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Athelia (*Sclerotium*) *rolfsii* in *Allium sativum*: potential biocontrol agents and their effects on plant metabolites

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ABSTRACT

Garlic (*Allium sativum* L.) plays an important role in popular culture due to its dietary and medicinal uses. It is also used to produce a wide range of pharmacologically interesting molecules. Several pathogens affect garlic plants, especially *Athelia* (*Sclerotium*) *rolfsii*, a fungus that is widespread and causes large economic losses. It causes direct damage to crops and leads to plant stress, which induces secondary metabolite production in plants. The use of microorganisms as biocontrol agents may induce the production of beneficial metabolites in plants that will protect it and promote resistance to pathogen attack. In addition to suppressing disease, biological control agents may have elicitor effects that could induce an increase in the production of useful bioactive secondary metabolites in plants, some of which may be of pharmacological interest. Therefore, the search for new biological control agents should also consider their potential as elicitor agents. This paper presents an analysis of the biological control of *Athelia* (*Sclerotium*) *rolfsii* by antagonistic microrganisms, the potential of yeasts and bacteria of the genus *Bacillus* for the biocontrol of phytopathogens, microrganisms influence in nutritional and bioactive compounds content of interest to the pharmaceutical industry.

Key words: biological control, phytopathogen, yeast, *Bacillus* spp., elicitation, garlic.

INTRODUCTION

Garlic (*Allium sativum* L.) is a member of the Alliaceae family. It is cultivated all over world and demand for it is high due to its medicinal properties. Brazilian production reached 126.157 tons in 2017, of which, 84.131 tons were exported (Agrianual 2018). Garlic contains high levels of starch and aromatic compounds, which means that it has a high flavor and medicinal value. It is also used as a herbal medicine due to its diverse pharmacological properties (Souza and Macêdo 2009, Resende et al. 2016)

The pharmacological properties of garlic are related to the presence of secondary metabolites, such as organosulfur compounds, which are responsible for its strong, characteristic odor. These compounds protect the plant against insects and

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phytopathogens (Kusano et al. 2016). Allicin is the main component responsible for the antibiotic, antiviral, and antifungal characteristics of the plant (Curtis et al. 2004, Ota et al. 2010, Borlinghaus et al. 2014). Phenolic compound contents are also high in garlic, especially the flavonoids: quercetin, apigenin, and myricetin (Lanzotti 2006), which play a role in plant resistance to pathogens (Lattanzio et al. 2006).

Garlic production can be affected by several phytopathogens, especially *Athelia* (*Sclerotium*) *rolfsii*, which causes white rot. In particular *A. rolfsii* causes bulb rotting, which means that it is an important disease that should be investigated further because it causes substantial economic production losses around the world. Garlic and onion losses can reach 65% in climate temperate countries, such as the United Kingdom and Canada, whereas the losses can be up to 100% in countries with a tropical climate, such as Brazil and Mexico (Kwon 2010, El-Nagar et al. 2013, Domingos et al. 2015). Therefore, there is a need to identify new methods that can be used to control phytopathogens, preferably ones that reduce the need to use pesticides and emphasize the use of biological control and resistance induction as efficient complementary measures.

Phytopathogen attacks influence secondary metabolite production in plants. The use of yeasts and bacteria to induce resistance also shares this principle because the plant starts to produce secondary metabolites that will protect it from pathogen attack. Therefore, we need to know about any alterations in the plant secondary metabolite contents and whether these are of biological and economic interest before yeasts and *Bacillus* spp. are used to biologically control *A. rolfsii* in garlic (*Allium sativum* L.). The aim of this study was to investigate whether yeast strains and *Bacillus* spp. could biologically control *A. rolfsii* in garlic when applied on a commercial scale and investigate their effects on plant metabolites.

GARLIC PRODUCTION AND *Athelia* (*Sclerotium*) *rolfsii*

Garlic (*Allium sativum* L.) is a species that originally came from Asia and belongs to the Alliaceae family (Souza and Macêdo 2009, Resende et al. 2016). Brazil is a major importer of garlic "in natura", and imports reached about 17,000 tons in 2016, corresponding to $26.9 million. However, its production in Brazil during the same year was approximately 130,407 tons over 11,334 ha. The average yield was 11.5 t/ha, and 274 kg (valued at $829,000) were exported between January and August, 2016 (Morozini et al. 2005, Fagundes 2016, IBGE 2016, Marques 2017). Furthermore, Brazil exported 7.0 tons of garlic powder, valued at $18.900, between January and August, 2016 (Fagundes 2016).

Garlic is a medicinal plant that has been used since ancient times to treat various diseases. Its main biological activities are fighting infections, lowering blood pressure and cholesterol, and cancer prevention. It is also has antibiotic, antifungal, antiviral, anthelmintic, light antihypertensive, antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, and wound healing properties (Gómez and Sánchez-Muniz 2000, Yeh and Liu 2001, Corzo-Martínez et al. 2007, Londhe et al. 2011, Borlinghaus et al. 2014).

The medicinal, dietary, and pest and disease control characteristics (Slusarenko et al. 2008) shown by garlic are due to its phytochemical composition. In particular, allicin and thiosulfates are responsible for a large number of the garlic medicinal properties, such as its antibiotic, antiviral and anti-cancer, antioxidant, hypotensive, reduction of endogenous cholesterol synthesis, and inhibition of platelet aggregation properties (Borlinghaus et al. 2014). Aliin, which has hypotensive and hypoglycemic properties (Gómez and Sánchez-Muniz 2000); ajoene, which is used to prevent clots, and has anti-inflammatory, vasodilator, hypotensive, and antibiotic effects (Corzo-Martínez et al. 2007); S-allyl cysteine, which is involved in the hypocholesterolemic properties of garlic (Yeh and Liu 2001); adenosine, which is a vasodilator, and has hypotensive and myorelaxant properties (Gómez and Sánchez-Muniz 2000); fructan, which is involved in the cardioprotective activity shown by garlic (Gómez and Sánchez-Muniz 2000); inulin, a reserve polysaccharide that acts as dietary fiber and a prebiotic (Dalonso et al. 2009); vitamins A, B, and C; proteins; and minerals, etc. (Ota et al. 2010) are mainly found in the garlic cloves. It is the part of the plant that is most often used to extract compounds because it is where the active constituent levels are greatest (Chagas et al. 2012).

Phytopathogen occurrence can reduce product quality. *A. rolfsii* is mainly a soil borne pathogen. It causes diseases in more than 500 species of mono and eudycotyledons, and is represented in 100 families (Kwon 2010, El-Nagar et al. 2013). It is polyphagous and can lead to rots in the roots, young plants, and seeds; and damages seedlings, leaves, and fruits (Faria et al. 2009). It is a fast-growing phytopathogen that produces persistent sclerotia, which makes it a difficult-to-control fungus that has been responsible large economic losses in the past (Ali et al. 2017). El-Nagar et al. (2013) observed virulence in three isolates of *A. rolfsii*, which had infection rates that varied from 56.7 to 73.3% in pre- and post-emergence garlic seeds. In the field, its incidence on garlic and onion crops seems to be highly correlated with sclerotia density in the soil during planting. The incidence of white rot caused by *Sclerotium cepivorum* is also related to sclerotia density (Domingos et al. 2015).

Product quality has an extremely important effect on garlic commercialization. Therefore, the control of pests and diseases throughout the production process is fundamental because they can reduce both garlic yield and quality (Moura et al. 2013).

BIOLOGICAL CONTROL OF *Athelia* (*Sclerotium*) *rolfsii*

Athelia (*Sclerotium*) *rolfsii* cannot be controlled by a single cultural or chemical method, such as the use of fungicides or cultural rotation with resistant plants (de Sousa and Blum 2013). Therefore, integrated pest management is important and one complimentary tool that can be used is biological control. Its advantages are that it is a safe, efficient, and economically viable method that causes less pollution than agrochemicals. Furthermore, it can be used in organic agriculture systems (Bettiol and Morandi 2009).

Isaias et al. (2014) verified the antagonistic effect exerted by fungi of the genus *Trichoderma* against *A. rolfsii*. They investigated this antagonism using a paired cultures test and by measuring the reduction in mycelial growth due to volatile, non-volatile and non-volatile thermostable metabolites. The results showed that the secondary metabolites produced by the antagonists in the genus *Trichoderma* had a significant effect on *A. rolfsii* growth (Table I).

An important action mechanism underlying the antagonistic microorganism effects on *A. rolfsii* is the production of hydrolytic enzymes that degrade phytopathogenic cells and, consequently, suppress their development through mycoparasitism of sclerodes and hyphae. This mechanism has been shown to be affective against actinobacteria in the genus *Streptomyces* (Li et al. 2017, Thampi and Bhai 2017). Mycoparasitism is one of the main mechanisms used by *Trichoderma* isolates to control *A. rolfsii* because they can produce chitinase and β-1,3-glucanase, which are involved in mycoparasitism (Hirpara et al. 2017, Rao et al. 2015). Bacteria in the genera *Pseudomonas* and *Bacillus* also produce chitinase, among other lytic enzymes. These enzymes can break down polymeric compounds, such as chitin, proteins, cellulose, and hemicellulose. Their main effect is to degrade the cell wall of the pathogen (Abou-Aly et al. 2015, Saritha et al. 2015, Janahiraman et al. 2016).

Antibiosis is another action mechanism that been shown to control phytopathogens. *Bacillus amyloliquefaciens* has biosurfactant activity and reduces the surface tension associated with the production of the lipopeptide iturin A. It shows 77.7% inhibition of the phytopathogen and has potential antifungal activity (Kumar et al. 2015). *Bacillus subtilis* also seems to use this mechanism against *Rhizoctonia bataticola* and *R. solani*. Electron microscopy images show that it reduces fungal growth and alters the morphology of the hyphae and sclerotia (Mnif et al. 2016).

The *Agrobacterium* and *Kluyvera* genotypes have been shown to promote the biocontrol of *A. rolfsii* by competing for iron. This limits various activities in the phytopathogen isolates and the nutritional composition of the growth medium (Pelzer et al. 2011). *Bacillus* isolates have also been shown to efficiently control *A. rolfsii* using antibiosis. They release extracellular compounds into the rhizosphere, and produce antimicrobial substances and plant hormones. These suppress plant diseases, promote growth, and improve plant nutrient absorption from the soil (Suneeta et al. 2016).

Hydrogen cyanide (cyanogenesis) and siderophore production may also inhibit pathogens. These methods are thought to be used by bacteria in the genera *Pseudomonas* and *Bacillus* to control *A. rolfsii*. Furthermore, they are organic compounds that could potentially replace pesticides (Abou-Aly et al. 2015, Kotasthane et al. 2017).

Mycorrhiza associated bacteria (MAB) help promote plant growth through the production of known and unknown metabolites, solubilization of nutrients in the soil, and suppression of pathogens. A previous study showed that *Pseudomonas putida*, in association with *Glomus mosseae*, could suppress *A. rolfsii*, using several potential control mechanisms (Saritha et al. 2015) (Table I). Saritha et al. (2015) investigated whether MABs could potentially be used for biocontrol and to manage soil-transmitted phytopathogens under field conditions.

The biocontrol of *A. rolfsii* has been studied using several microorganisms and crop types that are attacked by this pathogen, such as fruit, leguminous, tuberous, and herbaceous crops (Table I). The fungi and bacteria that have been tested include fungi in the genus *Trichoderma* (de Sousa and Blum 2013, Isaias et al. 2014, Rao et al. 2015, Dania et al. 2016, Hirpara et al. 2016, 2017, Pacheco et al. 2016, Islam et al. 2017), *Pseudomonas* bacteria (Saritha et al. 2015, Vaja et al. 2016, Kotasthane et al. 2017), and bacteria in the genus *Bacillus* (Abou-Aly et al. 2015, Kumar et al. 2015, Suneeta et al. 2016, Figueredo et al. 2017).

Further studies on biologically controlling *A. rolfsii* infection of *Allium sativum* are necessary because there have been few previous studies on this subject and they have only investigated *Trichoderma* (Table I). Yeasts and *Bacillus* spp. may be highly suitable biological control agents because *Bacillus* spp. have already been used to successfully control *A. rolfsii* (Table I) in other plants. Furthermore, yeasts have been shown to biologically control other phytopathogens using several different action mechanisms. However, the use of certain microorganisms for biological control could be restricted because the potentially useful microorganisms might not be able to adapt to different environmental and cultivation conditions. Selection pressure may also reduce their effectiveness. For these reasons, interest in identifying new alternatives for the biological control of *A. rolfsii* in *Allium sativum* is increasing. Future research should expand the number of biocontrol options and reduce the potential breakdown of existing control methods.

TABLE I
Antagonistic microrganisms in *Athelia* (*Sclerotium*) *rolfsii* control.

Culture	Antagonist	Action Mechanism	Reference
In vitro	*Trichoderma virens*, *T. koningii*, *T. viride* and *T. harzianum*	Mycoparasitism	Hirpara et al. (2017)
Solanum lycopersicum	*Trichoderma harzianum*, *T. virens* and *T. asperellum*	-	Islam et al. (2017)
In vitro	*Trichoderma virens* and *T. koningii*	Mycoparasitism and antibiosis	Hirpara et al. (2016)
Phaseolus vulgaris	*Trichoderma asperellum*, *T. harzianum*, *T. longibrachiatum* and *T. reesei*	-	Pacheco et al. (2016)
Amorphophallus paeonifolius	*Trichoderma harzianum* and *T. asperellum*	Mycoparasitism and antibiosis (diffusible metabolites)	John et al. (2015)
Nicotiana tabacum	*Trichoderma* Isolates	Mycoparasitism, antibiosis (volatile and diffusible metabolites), cyanogenesis and siderophores production	Rao et al. (2015)
Allium sativum	*Trichoderma harzianum*	-	de Sousa and Blum (2013)
In vitro	*Trichoderma*	Antibiosis (volatile and diffusible metabolites)	Isaias et al. (2014)
Dioscorea rotundata, *D. cayenensis*, *D. alata* and *D. dumetorum*	*Trichoderma asperellum*, *T. longibrachiatum*, *Bacillus subtilis* and *Pseudomonas fluorescens*	-	Dania et al. (2016)
Cicer arietinum	*Pseudomonas* Isolates	Siderophores and cyanogenesis production	Kotasthane et al. (2017)
In vitro	*Pseudomonas aeruginosa*	Siderophores production	Vaja et al. (2016)
In vitro	*Pseudomonas putida* associated to *Glomus mosseae* (bacterium associated with mycorrhiza)	Siderophores production, ammonia, cyanogenesis, protease, chitinase, urease and ACC deaminase, antibiosis	Saritha et al. (2015)
In vitro	*Pseudomonas fluorescens* and *Bacillus subtilis*	Activity chitinase, production of siderophores, cyanogenesis, antibiosis (volatile metabolites)	Abou-Aly et al. (2015)
Lycopersicon esculentum	*Delftia lacustris*, *Bacillus subtilis* and *B. cereus*	Increased production of β-1,3-glucanase, chitinase, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and catalase and induction of systemic resistance	Janahiraman et al. (2016)
Gerbera jamesonii (gerbera)	*Bacillus* Isolates	Antibiosis	Suneeta et al. (2016)
In vitro	*Bacillus amyloliquefaciens*	Biosurfactants and iturin A production	Kumar et al. (2015)
Arachis hypogaea (peanut)	*Bacillus* sp.	Systemic resistance induction (SRI)	Figueredo et al. (2017)

TABLE I (continuation)

Culture	Antagonist	Action Mechanism	Reference
In vitro	*Streptomyces globisporus*, *S. flavotricini*, *S. pactum* and *S. senoensis*	Antibiosis and mycoparasitism	Li et al. (2017)
Piper nigrum (black pepper)	*Streptomyces* sp.	Mycoparasitism, production of indoleacetic acid (IAA) and siderophores	Thampi and Bhai (2017)
In vitro	Rhizobacteria of genus *Agrobacterium* and *Kluyvera*	Competition for iron and antibiosis	Pelzer et al. (2011)

BIOLOGICAL CONTROL OF PHYTOPATHOGENS BY YEAST AND *Bacillus* spp.

Antagonistic yeasts are able colonize rapidly and grow in superficial lesions. Their faster growth rate means that they can inhibit the development of phytopathogenic fungi by successfully competing for nutrients and space. Yeasts are unicellular organisms, which mean they have a faster growth rate than multicellular phytopathogenic fungi, such as molds (Úbeda et al. 2014). *In vivo* tests have shown that *Pichia membraneefaciens* and *Kloeckera apiculata* significantly reduced (76.0% and 65.8%, respectively) the incidence of *Monilinia fructicola* rot in plums by competing with the phytopathogen for nutrients and space. They may also have produced volatile organic compounds with antifungal action (antibiosis) that caused mycoparasitism through the production of hydrolytic enzymes, such as chitinase and β-1,3-glucanase (Zhang et al. 2017).

Yeasts can also induce resistance in plants. Suspensions of *Candida oleophila* cells in grapefruit peel tissue increased ethylene biosynthesis and phenylalanine ammonia lyase activity. *Candida oleophila* inoculation also led to the accumulation of phytoalexins and increased levels of chitinase and protein β-1,3-endoglucanase (Droby et al. 2002).

Sun et al. (2018) applied preparations containing cell walls from the yeast *Rhodosporidium paludigenum* to pear fruits to investigate whether it had any activity against blue mold (*Penicillium expansum*). There was a reduction in disease incidence and lesion diameter at all tested concentrations. They also observed resistance induction in the fruits because *in vivo* spore germination decreased when the time difference between treatment with the cell wall preparation and inoculation with the phytopathogen was extended. For example there was 70% spore germination when inoculation took place at 2 hours after the cell wall treatment, but less than 10% spore germination when the inoculation time was 24 hours after the cell wall treatment. Induction of resistance caused a significant increase in the activity of defense-related enzymes (β-1,3-glucanase and chitinase) and the expression of specific protein genes related to pathogenesis (PR1-like, endoGLU9, endoCHI-like, and PR4).

The induction of a defense response in tomato by *Aureobasidium pullulans* increased β-1,3-glucanase activity and led to the production of bioactive volatile and non-volatile metabolites that were capable of inhibiting colony growth and causing morphological changes to the hyphae of *Phytophthora infestans* (Di Francesco et al. 2017).

Chi et al. (2015) reported that the yeast *Pichia kudriavzevii* formed a biofilm. Biofilms are morphologically more resistant to high temperatures and oxidative stress, and can be involved in biological control activity. Klein and Kupper (2018) reported a positive correlation between an increase in antagonistic activity and a rise in biofilm production. They emphasized that biofilm formation was an important biological control mechanism because the antagonist rapidly colonized the lesions and the film protected the plant against attack from phytopathogens.

Úbeda et al. (2014) suggested that the species *Pichia kudriavzevii* was a good candidate for the biocontrol and bioremediation of wastewater contaminated by heavy metals because it inhibited the molds: *Phaeomoniella chlamydospora*, *Neofusicoccum parvum*, *Diplodia seriata*, *Phaeoacremonium aleophilum*, and *Aspergillus niger*, and accumulated the highest proportion of the three tested metals: Cr (VI), Pb (II), and Cd (II). Kupper et al. (2013) evaluated whether *Saccharomyces cerevisiae* and *Bacillus subtilis* could be used to control *Penicillium digitatum*. They found that a large number of the bacterial isolates and all the yeast isolates were able to reduce *P. digitatum* mycelial growth. They also found that one of the bacterial isolates could inhibit conidial germination (72%) and that two *S. cerevisiae* isolates produced the best inhibition results (78 and 85.7% inhibition).

Bacillus is a genus of great interest to agriculture because some of its members promote plant growth and have a wide range of antagonistic mechanisms, which means that they are able to circumvent phytopathogen defenses (Lanna Filho et al. 2010). Bacteria in this genus colonize the roots and form microcolonies or biofilms at preferred root exudation sites. They act as rhizospheric agents that are capable of degrading substrates derived from fauna, flora, and compounds of organic origin. Furthermore, they promote nitrogen fixation and phosphate solubilization, which increases plant growth (Chen et al. 2007, Choudhary and Johri 2009, Kumar et al. 2012, Corrales-Ramírez et al. 2017). They also produce antipathogenic substances and/or induce resistance mechanisms in the plant (Chaurasia et al. 2005, Choudhary and Johri 2009, Huang et al. 2012, Kumar et al. 2012, Gond et al. 2015). In addition to these two biocontrol benefits, the genus *Bacillus* is also physiologically diverse due to the formation of endospores that allow it to survive for long periods in many different environments (Lanna Filho et al. 2010, Corrales -Ramírez et al. 2017).

Antagonistic microorganisms are already commercially available, such as the yeasts *Candida oleophila* and *Cryptococcus albidus*, and two strains of the bacterium *Pseudomonas syringae*, which are sold under the trade names Aspire (Ecogen Inc., Langhorn, PA, USA), YieldPlus (Anchor Yeast, Cape City, South Africa), and BIOSAVE-110 and BIOSAVE-111 (EcoScience, Orlando, FL, USA), respectively (Droby et al. 2002).

There are also several products on the market that contain commercial strains of bacteria from the genus *Bacillus* and they have been used to biologically control phytopathogens. For example there are Kodiak® and YieldShield® developed by the company Gustafson that contain strains of *Bacillus subtilis* and *B. pumilus*, respectively. Gustafson also developed BioYield®, which is a blend of *B. subtilis* and *B. amyloliquefaciens* strains. The products Rhapsody®, Sonata®, and Ballad® contain different strains of *B. pumilus* and are made by AgraQuest. There are also other products available that contain *B. subtilis*, such as Subtilex® (Becker Underwood), Serenade® (AgraQuest), and BioPro® (Andermatt Bioc.) (Bettiol and Morandi 2009).

INFLUENCE OF MICROORGANISMS ON PHYTOCHEMICAL COMPOSITION

One of the strategies used to increase the production of secondary metabolites that are of commercial interest is to create biotic and abiotic stress conditions (Jimenez-Garcia et al. 2013) because the plants then produce several secondary metabolites related to different stress adaptations (Shetty and Wahlqvist

2004). Phytoalexins are secondary metabolites produced by plants in response to stress, attack by microorganisms, and/or elicitor treatments. They usually have an antimicrobial action and could potentially be exploited as nutritional products that can be added to foods to give them antioxidant, anti-inflammatory, hypocholesterolemic, and anticancer activities (Boue et al. 2009).

Phytopathogen attacks cause plant stress and may induce the production of secondary defense metabolites. Pontin et al. (2015) found that garlic produced terpenes. These have antifungal properties and are produced in response to *Sclerotium cepivorum* infection. In addition, elicitor, biotic, or abiotic treatments can also be used. These treatments promote the induction of plant resistance against phytopathogens, which can lead to an increase in the production of certain compounds of interest by the plant (Baenas et al. 2014).

Resveratrol is a polyphenol produced by grapes and other plant species. It has antioxidant and chemopreventive activities against various types of cancer and cardiovascular diseases, which means it could be used to improve human health (Shankar et al. 2007). Resveratrol is a phytoalexin produced by grapes when they are under stress, such as excess UV radiation and phytopathogen attack. Jeandet et al. (1995) observed that the resveratrol content in grapes (*Vitis vinifera*) increased when they were attacked by *Botrytis cinerea* under natural conditions (Table II).

Yeasts are single-celled fungi that are part of the epiphytic, endophytic, and soil microbiota in the soil surrounding plants (Mello et al. 2011). The microbiota also includes bacteria (Bulgarelli et al. 2013). They compete for nutrients with pathogenic fungi, colonize wounds, and induce resistance (Mello et al. 2011). Yeasts, along with *Bacillus* spp. are increasingly being studied as tools that could be used in biological control plans (Punja and Utkhede 2003, Abraham et al. 2010, Janisiewicz et al. 2010, Machado and Bettiol 2010, Vargas et al. 2012, Kupper et al. 2013) and to induce plant resistance (Araujo and Menezes 2009, Zanardo et al. 2009, Bernardes et al. 2010). However, it is important to note that the use of yeasts and bacteria from the genus *Bacillus* to biologically control phytopathogens and/or induce plant resistance to phytopathogens can promote changes in their phytochemical composition.

Some studies have jointly cultivated plants with yeasts *in vitro* to increase the production of secondary metabolites, improve the production of phenolic compounds in *Malus domestica* and *Curcuma mangga* (Dias et al. 2016), and to increase the production of nicotine and serotonin in *Nicotiana rustica* (Karuppusamy 2009) (Table II).

Baenas et al. (2014) reported that elicitor treatments promoted defense responses in plants and may induce the synthesis of phytochemical compounds through primary metabolism and secondary metabolism. A yeast extract was included among the elicitor treatments because it has several components, such as chitin, N-acetylglucosamine, β-glucan, glycopeptide, and ergosterol oligomers, that are capable of eliciting defense responses in plants.

Studies have shown that arbuscular mycorrhizal fungi (AMF) can induce the accumulation of carotenoids, phenolic compounds, anthocyanins, and tocopherol in lettuce (*Lactuca sativa* L.) leaves (Table II). They seem to be a viable and supplementary alternative that can be used to improve the nutritional quality of lettuce (Baslam and Goicoechea 2012, Baslam et al. 2013). The AMF increased plant growth without diluting the plant bioactive compound contents, which indicates that mycorrhizal symbiosis can potentially increase the levels of compounds with antioxidant activity without the need to increase lettuce intake in the diet (Baslam et al. 2013).

Giovannetti et al. (2015) reported similar results for AMF and tomatoes (*Solanum lycopersicum* L.). They reported that the lycopene

TABLE II
Changes in plants bioactive and nutritional compounds by micr④rganisms.

Plant	Eliciting agents	Compounds	Variation	Reference
Malus domestica	Yeast extract with *Venturia inaequalis*	Phenolics compounds	Increase	Dias et al. (2016)
Curcuma mangga	Yeast extract and chitosan	Phenolics compounds	Increase	
Nicotiana rustica	Yeast gene ornithine decarboxylase	Nicotine and serotonin	Increase	Karuppusamy (2009)
Medicago truncatula	Yeast extract	Isoflavone	Increase	Saini et al. (2013)
Glycyrrhiza echinata	Yeast extract	flavanone 2-hydroxylase, isoflavone 2ž-hydroxylase and isoflavone	Increase	
Cucumis sativus	Yeast	N, P, K, Fe, Zn, Cu, Mn	Increase	Shehata et al. (2012)
Allium sativum	*Saccharomyces cerevisiae*	Organosulfur compounds	Increase	Kim et al. (2016)
	Lactobacillus plantarum	Organosulfur compounds	Increase	
	Mimulus pilosus	Organosulfur compounds	Increase	
Vitis vinífera	*Botrytis cinerea*	Resveratrol	Increase	Jeandet et al. (1995)
Lycopersicon esculentum	Polyversum® (*Pythium oligandrum*)	Phenolic compounds and carotenoids	Decrease	Cwalina-Ambroziak and Amarowicz (2012)
L. esculentum and Capsicum annuum	Polyversum® (*Pythium oligandrum*)	Pectin	Increase	
Solanum lycopersicum	*Glomus intraradices* (arbuscular mycorrhizal fungus - AMF)	Lycopene	Increase	Giovannetti et al. (2015)
		Ca, K, P and Zn	Increase	
Lactuca sativa var. Capitata	*Glomus intraradices* and *G. mosseae* (AMF commercial)	Phenolic compounds, anthocyanins and total carotenoids	Increase	Baslam and Goicoechea (2011)
Lactuca sativa var. Capitata	*Glomus intraradices* and *G. mosseae* (AMF commercial) and *G. fasciculatum* (AMF)	Neoxanthin, violaxanthin, anteraxanthin, zeaxanthin, lutein and β-carotene (carotenoids), lactucaxanthin and tocopherol	Increase	Baslam et al. (2013)

content increased by 18.5% compared to the non-inoculated control. Lycopene is a carotenoid and an important metabolite produced by tomato. It has high antioxidant activity and plays an essential role in disease prevention (Shami and Moreira 2004) (Table II). The AMF also increased the plant Ca, K, P, and Zn contents, which improved the nutritional value of the fruits (Table II). These results suggest that AMF could have a large number of potential applications in sustainable food production systems. They could also be used to improve the ecological balance in the plant rhizosphere. This would reduce damage to the environment and could lead to increases in the production of high quality food (Giovannetti et al. 2015).

Kuhn and Pascholati (2010) investigated whether a biotic inducer (*Bacillus cereus*) and an abiotic inducer (acibenzolar-S-methyl) could improve common bean resistance against *Xanthomonas axonopodis* pv. *Phaseoli.* They observed that both inducers protected the plant against the phytopathogen, but the abiotic inducer

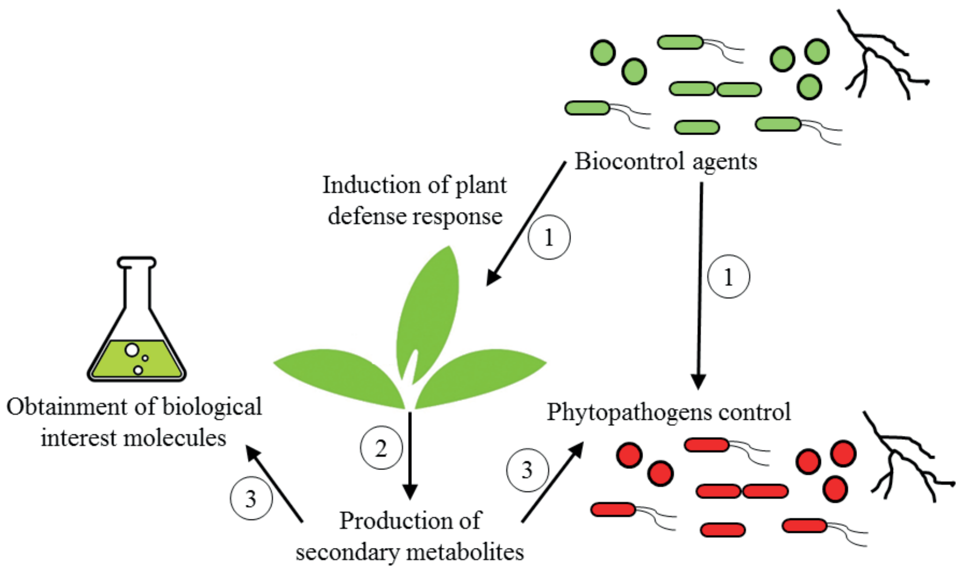

Figure 1 - Phytopathogens controls and obtaining secondary metabolites of interest through defense responses induction in the plant by biocontrol agents.

reduced the phenolic compounds content and the biomass of the plant, whereas the biotic inducer, the bacterium *B. cereus*, did not cause reductions in these parameters compared to the control.

Microorganisms can be used to fortify food. Kim et al. (2016) suggested fermenting garlic with the bacterium *Lactobacillus plantarum*, the filamentous fungus *Mimulus pilosus*, and the yeast *Saccharomyces cerevisiae* in an attempt to increase the contents of organosulfur compounds (Table II). These results suggest that there might be some compositional alterations when yeasts and bacteria are used to control *A. rolfsii* infection of garlic.

PERSPECTIVES ON THE USE OF YEASTS AND *Bacillus* spp. TO CONTROL *Athelia* (*Sclerotium*) *rolfsii* IN *Allium sativum*

Yeasts and *Bacillus* spp. are widely used to control several different phytopathogens. They have produced satisfactory results and commercial products are already available (Droby et al. 2002, Bettiol and Morandi 2009). The control of *A. rolfsii* in garlic is of great interest because this fungus causes considerable yield losses. Bacteria in the genus *Bacillus* are used to control *A. rolfsii* in other crops. Therefore it was important to investigate whether they could biologically control this phytopathogen in garlic. The *Bacillus* spp. and the yeasts use several mechanisms of action to control phytopathogens, which means that they could potentially control *A. rolfsii,* but further research on whether these species are suitable biocontrol agents needs to be undertaken.

Plants are able to defend themselves against potential pathogens using secondary metabolites. Their production is triggered by elicitor molecules or inducing agents. Secondary metabolites are molecules with an extensive range of biological activities, and are often of biological interest. Their resistance mechanisms include competition for nutrients, mycoparasitism, and antibiosis. Biocontrol agents can also act as elicitors. They induce defense responses in the host, such as the production of protective secondary metabolites (Punja and Utkhede 2003). Therefore biocontrol agents have considerable potential as disease control agents and many are also compounds of interest (Figure 1). These elicitor agents induce the production of secondary metabolites that could be potentially very valuable to the pharmaceutical and agricultural industries. Garlic is a plant that produces a wide range of molecules of biological interest that could increase the use of microorganisms with eliciting activity in biological control programs.

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