nematode *Nacobbus aberrans* Thorne and Allen [26]. Nematode control strategies are based on the use of chemicals; however, their high cost and principally their toxic effects have led to a search for more sustainable alternatives.

With a growing world population, currently, the main challenge for agriculture is to achieve food security; thus, food production has increased in recent years. However, pests and plant diseases, where *Capsicum* is no exception, have also increased as a result of changing climatic conditions, intensification of production systems and the opening of borders for the free transit of food, including fresh produce. In a conventional way, the high populations of insect pests and plant diseases

have been controlled through the use of chemical pesticides. Biological control through the use of antagonistic microorganisms, such as bacteria, fungi and viruses, is presented as an alternative to the use of chemicals. It has received more attention in recent years and arises in response to the search for ways to control pathogens, insects and nematodes in a sustainable manner. However, this type of control must meet some requirements such as being effective against the target organism, not causing problems to the health of people and animals, reaching adequate control levels in the field, the feasibility of being incorporated into integrated management practices and meeting with phytosanitary measures according to the enforced regulations in each country where they are used.

Considering the above, the objective of this chapter is to review the importance of microbial agents in the biological control of pests, plant diseases and nematodes in *Capsicum*.

2. Microbial biological agents

2.1 Biological pest control with microorganisms

In recent years, world markets have expressed increasing concern about the use of agrochemicals. Biological control, which is defined as the reduction of pest populations by natural enemies and usually involves human intervention, is presented as an alternative to the use of chemical pesticides [27]. Biological control agents are classified into predators, parasitoids and pathogens (microorganisms that cause diseases).

After being identified, isolated and reproduced, biocontrol microorganisms are applied in a directed way in dilutions or released on the insect pests and diseases of the crops so that they can carry out their colonising action, produce antagonism, and specific diseases in the agents that require control, with the purpose of reducing the incidence to inoffensive levels [28]. Many microorganisms have been used as biopesticides because they offer a number of additional benefits beyond their target function [29]. An antagonist microorganism used for biological control must meet certain requirements, such as being genetically stable, effective at low concentrations, undemanding in nutrients, adapted to different environmental conditions, effective for a wide range of pathogenic microorganisms, easily grown, easily manipulated, resistant to chemical pesticides, compatible with commercial processes, not phytotoxic and not harmful to humans [30].

Among the microorganisms that have been most studied and reported as antagonists of insect pests and plant pathogens of *Capsicum* are bacteria such as *Bacillus* spp. and *Pseudomonas* spp., and the fungi *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Trichoderma* spp. and *Clonostachys* spp., while the viruses of the Baculovirus group have proven to be effective insect controllers. The genus *Bacillus* is made up of species that have been widely used to control insect pests and plant diseases due to the morphological and physiological characteristics that allow them to be ubiquitous in nature. It is especially important to note that species of this genus produce metabolites with antimicrobial properties used for the control of plant pathogens. In addition, the species *B. thuringiensis* Berliner has important qualities for the control of nematodes and protozoa [31, 32]. Another important group used in the control of insect pests in *Capsicum* is the entomopathogenic fungi, which have been widely studied. For more than a century, Pasteur predicted the advantages of entomopathogenic fungi because of their role as bioregulators of pests, acting as parasites to insects that are harmful to plants. Currently, more than 700 species of fungi are known to affect insects of various orders and their use as

a biopesticide has increased during the last decade [33]. Mazón [34] indicated that the most important group of entomopathogenic fungi, with practical purposes for pest control, is constituted by *Metarhizium anisopliae* and *Beauveria bassiana*.

2.1.1 Entomopathogenic fungi

Entomopathogenic fungi (EPF) parasitize insects causing serious damage that can even lead to their death. The traditional mode of infecting insects with EPF involves the inoculation of conidia into the cuticle, followed by the formation of a germination tube and an appressorium which, through mechanical and enzymatic action, penetrates the cuticle and reaches the hemocele [35]. After the invasion of the hemocele, these fungi have the ability to re-cross the cuticle of the host and go outside, where they can continue to develop saprophytically on the insect, sporulating and turning them into new foci of dissemination of conidia [36].

EPF have potential for the control of whitefly in *C. annuum* cultivation. The effect of the *B. bassiana* strain M130 on *T. vaporariorum* adults was evaluated under greenhouse conditions reaching control levels of 45.3% in conidia applications to the foliage [14]. Its control of *B. tabaci* was also evaluated; applications of conidia in different concentrations applied to foliage had an effect on the mortality of eggs and nymphs of 37.8–59.04% and 38–75.9%, respectively. The highest percentages were reached with the highest concentrations (2×10^8 conidia/mL) of the Bb01 strain [37]. Flower thrips are an important pest in *Capsicum*; they affect its leaves, flowers and fruits, besides transmitting several viruses, as do white flies. For the control of flower thrips, several EPF have been evaluated as applications of *M. anisopliae* (Met52—Bioglobal Company) by spraying the foliage (flowers and leaves) proved to have the same efficacy on the number of adults of *F. occidentalis* as chemical insecticides [38]. The EPF *Fusarium subglutinans* (strain 12A), applied at concentrations of 1×10^6 conidia/mL, has proven to have the greatest lethal effect (58%) on the second instar nymphs [39]. Other EPF, applied individually or in combinations, have been evaluated in the control of this insect with different levels of efficacy [40]. These fungi have also presented biocontrol action against several aphids. Trials carried out by Curtis et al. [41], where the use of different additives in the formulation of the EPF *Verticillium lecanii* (Zimmerman), now classified as *Lecanicillium lecanii*, was evaluated in applications to pepper foliage for the control of *Myzus persicae*, determined that the formulations did not manage to increase the efficacy of the fungus since the applications of conidia in water suspensions reached the lowest infection rates (<5.0%). The controlling effect of *Lecanicillium attenuatum* (Petch) (CS625) on *Aphis gossypii* has also been evaluated in laboratory trials. The evaluated strain negatively affected aphid populations by decreasing their life expectancy and total fertility, in addition to the direct effect on nymphs [42]. Moreover, these fungi have also proven to be effective in controlling mites. Nugroho and Bin Ibrahim [43] determined that under field conditions, *B. bassiana* was the most effective in suppressing *P. latus* populations (eggs per outbreak) in chilli peppers, in comparison with strains of *M. anisopliae* and *P. fumosoroseus*.

2.1.2 Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are widely distributed throughout the world and have a large number of host insects [44]. They are soft-bodied, non-segmented vermiform organisms that are sometimes forced or opt to parasitize insects. They use insects as feeding, dispersal and propagation substrates, characteristics that have allowed them to be used for the biological control of insect pests. Naturally found in the soil, they are able to pursue and locate their host insect,

responding to carbon dioxide emissions, vibrations and chemical signals. They have potential for use in crop protection because they are easy to mass produce and harmless to vertebrates and plants [45, 46]. The genera most used in the biological control of insect pests are *Steinernema* spp. and *Heterorhabditis* spp. These nematodes establish a mutualistic association with bacteria of the family Enterobacteriaceae, which are Gram-negative and facultatively anaerobic. Nematodes of the genus *Steinernema* are associated with bacteria of the genus *Xenorhabdus*, while those of the genus *Heterorhabditis* are associated with *Photorhabdus* [47].

Studies by Uhan [48] showed that nematodes of the genus *Steinernema* caused between 23.9 and 78.3% mortality of *S. litura* in pepper plants. The effect of native strains of *Steinernema feltiae* (Filipjev) (Rhabditida: *Steinernematidae*) and *S. carpocapsae* (Weiser) (Rhabditida: *Steinernematidae*) against *S. littoralis* and *Agriotes sordidus* Illiger (Coleoptera: *Elateridae*) larvae and *Ceratitis capitata* (Wiedemann) (Diptera: *Tephritidae*) pupae has also been studied at laboratory and greenhouse levels using pepper plants, with interesting levels of control that varied depending on the strain and soil texture [49]. Although EPNs have been used in *Capsicum* mainly for insects that have larval stages in the soil, they have also been evaluated against pests that spend their entire life cycle on the aerial part of the plant. Studies carried out by Rezaei et al. [50] on pepper plants determined that strains of *S. feltiae* and *H. bacteriophora* (Koppert Biological Systems) had a biocontrol effect on nymphs (second stage) and adults of *T. vaporariorum* in foliar applications.

2.1.3 Entomopathogenic bacteria

One of the most widely used bacteria for insect control is *Bacillus thuringiensis*, a Gram-positive bacterium, which has the ability to form spores as well as synthesise crystalline inclusions containing one or more proteins, some of which are toxic to a significant number of insect pests of the orders Lepidoptera, Diptera, Coleoptera and Hemiptera [51–53]. Evaluations conducted under laboratory conditions determined that 17 of 40 *B. thuringiensis* strains achieved mortality rates of *M. persicae* ranging from 64.4 to 88.9% [54]. On the other hand, some researchers have evaluated the effect of *B. subtilis* strains on this insect; however, no evidence was found of the direct effect of the biocontrol, rather the bacteria was found to promote plant growth by indirectly mitigating the damage caused by the insect [55, 56].

The Gram-negative bacterium *Chromobacterium* subtsugae presents insecticide activity against several orders, highlighting its biocontrol effect on *B. tabaci*, an important pest of *Capsicum*. Its broad-spectrum activity in insect control is related to multiple modes of action that probably involve different chemical compounds [57]. This bacterium could also control populations of *F. occidentalis* [58]. Recently, other bacteria with entomopathogenic action have been studied, such as *Serratia marcescens*. This bacterium as well as *B. cereus*, *B. pumilus* and some entomopathogenic fungi produce the enzyme chitinase, which has an important action in the pathogenicity of insects. Studies carried out by Aggarwal et al. [59] showed that *S. marcescens* (SEN strain) has an important insecticide activity against all stages of development of *S. litura* larvae; the ingestion of this bacterium produced larvae and pupae with less weight, as well as negatively affecting the fertility of the eggs.

Bacteria have also been evaluated for the control of mites. Aksoy et al. [60] investigated the effect of the biocontrol *Pseudomonas putida* (Biotype B) on *T. urticae* at the laboratory level. The bacterium was applied by spraying and dipping newly emerged females, achieving a decrease in the total number of eggs and hatching, compared to their respective controls. Spraying was the most effective method of application. On the other hand, *Burkholderia* spp. (strain A396), after a heat treatment, showed oral toxicity and contact effects against *S. exigua* and *T. urticae*;

its insecticidal action could be related to the production of different bacterial metabolites [61]. There is another group of entomopathogenic bacteria that includes the endosymbionts of the entomopathogenic nematodes, of which the genera *Xenorhabdus* and *Photorhabdus* are especially important [62]. These bacteria can produce metabolites that allow them to colonise and reproduce inside insects.

Although entomopathogenic bacteria have great potential to control insects affecting the cultivation of *C. annuum*, studies with this species are still scarce.

2.2 Disease control with microorganisms

One of the main problems that negatively affect the cultivation of *Capsicum* is the presence of a phytopathogenic complex formed by the genus *Fusarium*, together with others, such as *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia* spp., which are agents that cause seedlings to fall. Together, these can cause losses of between 60 and 100% in production [63]. Nonetheless, conventional control methods have been insufficient and difficult to apply, in addition to the problems associated with soil contamination, phytotoxicity and resistance production in the target pathogen [64]. Considering the above, the use of beneficial microorganisms is presented as an alternative for the control of these pathogens, not only for soil-borne diseases but also for pathogens that can proliferate in the aerial part of the plant and can also compensate for the negative environmental impacts caused by chemical pesticides [65]. Studies on the biological control of diseases through the use of microorganisms have recently increased, and the role of the interactions of beneficial microorganisms with the plant and/or the pathogen is receiving more and more attention, not only because of their antagonistic action but also because of their potential to promote plant growth, thus contributing to a more sustainable production over time [66].

Among the most commonly used microorganisms for the control of pathogens are fungi of the genera *Trichoderma*, *Clonostachys*, and *Penicillium*; and the bacteria *Pseudomonas* and *Bacillus*. In relation to their antagonistic activity, these present diverse mechanisms of action that can act independently or jointly. If the antagonist can express different mechanisms of action, the likelihood of developing resistance in the pathogen is reduced. The mechanisms of action described include competition for nutrients and space, antibiosis, parasitism (considered as an antagonistic symbiosis between organisms) and activation of systemic resistance. The use of these control agents is projected as a more efficient control alternative; however, there are several aspects that should be studied so that they can express their full potential, such as the form of application, the combined use of antagonists, formulation and conditions during application [67]. Moreover, the possibility that beneficial microorganisms may persist in the soil could be an important advantage over the use of pesticides. An additional advantage is that if they remain in the rhizosphere they are in the first line of defence against attack by soil pathogens.

2.2.1 Antagonistic fungi

A diverse group of fungi have been shown to antagonise a significant number of pathogens affecting *Capsicum,* such as *Phytophthora* spp., *Colletotrichum* spp., *Rhizoctonia* spp., *Fusarium* spp., and *Botrytis cinerea*, among others [68–72]. One of the most cited examples in the literature are the fungi of the genus *Trichoderma*, which due to their diversity, versatility, adaptability and easy handling have proven to be good candidates for commercial development as a biofungicide. Moreover, these fungi produce three types of propagules, hyphae, chlamydospores and conidia, all of which are active against pathogens in different phases of their life cycle, along with presenting an interesting activity promoting plant growth.

Studies carried out by Ahmed [68] indicate that *T. harzianum* presents an antagonistic effect against the pathogen *P. capsici* because it significantly reduces stem necrosis compared to values obtained in plants inoculated only with the pathogen. Applications of *Trichoderma* spp. in jalapeño peppers were able to decrease the growth rate of *P. capsici* in addition to increasing plant growth [73]. Another soil fungus controlled by *Trichoderma* is *Fusarium* spp., which was confirmed in studies carried out by Sinha et al. [74] on chilli peppers under greenhouse conditions, which showed that strains of *T. viride* and *T. harzianum* reduced the incidence of *F. oxysporum* f. sp. *capsici* by 83.93–87.5%. *Trichoderma* has also been used to control anthracnose, which is caused by several species of *Colletotrichum*. Applications of strains of *T. harzianum* (BHUF4) and *T. asperellum* (T16A) promote the accumulation of the phenol that stimulates the expression of the defence gene, both of which have proven to induce and acquire systemic resistance, providing a solid protection against *C. truncatum* [72]. Studies carried out by Rahman et al. [71] also determined that *Trichoderma* spp. had a biocontrol effect on *C. capsici* in chilli peppers since they managed to decrease the percentage of infection in fruit, although the strain *T. harzianum* (IMI-392433) presented the best antagonistic effect against this pathogen, all the strains (*T. harzianum, T. viride* and *T. pseudokoningii*) presented a promoter effect in plant growth and higher fruit yield.

The *Clonostachys* fungus also presents important characteristics in the biological control of pathogenic fungi. Nobre et al. [69] determined that strains of *C. rosea* were able to suppress more than 80% of the sporulation of *B. cinerea* in chilli peppers and tomatoes. Another fungus studied for disease control in *Capsicum* is *Xylaria poitei*. The antagonistic activity *in vivo* and the protection that *Xylaria poitei* provided chilli pepper seedlings against the pathogen *P. capsici* were evaluated. This antagonist allowed the survival of 58.3% of the seedlings, while seedlings inoculated only with the pathogen showed 100% mortality [75].

2.2.2 Antagonistic bacteria

Several species of *Bacillus* are known for their antagonistic capacity against pathogens that cause disease in plants; many of these are also considered because they could have a direct action in disease control as a result of the production of metabolites or indirectly by promoting growth. These bacteria act not only on soilborne diseases but also on leaf and fruit pathogens [76–78]. Diverse studies have provided evidence that plant growth-promoting bacteria have the ability to activate systemic resistance induced by the activation of physical or chemical defences of the host plant by an inducing agent [79].

Studies carried out by Yu et al. [77] determined that applications of *B. subtilis* (CAS15 strain) significantly suppressed spore germination of *F. oxysporum* f. sp. *capsici* by 8–64%. In addition, the incidence of the disease was much lower in plants treated with the bacteria compared to the control (12.5–56.9%); these results are attributed to the induction of systemic resistance. This bacterium has also been used in *Capsicum* for the control of the anthracnose disease in chilli plants caused by *Colletotrichum gloeosporioides* (OGC1 strain). Seeds treated with the bacterium showed a 65% decrease in disease incidence compared to seeds treated with the pathogen alone [78]. Some *Bacillus* species have also been evaluated in the control of diseases caused by pathogenic bacteria, such as pepper bacterial wilt caused by *Ralstonia solanacearum*, an important *Capsicum* disease [79]. The application of *B. amyloliquefaciens* (strain Bg-C31) and its metabolites significantly reduced the percentage of plants affected by *R. solanacearum* at the field level and in pepper pots [80].

Pseudomonas is another group of bacteria used for disease control in *Capsicum*; among these, the most reported is *P. fluorescens* which has been shown

to have a biocontrol effect on pathogens and also some phytoparasitic nematodes [81, 82]. Basu [83] evaluated the effect of *P. fluorescens* on *Ralstonia solanacearum* in chilli pepper plants and determined that combined applications of the antagonist to the seed and the soil resulted in the lowest percentages of disease incidence (0.83–10.82%), compared to the control which was inoculated only with the pathogen (9.99–44.96%).

Many bacteria have demonstrated an important potential for the control of diseases caused by fungi and bacteria; nevertheless, many of them have been evaluated under controlled conditions, which makes it necessary to concentrate efforts to increase the number of field level trials, and thus determine the real potential of being incorporated into the cultivation of *Capsicum*.

2.3 Nematodes with microorganisms

Infestation of peppers by nematodes, such as *Meloidogyne* spp., is one of the major problems for pepper production worldwide [84]. They attack the roots, leading to root system dysfunction, reduced rooting volume, inefficient use of water and nutrients, reduced crop and plant growth and yield [85]. Another nematode that causes damage in *Capsicum* is the false root-knot nematode, *Nacobbus aberrans*, which is associated with *F. oxysporum* and *P. capsici*, and can cause up to 100% crop loss [86]. For its control, chemical nematicides are applied; however, in the cultivation of *C. annuum*, its use has been restricted due to its toxicity levels, residual elements in the environment and the selection of resistant nematode populations [87].

An alternative for the biological control of phytoparasitic nematodes is nematophagous fungi. These ubiquitous microorganisms are capable of modifying their saprophytic behaviour and can feed on nematodes in unfavourable nutritional conditions. They are natural enemies of nematodes and have developed highly sophisticated infection strategies [88]. They are classified into three groups according to their predation characteristics. First are the nematode traps or predators (their modified hyphae form traps and through a chemical and mechanical process they digest the nematode), second are the opportunists or ovicides (they form traps to catch eggs, cysts and females) and finally the endoparasites (forced parasites of the nematodes that use their spores as structures of infection, which can adhere to the cuticle of the nematode or be ingested) [89, 90].

Paecilomyces lilacinus have a negative effect on the root nodulation produced by *M. incognita* in peppers [91]. Another trial used *B. bassiana* strains and determined that applications of this fungus to the substrate in pepper plants decrease the number of root nodules caused by *M. incognita* [92]. The combination of nematophagous fungi has also been evaluated on *M. incognita*. Studies carried out by Requena Candela [93] demonstrated the antagonistic effect of the combination of *P. lilacinus* and *T. harzianum* by reaching decreases in the incidence of nematodes close to 70%. Pérez-Rodríguez et al. [94] evaluated the nematophagous action of the fungus *Pochonia chlamydosporia* for the control of *N. aberrans* nematodes in broad chilli peppers and determined that the combination of vermicompost with the nematophagous achieves a reduction in the number of juvenile nematodes, the number of eggs and females per gram of roots, as well as increasing the dry matter of the plants.

2.4 Endophytic microorganisms for the control of insect pests and plant diseases

Endophytes are defined as microorganisms that spend most or all of their life cycle colonising host plant tissues without causing obvious damage [95].

Endophytes are associated with most plant species, are naturally found in the ecosystem and are considered an extremely important partner for plants [96, 97]. These microorganisms have been of interest for study during the last few years due to the beneficial characteristics they are able to bestow onto their hosts [98], among which are the promotion of plant growth, inhibition of pathogenic organisms, control of insect pests, removal of contaminants from the soil and increased tolerance to extreme conditions of temperature, water availability and salinity [99–101]. Consequently, they have an important future in agrifood production.

Currently, several studies have shown that endophytic fungi can protect host plants against pathogens and herbivores [102, 103]. Some endophytes, when artificially inoculated, can confer the beneficial characteristics mentioned above to their hosts, as they can influence key aspects of physiology. The host plant receives multiple benefits from its interaction with the endophyte in exchange for carbon-based resources [104]. The type of interaction used by endophyte biological controllers is not yet clear [105], but it is believed that this type of interaction is established through a process of co-evolution [106].

These fungi generate interspecific interactions and protection against the incidence of pathogens produced by direct mechanisms such as competition, parasitism, antibiosis and indirect mechanisms such as induction of resistance [97, 104]. The activation of systemic resistance in the plant may be due to the presence of endophytic fungi, a mechanism that has already been shown to be effective against other fungal pathogens [107]. On the other hand, among the most important reported mechanisms for pest control are parasitism by ingestion [108], antagonism by the action of metabolites [109, 110], systemic resistance [111] and a tritrophic action [112].

Works carried out by Barra-Bucarei et al. [113] present the first report of the endophyte *B. bassiana* with antagonistic action against *Botrytis cinerea* in chilli pepper plants. Five native strains were evaluated, and the percentage of leaf area affected by the pathogen (PSAP) was determined. Plants inoculated with endophytes showed 2 to 18% of PSAP compared with plants treated only with the pathogen, which exhibited early symptoms of the disease with a PSAP of 63%. Another study conducted by the same authors (unpublished data) demonstrated that the same strains are endophytic (**Figure 1**) and reduced the symptoms caused

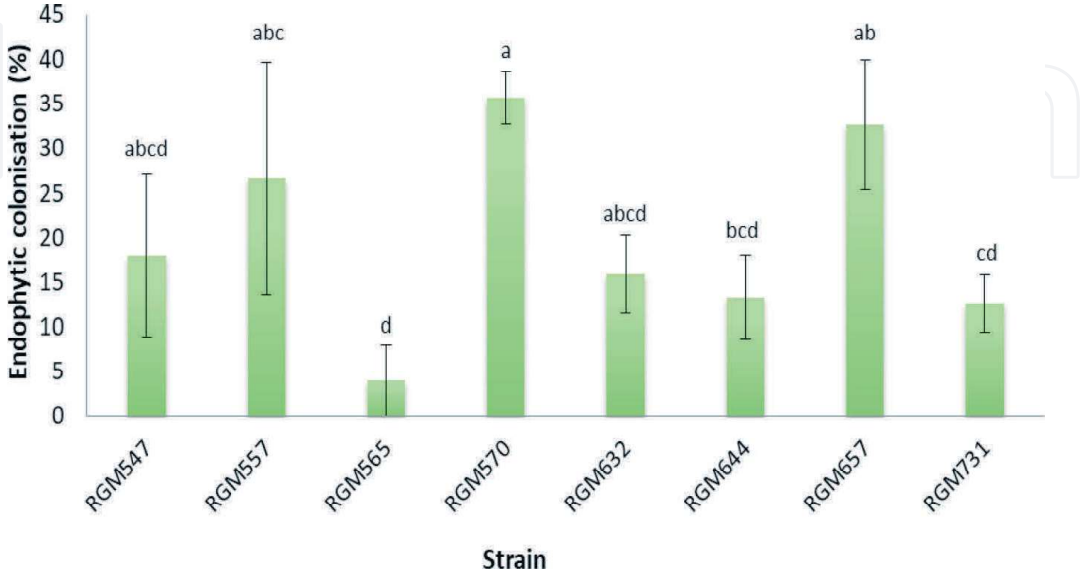


Figure 1.
Endophytic colonisation of B. bassiana *strains in chilli pepper plants (leaves, stems and roots), 30 days after inoculation (n = 5). Data represent the mean standard error. Different letters over the bars represent significant differences among the treatments according to the Fisher's LSD test (p < 0.05).*

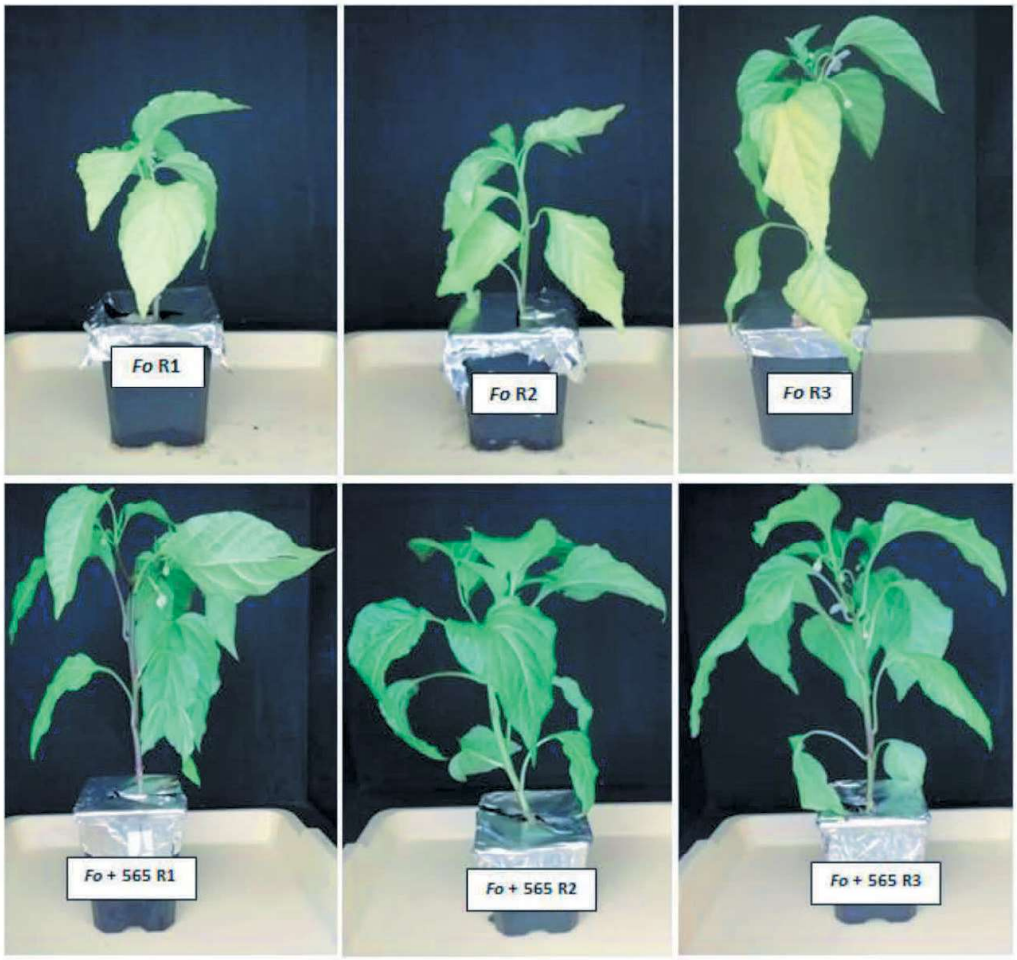


Figure 2.
In vivo *antagonism of* B. bassiana *(565) against* F. oxysporum *(*Fo*). Plants inoculated with* F. oxysporum *(*Fo*) showed chlorosis and plants inoculated with* B. bassiana *RGM565 +* Fo *showed no symptoms, 45 days after inoculation (n = 10).*

by *F. oxysporum* in chilli pepper plants, increasing dry matter and decreasing the level of leaf chlorosis in adult plants, making the plants more resistant to the attack of this pathogen (**Figure 2**). These studies coincide with research conducted by Jaber and Alananbeh [103], in pepper plants which provided evidence that commercial strains of *B. bassiana* (Naturalis) and *M. brunneum* (Bipesco5) can inhibit the growth of several species of *Fusarium* (*F. oxysporum*, *F. culmorum* and *F. moniliforme*) *in vitro* and in potting trials.

3. Conclusion

With the increase of the world population, it is urgent for the development of sustainable strategies to improve food availability. *Capsicum annuum* is an excellent source of natural health-related compounds, such as micronutrients and antioxidants; its fruits have been used for fresh and cooked consumption. The crop of this vegetable can be negatively affected by biotic factors, provoking decreased yields. In this chapter reviewed, we have provided evidence of the potential that microorganisms present for the control of insect pests, plant diseases, and nematodes in the *Capsicum* crop. There are a significant number of investigations carried out under laboratory conditions with good biocontrol results; however, it is necessary to increase the number of field studies and application methods, since environmental

conditions could condition the behaviour of microorganisms and not reach the same levels of efficacy obtained under controlled conditions. In addition, it is necessary to study the ecological behaviour of beneficial microorganisms and their interaction with others. The biocontrol microbial agents can fulfil diverse functions in plants and give an eco-friendly approach to promoting sustainable agriculture.

Conflict of interest

The authors declare no conflict of interest.

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