Türkiye Tarımsal Araştırmalar Dergisi
dergipark.org.tr/tutad
Review
Turk J Agric Res
2022, 9(1): 118-135
© TÜTAD
ISSN: 2148-2306
e-ISSN: 2528-858X
doi: 10.19159/tutad.1004550

Rhizoctonia solani and Its Biological Control

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Received: 04.10.2021 **Accepted:** 31.01.2022

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Abstract: *Rhizoctonia solani* is a soil- and seed-borne fungus belonging to class basidiomycete and capable of living free and as a saprophyte in the soil. It is divided into 14 anastomosis groups (AGs), which are incompatible with each other in terms of reproduction and proliferation. It has the potential to cause disease in various annual and perennial fruits, vegetables, and industrial and cereal crops. Plant diseases are mostly controlled by cultural methods; however, sometimes chemical control is also employed for this purpose. However, these control methods are mostly insufficient due to the soil-borne and saprophytic nature of the fungus. Therefore, researchers are working on other alternative methods. Biological control is one of the important ones among these methods. Antagonists used in biological control directly interact with *R. solani* through hyper-parasitism, antibiosis, or competition. Furthermore, antagonists trigger the defense reaction of host plants, which improves the control over the pathogens. The most important fungi species among these antagonists are; *Trichoderma spp*., *Gliocladium spp*., *Verticillium biguttatum*, and *Stachybotrys elegans*. The most important bacteria species used for the management of *R. solani* are; *Fluoresent pseudomonas*, *Bacillus* spp., *Streptomyces* spp., *Pseudomonas fluorescens*, and *Erwinia spp*. This review is focused on the biology, anastomosis groups, and biological control of *R. solani*. The antagonists used in biological control and studied mechanisms have been explained with examples. The review has been concluded with the latest developments in the literature.

Keywords: *Rhizoctonia solani*, antagonism, biological control, biofomulation, *Trichoderma* sp., *Fluoresent pseudomonas*

1. Introduction

Rhizoctonia possesses a wide host range and is capable of causing diseases in many crop plants. It causes significant economic losses globally (Sneh, 1996). It is sometimes challenging to calculate the yield losses caused by *Rhizoctonia* infection on a single plant since many distinctive symptoms occur below the soil surface. Furthermore, the effect of *Rhizoctonia* infection cannot be visible in some plants until harvest. For example, *Rhizoctonia* affects belowground organs, causes root rot, infects stolon, and forms sclerotia on the tubers in potato crop (Boosalis and Scharen, 1959; Campion et al., 2003; Truter and Wehner, 2004; Aydın et al., 2011; Aydın and Turhan, 2013; Demirer Durak, 2016). It has been reported that yield losses may reach up to 15-50% in potatoes and rice crops (Kumar et al., 2013). This disease is difficult to control due to its soil- and seed-borne nature and easy transport with some plant organs such as tubers. The main reasons for the difficulties faced in the management of this pathogen are its large host range. This pathogen causes huge yield losses in >100 field and horticultural crops every year (Chen et al., 2016). Nevertheless, it can survive for years as mycelium in organic material and as sclerotia in soil (Boosalis and Scharen, 1959). Generally, it causes seed, root, stem, and fruit rot, stem and crown cancer, leaf and scabbard blight, and dwarfism in several plant organs. Furthermore, the pathogen causes devastating diseases in the seedlings of various plant species (Carling et al., 1994). The main annual host species of the pathogen are barley, pepper, wheat, tomatoes, beans, carrots, cloves, cauliflower, chickpeas, potatoes, sugar beets, soybeans, tobacco, rice, etc. Fruit trees such as pistachio and apricot, and forest trees are among the perennial host species of the pathogen (Sneh et al., 1991; Bolton et al., 2010; Taheri and Tarighi, 2011; Hane et al., 2014;

Aydın and Ünal, 2021). There are 14 anastomosis groups of *R. solani*, can cause disease in different hosts (Carling et al., 2002). These groups differ in geographic origin, host sequence, morphology, and pathogenicity.

Various management methods are used to control the diseases caused by *R. solani*. These include chemical, physical, cultural measures, crop rotation, resistant varieties, and biological control (Secor and Gudmestad, 1999; Bains et al., 2002; Tsror, 2010). The inadequacy of these methods for managing the disease when applied alone or associated problems with them has necessitated alternative management methods (Pal and Gardener, 2006). Biological control program is the most important among these alternative methods (Trillas et al., 2006). Biological control is regarded as an eco-friendly alternative method to protect plants from soil-borne pathogens. Numerous studies have reported that some antagonists used in biological control are effective in controlling *R. solani* (Roy, 1989; Brewer and Larkin, 2005; Aydın, 2015)

The information on *R. solani* -an important soil-borne pathogen- has been compiled and progress on its biological control is discussed in this review.

2. *Rhizoctonia solani* as a Pathogen

Rhizoctonia is divided into three groups based on genus and number of nuclei present in their hyphae. They are grouped as multinucleate, binucleate, and uninucleate. Anamorphic *R. solani* belongs to multinucleate group. *Thanatephorus* spp. and *Waitea* spp are telomorphic (Ogoshi, 1996). Multinucleated *R. solani* is the most studied and widely known species among these groups. It belongs to the phylum Basidiomycota and considers as a sterile mycelial fungus, which does not form conidia (Binder et al., 2005). The fungus survives under as adverse conditions as sclerotium and rhizomorph, which are resistant structures. It also reproduces by sexual spores known as basidiospores under some environmental circumstances. Therefore, sexual period is known as *Thanatephorus cucumeris* [FR] Donk (Sneh et al., 1991).

The hyphae are distinctly segmented and change color from light to dark with age. They form a 90-degree angle during branching and narrowing is observed at the base of the hyphae branch (Figure 1a). The fungi colonies grew on the medium in the laboratory form a light brown appearance, which turns to camel hair colored at later stages (Figure 1b).

Rhizoctonia isolates are identified and classified using classical hyphal development, biochemical and molecular methods. Sequence analysis of the internally transcribed spacer (ITS) region of ribosomal DNA genes (rDNA) is considered the most convenient and reliable identification method for the isolates (Sharon et al., 2008).

Rhizoctonia solani hyphae form subgroups that are compatible with each other and fuse upon contact. These groups are called anastomosis (Vilgays and Cubeta, 1994). There are 14 anastomosis groups of *R. solani*, i.e., AG1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and AGB1 (Carling et al., 2002; Sharon et al., 2008). Depending on geographical origin, host sequence, morphology, pathogenicity, and molecular techniques, these subgroups can cause disease in different hosts. The AG-1 among these groups is observed in many countries and different regions. The group AG-1 is sub-divided into IA, AG-1 IB, AG-1 IC, AG-1 ID, AG-1 IE and AG-1 IF (Priyatmojo et al., 2001; González et al., 2012). The AG-1 IA sub-divide

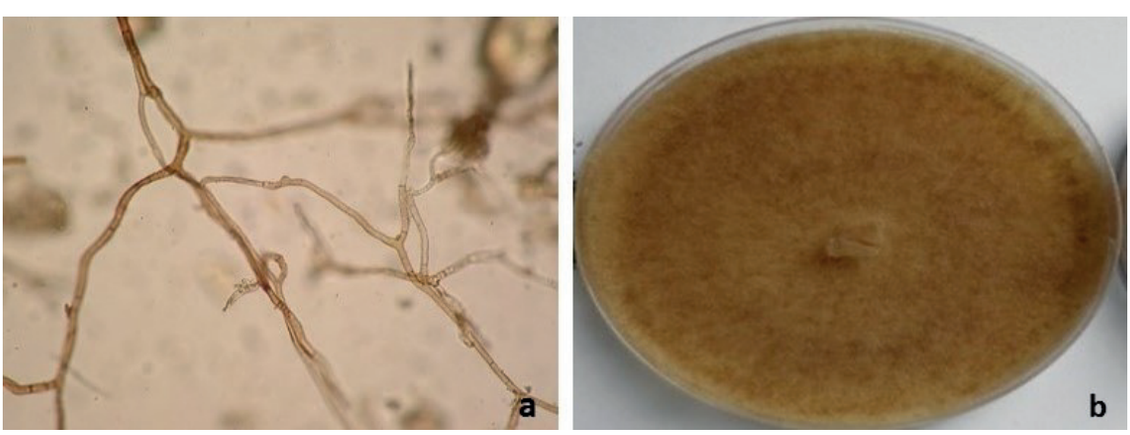

Figure 1. Branching and branching habit of *Rhizoctonia solani* under microscope (a) and fungal colony grown on potato dextrose agar (b)

group is a pathogen that can spread through areal dispersal pathways. It causes leaf blight in many host species, especially sheath blight in rice, and brown spot on grass (Rosewich et al., 1999; Quadros et al., 2019). However, the AG-1-IC subgroup is soil-borne and causes damping-off disease in many host species.

The group AG-2 is also widespread and subdivided into seven groups, i.e., AG-2-1, AG-2-1 Nt, AG-2-2 IIIB, AG-2 IV, 2-2 LP, 2-2 WP and AG-2-3 (Kuramae et al., 2003; Pannecoucque et al., 2008). This group cause damping-off, root and collar rot in many host species. It has been reported that this group causes damping-off disease in the members of Cruciferae family during seedling stage (Kataria and Verma, 1992). Furthermore, it causes leaf blight, root rot and formation of empty areas in lawns (Burpee and Martin, 1992). In a study conducted in the Northern region of the USA, AG 2-2 was reported as the most aggressive group on beans (Engelkes and Windels, 1996).

The AG-3 was previously known as a homogeneous group without subgroups; however, it was divided into three subgroups based on DNA sequence studies. These groups are termed as, potato type (PT), tobacco type (TB) and tomato type (TM) (Kuninaga et al., 2007). The isolates of AG-3 grow slowly on nutrient media compared to other groups. Furthermore, this group is more tolerant to cold climates compared with other *Rhizoctonia* AG groups. The most important hosts are potatoes, tobacco, and tomatoes in the same family. It causes root and crown rot, and tuber black scurf disease in potatoes (Sneh, 1996; Wicks et al., 1996; Aydın et al., 2011). These are frequently observed in the potato-growing regions around the world (Campion et al., 2003; Tsror, 2010). Nonetheless, these cause significant yield and economic losses in tomato, papper and tobacco crops (Misawa and Kuninaga, 2010; Demirer Durak, 2018).

The AG-4 group of *R. solani*, like the other groups is frequently observed and causes disease in many plant species as described above for other groups. Although there is no difference according to the anastomosis reaction, this group is divided into 3 subgroups (AG-4 HGI, AG-4 HGII, AG-4 HGIII) depending upon DNA-based diagnoses (Stevens Johnk and Jones, 2001). It causes damping-off and stem necrosis in potatoes (Sneh, 1996; Aydın and Turhan, 2013). It has been recorded that it is more adapted to temperate conditions than many other anastomosis groups; thus, it may be more important at low altitudes (Anguiz and Martin, 1989). There are several host species of *R. solani* AG-4 group other than potato. It causes seedling diseases in a variety of crops, including broccoli, cotton, melons, peanuts, potatoes, spinach, soybeans, and tomatoes (Ajayi-Oyetunde and Bradley, 2018; Demirer Durak and Ok, 2019). The AG-4 group of *R. solani* also causes root and collar rot in perennial plant species. It has been reported that AG-4 causes root and stem rot in pistachio orchards, particularly in nurseries and causes serious economic losses (Ashkan and Abusaidi, 1995; Ilkhan et al., 2011; Aydın and Ünal, 2021).

Some other anastomosis groups of *R. solani* cause diseases with varying virulence in various plant species. For example, the AG-5 group was mostly observed in potatoes and cereals (Bandy et al., 1984; Woodhall et al., 2012). It causes root and stem rot in potato; however, its virulence is lower than AG-3 (Campion et al., 2003). The AG-5 group is known to cause root rot in wheat and barley crops and considered as the most dominant group (Demirci, 1988; Ünal et al., 2015). Some anastomosis groups of *R. solani* are not pathogenic and have positive impacts on plant development. The isolates belonging to AG-6 and AG-12 groups of *R. solani* improved the development of some orchid species through mycorrhizal properties in Australia (Pope and Carter, 2001). Among other groups, AG-7 is known to cause damping-off in cotton crop (Abd-Elsalam et al., 2010). The AG-8 is a pathogen in wheat to some extent, while known as an effective pathogen causing diseases in other cereals (Sneh et al., 1991; Ünal et al., 2015). It is assumed that the remaining groups that are described above are mostly saprophytic, weak or non-pathogenic.

Mostly, the studies conducted on *R. solani* in the world are centered on the detection of anastomosis groups and their virulence on plants. The isolates obtained in this regard mostly belonged to AG-3 and AG-4 groups. Symptoms and sclerotia formation on carrot and potato plants by AG-3 group was reported for the first time from Sweden. It was reported that this situation might have resulted from the intensely practiced potato-carrot rotation and there might have been a transition of the pathogen from potato to carrot (Marcou et al., 2021).

3. Management of *Rhizoctonia solani*

Several management strategies are selected to control various diseases caused by *R. solani*. Fungicides are applied to some host species, i.e., potato tubers (Bains et al., 2002). Furthermore, resistant varieties are used for some plant species, and economic losses are prevented by cultural and crop rotation practices (Tsror, 2010). For example, some cultural measures against *R. solani* in the

potato crop are long-term rotation with cereals, early harvesting, and planting in warm and dry conditions to encourage tuber sprouting. The purposes of these measures are to reduce the disease by minimizing the contact time of the plant with the pathogen (Secor and Gudmestad, 1999).

Physical and chemical measures opted in greenhouses and nurseries for the eradication of the pathogen are not considered economical. In addition, problems caused by the application of chemicals (environmental, residues in foods, etc.) or inadequacy of the applied control methods have brought up alternative control opportunities (Pal and Gardener, 2006). Biological control is one of the most important among alternative control strategies. Biological control is briefly suppressing a pathogen with a living microorganism that does not cause disease in plants. *Trichoderma spp*., *Gliocladium spp*., binucleate *Rhizoctonia*, *Pseudomonas spp., Bacillus spp*. and *Streptomyces spp*., are used as antagonist microorganisms against *R. solani* (Trillas et al., 2006). Biological control is an environment-friendly alternative to protect plants, especially from soil-borne pathogens. Several studies have reported some antagonists used in biological control are effective against *R. solani* (Roy, 1989; Brewer and Larkin, 2005; Aydın, 2015). Compared to chemical control, this control method is thought as a good alternative for the control of soil-borne pathogens (Akrami et al., 2013).

3.1. Biological control of *Rhizoctonia solani*

Rhizoctonia solani is a good candidate for biological control than other alternatives due to its widespread environmental adaptation, large number of hosts, and the high number of sclerotia produced in the soil (Ganeshamoorthi and Dubey, 2015). It is one of the most studied microorganisms as pathogens and many antagonists for this pathogen have been identified.

The first work on the use of microorganisms as antagonist were interactions of *Trichoderma* and *Gliocladium* with *R. solani* (Weindling and Emerson, 1936; Weindling, 1937). These works isolated Gliotoxin from *Gliocladium* fungus and tested its efficacy. Biological control studies have been accelerated since the 1980s. *Fluoresent pseudomonas* and *Bacillus* sp. are the most prevalent bacteria species in soil and rhizosphere and have the potential to promote plant growth and suppress plant diseases, *Trichoderma sp*. and *gliocladium* fungal antagonist were the most frequently used species in the biological control. One review article was published in 1989. The works conducted from 1989 to date have been reviewed and pathogen antagonists are listed in this manuscript (Roy, 1989). These antagonists include; *Aspergillus clavatus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Epicoccum nigrum*, *Fusarium solani*, *Gliocladium roseum* also known as *Clonostachys rosea* f. *rosea*, *Laetisaria arvalis*, *Myrothecium sp*., *Penicillium cyclopium*, *P. ehrlichii*, *P. funiculosum*, *P. vermiculatum*, *Penicillium sp*., *Pseudocercosporella herpotrichoides*, *Pseudoeurotium multisporum*, *Pythium oligandrum*, *Trichoderma aureoviride*, *T. hamatum*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. polysporum*, *T. pseudokoningii*, *T. viride*, *Trichoderma spp*., *Verticillium biguttatum*, *V. lammellicola*, *V. lecanii*, *V. nigrescens*, *V. psalliotae*, *V. sphaerospermum* and *V. tenerum*. Some scientists have identified new antagonists of *R. solani*. These were; *Streptomyces ochraceiscleroticus*, *S. nobilis*, *Neocosmospora vasinfecta* var. *africana*, *Acrophialophora levis*, *Botryotrichum piluliferum*, *Coniothyrium sporulosum, Dicyma olivacea*, *Gliocladium catenulatum*, *Stachybotrys chartarum*, *S. elegans*, *Stachylidium bicolor*, *Verticillium chlamydosporum*, *Cylindrocarpon olidum* (Turhan, 1981, 1994; Turhan and Grossmann, 1989). In another study, they identified 5 species of *Myrothecium* species (*M. carmichaelii*, *M. cinctum*, *M. roridum*, *M. tongaense ve M. verrucaria*) which had antibiotic and mycoparasitic effects on *R. solani* (Turhan and Grossmann, 1994). Benyagoub et al. (1994), reports that *Stachybotrys elegans* is an effective antagonist of *R. solani*. In another comprehensive study to identify antagonists of *R. solani*, soil samples were collected from different geographic regions and 14 *Trichoderma* species were isolated from these soil samples. These species were *T. asperellum*, *T. atroviride*, *T. crassum*, *T. croceum*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. inhamatum*, *T. neokoningii*, *T. spirale*, *T. strigosum*, *T. tomentosum*, *T. virens* and *T. viride*. These species were proved as effevtive antagonists of *R. solani* both in *invivo* and *invitro* studies. These species except *T. harzianum, T. viride, T. hamatum* and *T. virens (=G. virens)* were the firsts report from Turkey (Aydın and Turhan, 2009; Aydın, 2015).

Several studies are reporting that *Trichoderma* (Teleomorph: Hypocrea) genus is effective in biological control of *R. solani*, stimulating plant growth and defense reaction, and producing antibiotics and enzymes (Howell, 2003; Druzhinina et al., 2011; Aydın, 2015; Abbas et al., 2017). Many *Trichoderma* species, especially *T.harzianum*, are experienced on potatoes, rice, chickpeas, beans, cotton, tomatoes, peppers, melons, cucumbers, etc. against *R.solani*, which causes disease in plants, and is currently used as a bioformulation.

Gliocladium spp. is another important antagonist for the biological control of *R. solani*. It is a prevalent soil fungus. It can live as saprophytic or parasitic on other fungi species (Castillo et al., 2016). The most important species of the fungus are *G. catenulatum*, *G. virens* and *G. roseum*. These species are hyper-parasites of *R. solani*. For example, *G. virens* suppressed damping-off in cotton by 11-55% and reduced the number of viable *R. solani* sclerotia by 63% (Howell, 1982). In another study, *G. virens* decreased the severity of *Rhizoctonia* root rot in beans in soil contaminated with *R. solani* with increasing concentration resulting in the higher suppression (Tu and Vaartaja, 2011). These studies show that *Gliocladium* species are promising biological control agents against *Rhizoctonia* root rot in some plant species.

Some bacteria species having antagonistic potential against *R. solani* have also been reported. These species include *Fluoresent pseudomonas, Bacillus* sp., *Burkholderia* (*Pseudomonas*) *cepacia*, *Streptomyces sp., Pseudomonas fluorescens* and *Erwinia sp.* (Tariq et al., 2010; Huang et al., 2012; Donmez et al., 2015). These bacteria generally persist for a long time in plant roots and colonize well over there. These bacteria species compete for food when interact with pathogens. Furthermore, they secrete antifungal metabolites. These features enabled these bacteria species to be effective against pathogens in biological control. These bacteria can suppress some soil-borne pathogens, including *R. solani*, due to their high competitiveness against target pathogens, provide systemic resistance to the plants, secrete mucolitic enzymes and produce metabolites such as antibiotics or siderophores (Weller, 1988; Yin et al., 2013).

3.1.1. Mechanisms of action of *R. solani* antagonists in biological control

The biocontrol mechanisms involved in the interaction between pathogen and antagonist in a plant or environment include (a) antibiosis, (b) competition, (c) mycoparasitism, (d) pathogen cell wall disrupting enzymes, and (e) induced resistance and hypovirulence (Adams 1990; Djonovic et al., 2007; Aydın, 2015). These mechanisms can work in concert with each other and can be used by the antagonist alone or in combination.

The antagonist effects between biological control agent and the pathogen can occur directly. For example, some *Trichoderma* species secrete ß-(1-3)-glucanase and chitinase against *R. solani* and kill pathogen hyphae by dissolving them. This event is defined as mycoparasitic effect (Van Den Boogert, 1996). Mycoparasites are effective against *R. solani* either by directly parasitizing their host hyphae or by producing antibiotics, especially in the form of antagonism and dissolving the outer layer of pathogen's cells with secreted enzymes (Elad et al., 1980). When *Trichoderma* comes in contact with *R. solani* in the nutrient medium, it first recognizes the host and then directs its hyphae and the pathogen spreads afterwards (Figure 2a). An effective antagonist must spread rapidly on the pathogen after the start of competition. *Trichoderma* species parasitize the aerial hyphae of *R. solani*, usually with extensions from the main hyphae. Parasitism occurs by coiling or parallel development along the host hyphae (Figure 2b). Antagonist selection in terms of mycoparasites should be made by considering these antagonist selection criteria under laboratory conditions. Apart from *Trichoderma*, antagonists with high mycoparasitic potential against *R. solani* have also been detected. For example, *Stachybotrys elegans* have been reported as a destructive and effective mycoparasite of *R. solani* (Benyagoub et al., 1994). *Verticillium biguttatum* has also been reported as an effective mycoparasite of *R. solani* (Morris et al., 1995). This mycoparasite is effective in dissolving cell walls of *R. solani* by producing enzymes such as chitinase, glucanase and protease. Wicks et al. (1995) reported that application *V. biguttatum* spore suspensions to potato tubers in the form of spray or branch application reduced the viability of sclerotia by 90%. Although *V. bigittatum* is mostly successful against potato black scurf disease, some special conditions are required for its effectiveness. For example, temperature should be at least 13 °C and the minimum air humidity should be 90% and above. The relationship of two important mycoparasites, i.e., *T. harzianum* and *S. elegans* with *R. solani* is shown in Figures 2 and 3.

The other form of impact are indirect effect. Some bacteria are responsible for indirect effects in the biological control against *R. solani*. The antagonist acts against the pathogen by competing for space and food (Weller, 1988). Induced resistance is another mechanism observed in plant because of the antagonist. In this case, antagonist makes host plant's defense system sensitive, so that the host is ready for pathogen attack (Bora and Özaktan, 1998). *Bacillus* and *Pseudomonas* species have been reported to successfully used for the control of *R. solani* (Saikia et al., 2006; Anitha and Das, 2011; Aşkın Şenocak et al., 2019). These studies reported that *Pseudomonas* species successfully adapt rhizosphere, grow rapidly and promotes systemic resistance in the plants. Siderophores of *Pseudomonas* have been reported to several secondary metabolites, including pseudobactin, pyochelin, pyoverdine, ferribactin,

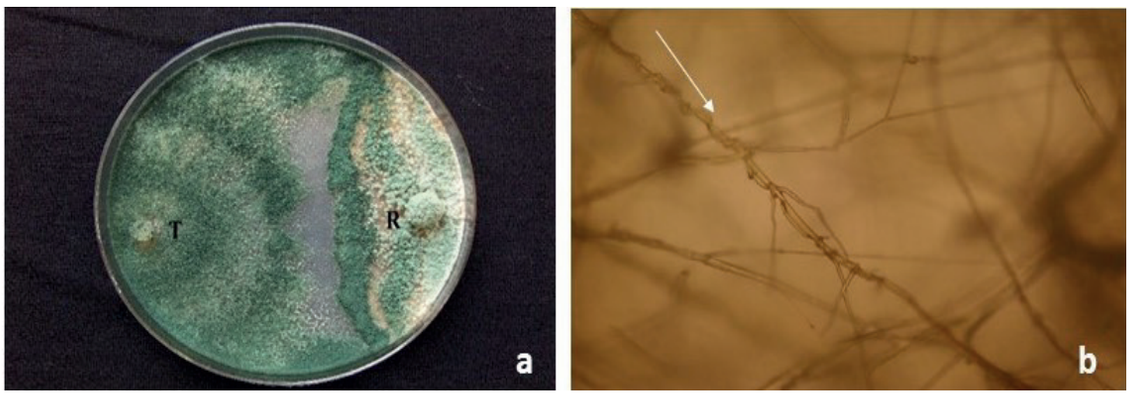

Figure 2. Spread (a) and hyphae wrapping under microscope (b) of *Trichoderma* (T) on *R. solani* (R)

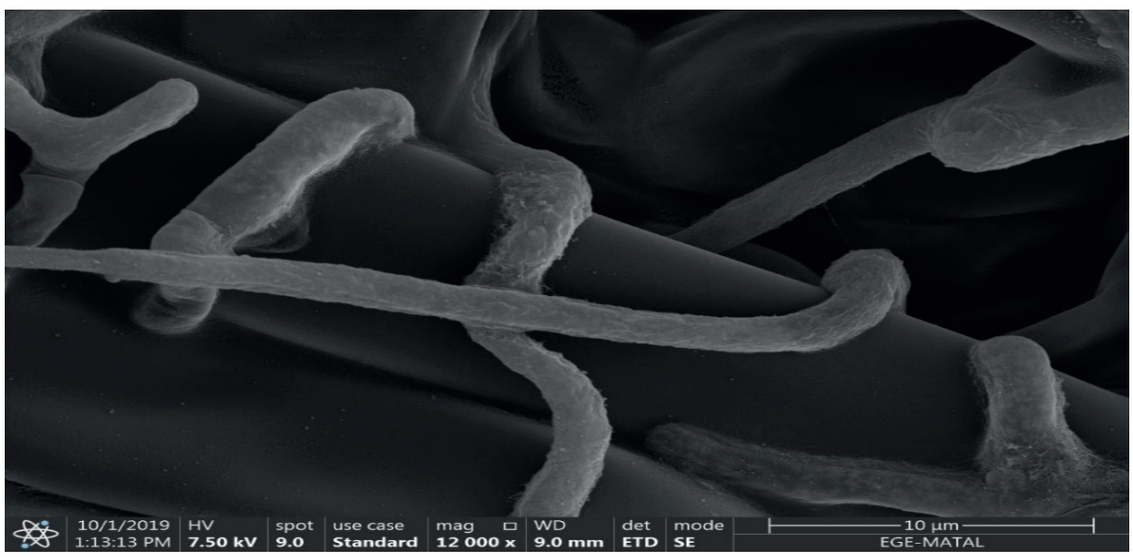

Figure 3. *Stachybotrys elegans* enveloping *Rhizoctonia solani* hyphae (Photograph courtesy of G. Turhan, Ege University)

ferrichrome, phytosiderphores, antibiotics such as phenazines, pyoluteorin, tropolone, pyocyanine, hydrogencyanide, phenazine-1-carbosylic acid (PCA), ionomycin acid, oomycin-3 Aomycin, β-1,3 glucanase and laminariase. These bacteria act as effective broad-spectrum antagonists against *R. solani* by producing many antibiotics, siderophores, and several toxins (Saikia et al., 2006).

Antibiosis is another important mechanism involved in biological control. Briefly, it is the process of inhibition or destruction of one organism by the metabolites of another organism. These metabolites are known as antibiotics. They are generally low in molecular weight, volatile or non-volatile toxic substances capable of spreading in the environment. Antibiotic substances produced by different microorganisms adversely affect the pathogenicity of *R. solani* (Braun et al., 2010). *Actinomycetes* and *Trichoderma* species are a very important group among antagonists producing antibiotic substances. For example, *T. viride* can produce several antibiotics, including Gliotoxin, Viridin, Trichodermin, Trichodermycin and Trichodermol. (Weindling, 1941; Vinale et al., 2014). Below is the inhibition zone formed by producing antibiotics due to interaction of the pathogen and the antagonist *Trichoderma* in the medium (Figure 4).

Pseudomonas and *Bacillus* spp. species among bacteria produce both antibiotics and stimulate plant growth (Abbas et al., 2019). *Bacillus* spp. has become a remarkable microorganism as it produces highly resistant structures called endospores that allow it to survive under adverse environmental conditions. It promotes plant growth and stimulates systemic resistance. It also exhibits antagonist properties by secreting various types of antimicrobial compounds (Huang et al., 2012). It is reported that the BL915 strain of *Pseudomonas fluorescens* was effective on *R. solani* through the production of pyrrolnitrin (Hill et al., 1994).

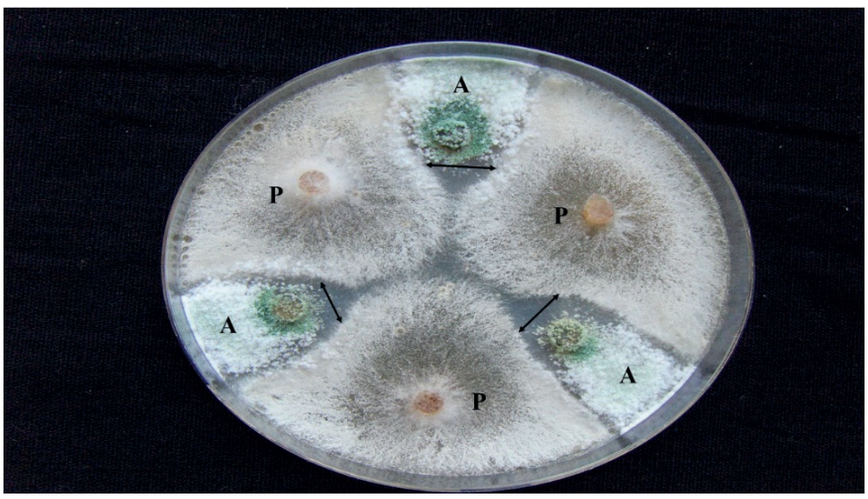

Figure 4. Growth and inhibition zone formed by *Trichoderma* and *Rhizoctonia* isolates in PDA medium (A= Antagonist, P= Pathogen)

The antibiotics can inhibit various pathogens, especially *R. solani*. These antibiotics are purified, multiplied, and used in the pharmaceutical industry. Nonetheless, biological control potential efficiency of some microorganisms has been increased through molecular studies. For example, *Pseudomonas putida* WCS358r strains have been genetically engineered to produce phenazine and DAPG antibiotics, which suppress some diseases in wheat crop (Glandorf et al., 2001).

Trichoderma is one of the microorganisms having the most effective mechanisms of action against pathogen. Furthermore, *Trichoderma* species can control plant growth through production of plant hormones and exhibit soil conditioning activity by producing enzymes (Druzhinina et al., 2011; Mukherjee et al., 2012). Numerous studies to date have reported that *Trichoderma* species can control important soil-borne phyopathogenic fungi such as *R. solani* (Elad et al., 1980; Chet and Inbar, 1994; Kubicek et al., 2001; Aydın and Turhan, 2009, 2013; Aydın et al., 2011; Aydın, 2015).

An important part of the biological control studies against *R. solani* has been conducted on potato crop. There are also studies showing that some biological agents are effective in controlling *R. solani* infestation in potato crop (Wicks et al., 1996). *T. harzianım* and *T. virens* (=*Gliocladium virens*) are reported to suppress *R. solani* under field conditions (Lewis and Larkin, 1997; Lewis et al., 1998). The inoculum source of *R. solani* is found freely in soil and as sclerotia in potato tuber (Wicks et al., 1996). The inoculum on the tuber is important factor involved in the spread of the disease. Since antagonist' application to potato tuber is more practical than other plant materials, it can be easier to get effective results. Aydın et al. (2011) applied some *Trichoderma* species to tubers infected with sclerotids of the pathogen and found that *T. viride* VG18, *T. gamsii* VG47, *T. strigosum* LO43, *G. roseum* LO41 and *T. asperellum* TZ17 isolates inhibited sclerotid viability by >70%.

Pseudomonas fluorescens bacteria is another important biological control agent of *R. solani.* Some *Pseudomonas fluorescens* strains stimulate plant growth by producing siderophores against *R. solani*, and suppress disease by producing antibiotic substances (Boruah and Kumar, 2002). In short, siderophores and antibiotics produced by these bacteria are important in their use against *R. solani*.

Hypovirulence is another mechanism operating in the biological control of *R. solani.* It results through hybridization and hyperparasitism between a virulent and a less virulent individual, which results in virulence decline. Loss of pathogenicity (hypovirulence) in *R. solani* is a disorder of cytoplasmic inheritance. In weak pathogenic isolates, declined rate of hyphal development and ability to form sclerotia, and disappearance of dark pigmentation in the mycelium is observed along with decreased pathogenicity. In other words, pathogen isolates of *R. solani* usually have dark pigmentation (Sneh, 1996). Figure 5 below shows dark-colored (*R. solani*) and light-colored (Np-*Rhizoctonia*) isolates.

Low or avirulent isolates are considered hypovirulent or nonpathogenic *Rhizoctonia* (Np-R) isolates (Van Alfen, 1982). These *Rhizoctonia* species are binucleated. These are not pathogenic to plants. This binucleate *Rhizoctonia* is differentiated according to the anastomosis groups (AG) between its hyphae like *R. solani*. These are known by words from AG-A to AG-W) (Yang et al., 2015). The Np-R could be found among all populations of AGs

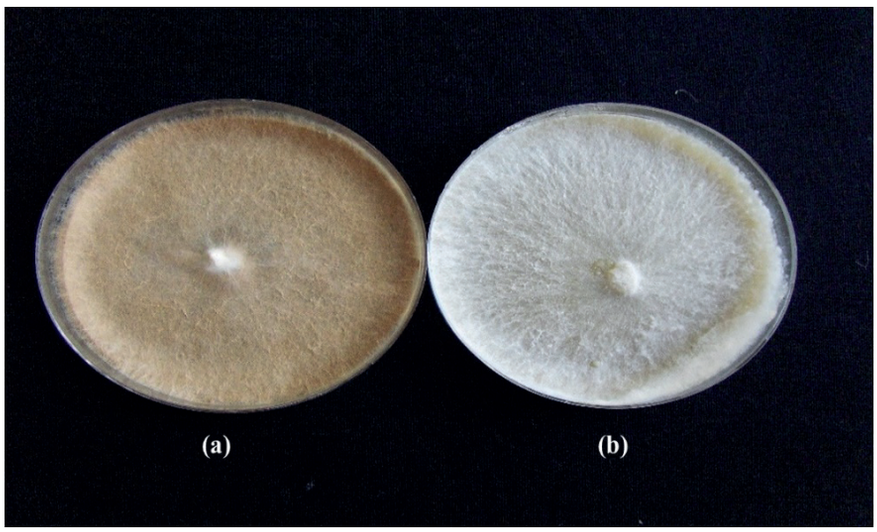

Figure 5. *Rhizoctonia solani* (a) and Np-*Rhizoctonia* (b) isolates grown on PDA medium

(Sneh, 1996). Hypovirulent or non-pathogenic *Rhizoctonia* isolates are found in many areas and constitute 10-30% of total *Rhizoctonia* population (Ichielevich-Auster et al., 1985). These non-pathogenic isolates of *Rhizoctonia* are also effective in biological control (Sneh et al., 2004). For the realization of this activity, hypovirulent or Np-R isolates must have some similar characteristics with other virulent isolates. They also need to have the same ecological niche coverage on the plant surface and competitiveness for colonization. Np-R isolates are capable of intense colonization on plant surface, underground plant organs, stems and leaves. This indicates that Np-R isolates enter into physical competition with virulent isolates by covering the infection site or competing for nutrients. Root exudates are essential for the pathogenicity of *Rhizoctonia*. Thus, competition for nutrients is an appropriate mechanism for the protection of seedlings by Np-R isolates (Freeman and Rodriguez, 1993; Sneh and Ichielevich-Auster, 1998; Sneh et al., 2004). Besides, some isolates of these species stimulate plant growth, increase crop yield and impart drought tolerance to plants (Sneh et al., 1986; Sneh and Ichielevich-Auster, 1998). A total 206 *Rhizoctonia* spp. were isolated from soil in New Zealand and 55% of the isolates were pathogenic in radish seedlings, and 13 of 92 hypovirulent isolates prevented >50% of the damping-off disease caused by *R. solani* in radish seedlings (Sneh et al., 2004). In a study conducted in the USA, 153 *Rhizoctonia* isolates obtained from soil samples collected from different fields consisting of hypovirulent or non-pathogenic isolates in cabbage plant. Fourteen isolates among these provided cabbage seedlings with >60% protection against *R solani.* Furthermore, 8 isolates protected the cucumber seedlings by 73-95% from *R solani*. Nonetheless, Np-R colonized roots at low soil moisture, which improved drought tolerance of seedlings (Sneh and Ichielevich-Auster, 1998). In conclusion, development of microbial preparations from Np-R isolates with high activity against *Rhizoctonia* diseases can be of great value in the agricultural biotechnology industry and safe sustainable farming technique, especially with their ability to protect seedlings from moisture deficiencies and increase yield.

3.1.2. The effect of antagonist microorganisms on the host plant in biological control

Biological control agents exert direct effects on the pathogen and indirect effects on the host. The indirect effects stimulate plant development and growth, making it more resistant to pathogens (Sani et al., 2020). The most important of these agents is *Trichoderma* as described above. Some of them are saprophytic and others are pathogenic on other fungi (Aydın, 2015). In addition, they have bio-stimulant effect on the plant (Yanpo et al., 2015). The disease can be prevented by applying *Trichoderma* to plants. In addition, *Trichoderma* increases yield and plant development to significant extent (Harman, 2006; Woo et al., 2014). *Trichoderma* applied to potato root area indicated that the effect of some diseases, especially *R. solani* were decreased. It also promoted growth, yield characteristics and quality of potato crop. The effect of the antagonist here has been evaluated as a biofertilizer, increasing the activity of nutrients in the potato root zone (Molla et al., 2012; Purwantisari et al., 2018).

Bacteria that increase plant growth and also protect plants from diseases are called plant growth-promoting bacteria (PGPB) (Compant et al., 2010).

Members of *Bacillus* species are bacteria that protect plants from biotic and abiotic stresses and promote plant growth (Radhakrishnan et al., 2017). The PGPBs suppress soil-borne pathogens such as *R. solani* and accelerate plant growth. They also produce antibiotics and impart systemic resistance to plants against plant pathogens.

Consequently, biological control agents such as *Trichoderma* and PGPB bacteria had direct effects on pathogens, including hyperparasitism, and antibiotic production in addition to positive effects on plant development, growth and yield. Healthy plant growth makes it more resistant to diseases.

3.2. Methods of obtaining *R. solani* antagonists

Fungal or bacterial antagonists isolated from different organs of plants, root, fruit, leaf etc. as well as from the soils with different physical and chemical properties in different geographies through using different methods. Sometimes, effective antagonists are obtained through a simple and inexpensive method. For example, antagonist can be obtained by transferring the root parts taken from the plant selected in the field to a pre-prepared selective nutrient medium (Figure 6). In the same way, especially bacterial antagonists are obtained by centrifuging the parts taken from the plant root surface in a liquid suspension, diluting and transferring them to special media (Berg et al., 2002).

In Figure 6, a piece of the root part of the potato plant slightly contaminated with *R. solani* was taken and transferred to Potato Dextrose Agar (PDA) nutrient medium and incubated at 22-24 °C. Two different *Trichoderma* species and the pathogen *Rhizoctonia* developed from the piece of root. Thus, two effective antagonists were isolated by following a biological control process that works spontaneously in nature.

The *R. solani* antagonists are mostly isolated from the soil. The “serial dilution plate technique”, “pour plate”, “differential centrifugation technique”, “the slide-trap method” and “the dilution plate count technique” is used for the isolation (Hopkins et al., 1991; Vargas Gil et al., 2009; Aydın and Turhan, 2017). These are classical and most known methods in isolation from soil. For the isolation of *Trichoderma* species, soils that are suspected to contain antagonists are inoculated on various nutrient media (such as PDA, malt extract agar (MEA), rose bengal agar, oatmeal agar) and left in the incubator at 24-28 °C for the development of antagonists (Küçük and Kıvanç, 2003). Rose Bengal, pentachloronitrobenzene (PCNB) and captan are mixed in such nutrient media at certain rates to prevent the development of unwanted microorganisms (Elad et al., 1980). Antagonists are isolated by serial dilution technique of soil with sterile water by using special media (Elad and Chet, 1983). Different antibiotics and chemical drugs are added to the media depending on their purpose. A comprehensive study for the identification and isolation of *R. solani* antagonists has been conducted. "Soil isolation method", "antagonist isolation of *Rhizoctonia* sclerotia from rinsing water", "antagonist isolation by using Pathogen culture as trap" and "antagonist isolation from *Rhizoctonia* sclerotia on tuber" methods were used in the study. Among these methods, "the antagonist isolation method by using the pathogen culture as a trap" has been developed and effective results are obtained. Researchers reported that this method is simple to work with and abundant antagonists are isolated (Aydın and Turhan, 2009, 2017). In this method, soil samples are spread over the colonies of *R. solani* grown in PDA medium in Petri dishes and incubated for two weeks. The Soil on the cultures was shaken and the pathogen colony below was thoroughly washed with sterile water at the end of

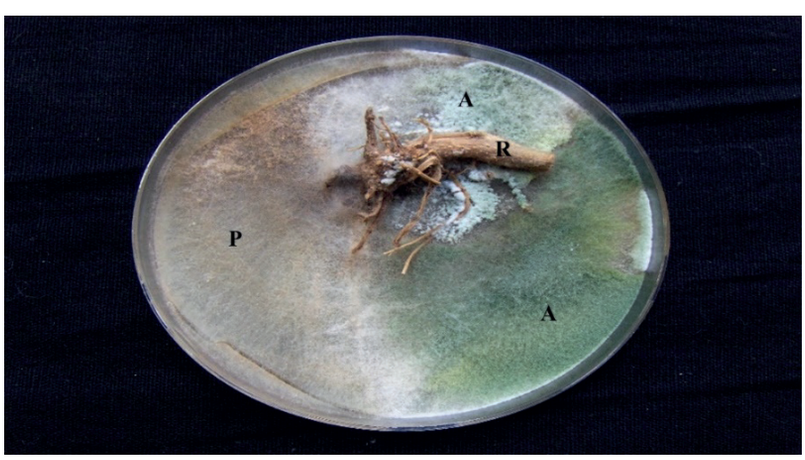

Figure 6. Pathogen and antagonist fungi grown from plant roots in PDA medium (P= Pathogen, A= Antagonist, R= Root)

incubation period. Afterward, discs cut from the colony were transferred to Rizolex (Tolclofos-methyl) doped PDA medium and put into the incubator. It was observed that antagonist candidates with hyperparasitic character developed in a short period of time, i.e., 3-5 days (Figure 7). The most important result of this method is that it is particularly suitable for the isolation of hyperparasitic *Trichoderma* species from the soil.

Bacteria are another important antagonist group of *R. solani*. Different methods are used for the isolation of antagonist bacteria. Bacterial antagonists are obtained from the root zone or soil of the selected plants, or the vegetative parts of the plant using suitable media. For example, *fluorescent pseudomonas* is mostly isolated from the root zone of healthy plants (Geels and Schippers, 1983). It is important to select the suitable media for bacterial antagonists. For example, King-B is used for isolating the *Pseudomonas* group, Chitin-agar medium for actinomycetes group, and CVP and MS medium are used for the isolation of *Erwinia caratovora* and *E. herbicola* (Harman et al., 1981; Geels and Schippers, 1983; Andrews, 1991). Selective media with the known suspension dilution technique are used in the isolations of such species from the soil (Sanders, 2012).

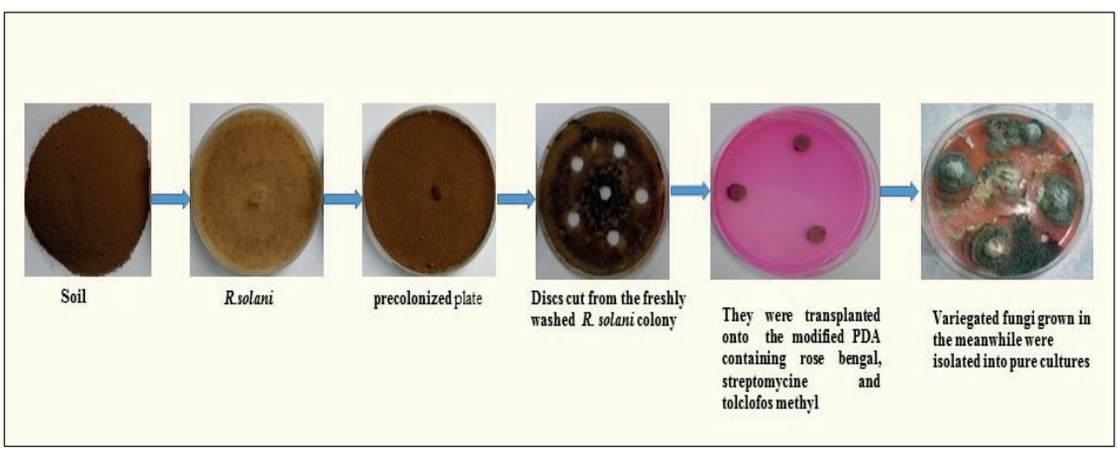

Figure 7. Schematic diagram of antagonist isolation method using pathogen culture as trap

3.3. Bioformulation process

Chemical control is the most prevalent method used to manage plant diseases. But, results in several problems. These, include residues in soil and produce, the evolution of resistant pathogen strains due to frequent chemical use, and environmental pollution etc. In addition, alternative methods have gained importance due to the spread of some diseases caused by soil-borne pathogens such as *R. solani*. Thus, biological preparations were developed and their use was started to lower the negative concerns of chemical control. The number of these commercial preparations and their use in the world is constantly increasing. These preparations are mostly found in greenhouse and seedling nurseries to control soil-borne pathogens, including *R. solani*, *Pythium* spp., *Phytphthora* spp., *Fusarium* spp. and *Sclerotinia*. Some commercial preparations developed against soil pathogens, including *R. solani* and their properties are given from Table 1 below.

It is seen that the antagonist fungi are mostly *Trichoderma* spp. It is further noticed that bacterial antagonists are mainly *Bacillus* species, *Pseudomonas fluorescens* and *Burkholderia cepacia*. *Trichoderma* species account for ~60% of registered bio-fungicides worldwide. Therefore, these accepted as the most successful bio-fungicide used in today's agriculture. Solid and liquid formulations are used in the bio-formulations of *Trichoderma.* These formulations contain hyphae, chlamydospores, or mostly conidia of the fungus (Howell, 2003). For liquid formulation, deep tank fermentation system is preferred. Inexpensive growth media such as molasses and brewer's yeast are used. The solid formulation is an alternative method for inoculum production. Agricultural residues such as wheat and paddy straw sugarcane pulp, corncob, sawdust, and rice bran are used. This formulation method is applicable for low-cost, small-scale production. These commercial preparations are used in the form of direct mixing, spraying or injection into the soil, application to lesions, covering the seed or tuber with suspension, applying to the soil with drip irrigation, immersing the root and stem suspension followed by planting (Aydın, 2015). Solid formulations generally give better results than liquid ones in the commercial production of *Trichoderma*. Talc-based

Table 1. Some commercially produced and used biological preparations against *R. solani*

Name	Trade names	Important crop/Use	Licensed Country
Bacillus subtilis (GB03)	Kodiak, Companion	Cotton, Beans	USA
Bacillus subtilis (MBI 600)	Q4000, System 3, Subtilex, Pro-Mix, HiStick NT	Beans, Cotton, Cereals, Soybean, Groundnut	USA, Canada
Bacillus subtilis	Epic	Cotton, Cereals	USA
Streptomyces griseoviridis K61	Mycostop	Damping-off of seeds and seedlings	Canada
Bacillus subtilis	Defender	Used against soil-borne pathogens	South Africa
Bacillus pumulis (GB-34)	Yield shield	Cereals	USA
Pseudomonas fluorescens	ABTEC Pseudo, Biomonas Esvin Pseudo, Sudo	Soil-borne pathogens	India
B. subtilis subsp. *amyloliquefaciens* FZB24	Taegro	Diseases caused by *Fusarium* and *Rhizoctonia* in plants	USA
Burkholderia cepacia	Blue Circle	Maize, Melon, Cotton and soil-borne pathogens of beans	USA
Streptomyces griseoviridis K61	Mycostop	Soil-borne pathogens in different plant species	England
Burkholderia cepacia	Intercept	Soil-borne pathogens of maize and melon	USA
Burkholderia cepacia	Wisconsinstrain M54	Damping-off of vegetable seedlings	USA
Burkholderia cepacia	Wisconsin strain J82	Damping-off of vegetable seedlings	USA
T. harzianum	Th-Derma	Root rot and wilt pathogens in greenhouses and nurseries	India
T. viride	Guard	Soil-borne pathogens in various plants, seedlings, seeds	India
T. harzianum	Root Shield, BioTrek 22G, Supresivit, T-22G, T-22HB	Soil-borne pathogens in different plant species	USA, Europe
Trichoderma harzianum	Trichosav-34	Soil-borne pathogens in vegetables and ornamentals	Cuba
Trichoderma spp	Promot, Trichoderma 2000, Biofungus	Soil-borne pathogens in vegetables	USA, Belgium
T. viride	Trieco, Trieco	Seed-borne pathogens in vegetables and orchards	India
Gliocladium catenulatum J1446	Prestop	Damping-off of seeds and seedlings	Canada
T. asperellum ICC 012, *T. harzianum* ATCC080	Tenet, Bioten, Remedier	Soil-borne diseases in various plants	USA
T. harzianum	Trichoderma 2000	Soil-borne pathogens in different plant species	Israel
Trichoderma atroviride I-1237	Esquive	Soil-borne pathogens in different plant species	England
T. harzianum	Tusal	Soil-borne pathogens	Spain
T. harzianum, T.viride	Trichodowels, Trichoject, Trichoseal	Soil-borne pathogens in different plant species	New Zealand
Trichoderma sp.	F.Stop	Damping-off disease in pea and maize	USA
Trichoderma gamsii	Remedier	Soil-borne pathogens in different plant species	England
Trichoderma harzianum	Biozim, Phalada 105, Sun Agro, Derma H	Soil-borne pathogens	India
Trichoderma asperellum	Trichotech	Soil-borne pathogens in different plant species	Kenya
Trichoderma harzianum	Ecotrich (not registered) Trichodermil SC 1306	Soil-borne pathogens	Brazil
T. harzianum	AG-2	Soil-borne pathogens in different plant species	USA
Trichoderma harzianum	Gliocladin, (Trichodermin T, Z)	Different fungal diseases	Russia
T. harzianum	Trichoderma 2000	Soil-borne pathogens	Israel
T. harzianum, *T. polysporum*	Binab T	Soil-borne pathogens in lawns	Sweden
T. lignorum	Trichodermin-3	Soil-borne pathogens in different plant species	USA
Trichoderma harzianum	Eco-77	Soil-borne pathogens in different plant species	South Africa
Gliocladium virens	GlioGard	Soil-borne pathogens in seedlings and nurseries	USA
T. harzianum	Trichodex	Different fungal diseases	Israel, Europe
Gliocladium virens	Soilgard	Soil-borne pathogens in ornamentals and seedling nurseries	USA

bioformulation is found as the best material for maintaining the maximum number of live propagules. In addition, isolates can maintain their viability for up to 120 days under all conditions. It was found that seed treatment with the bioformulation of *T. harzianum* can increase seed germination, root and shoot length, and seedling viability over untreated ones. Compared with control and other isolates, the application of bio-formulated *T. harzianum* was more effective in reducing wilt disease caused by some pathogens. (Shahid, 2014).

Liquid culture is more preferred in bioformulation of bacteria unlike fungi. Products obtained from liquid fermentation are generally formulated as powders, wettable powders (wp), granules, pellets, gels and emulsified liquids similar to the formulation of synthetic chemicals (Bora and Özaktan, 2008).

3.4. Use of biological control agents with some other applications

While using antagonists against *R. solani* and some other soil-borne pathogens, some other applications may be more effective in combating diseases and increasing the expected effects of antagonists. For example, approaches such as using antagonist and chemical pesticides together, combining the antagonist with physical applications such as solarization, applying cultural measures to reduce disease inoculum in the soil could increase the effectiveness of the antagonist and are considered within the framework of integrated control principles. In addition, antagonists can be applied in combination with their complementary mechanisms according to the characteristics of the pathogen.

The purposes of combining antagonist with chemical pesticides are a) to increase the effectiveness of the antagonist and pesticide which are not effective against the disease, and b) to keep the environment safe from the negative effects of pesticide by using pesticide at lower than the recommended dose. It is necessary to reveal the sensitivity of the antagonist to the pesticide with some tests. For example, pesticides recommended against *R. solani*, which causes black scurf and stem canker necrosis in potatoes, are highly effective against *R. solani* in vitro, but some *Trichoderma* spp. were less effective against other species. *Trichoderma* were applied together with ¼ dose of pesticide and black scurf disease on the tuber was prevented more than the sole application of *Trichoderma* (Aydın and Turhan, 2013). Duffy (2000) investigated the efficacy of 2-79 strain of *P. flourescens* (non-pathogenic species of *Pseudomonas* group) and pesticide Pencycuron (Monceren®) against *Rhizoctoni* root rot and take-all disease in summer wheat under *in vitro and in vivo* conditions. Pencycuron inhibited *R. solani* under in vitro conditions, protected plants against *Rhizoctonia* root rot and take-all by seed application; however, could not prevent the colonization of biocontrol strain 2-79 of *P. flourescens*. When both were applied together to seed, higher disease suppression was recorded compared to sole application of both.

Antagonists can be applied in combination with solarization in greenhouses. The method of application is the application to the with or after solarization. Promising results have been obtained with this application for the control of soil-borne diseases, especially *R. solani*. One of the earliest studies on this subject was conducted by Chet et al. (1982). They stated that the use of solarization together with *T. harzianum* against *R. solani* was effective in preventing the pathogen. In another study, alternative control methods against soil-borne pathogens with methyl bromide were investigated in pepper and eggplant in plastic greenhouses and tunnels and strawberry in open fields in the Eastern Mediterranean region of Turkey. Along with some other applications (solarization + fresh cattle manure, solarization + low dose chemical), the seedlings inoculated with the antagonist microorganism *T. harzianum* (T-22 Planter Box) were planted in the solarized soil. While the disease incidence was 12.5-21.5% in solarization + *Trichoderma* application, it was 25.0-38.7% in the control plots (Yücel et al., 2007). These studies show that *R. solani* and other soil-borne pathogens can be suppressed by *Trichoderma* combined with some other practices.

Sometimes two or more antagonists are used together to increase the efficacy of biological control. Commercial preparations are also available for these antagonists. The aim here is to make the antagonists more effective against the pathogen through different mechanisms. For example, synergistic interaction can be found among *Trichoderma* spp. strains and bacterial antagonists such as *Pseudomonas syringae* for the control of plant pathogens (Whipps, 1997).

4. Conclusions

Rhizoctonia solani is an economically destructive plant pathogen of many plants, especially in field, garden, ornamental and forest plants. *Rhizoctonia solani*, a soil-borne fungus, is the most important species of the *Rhizoctonia* genus in terms of morphology, virulence and host species. It is divided into genetically distinct, intraspecific anastomosis groups (AGs). The morphology,

virulence and hosts of these AGs are different from each other. Today, there are 14 known AGs (AG-1, AG-13 and AGBI) and some subgroups of AGs. These groups were previously identified with test strains that provided hyphal fusion. However, this diagnostic method requires the isolation and maintenance of all test isolates. Again, the work takes a long time and requires intensive labor. In addition, difficulties are faced in the diagnosis of advanced subgroups of *R. solani* with these classical methods. In a study conducted with *Rhizoctonia* isolates causing seedling wilt in pistachio, classical and modern methods were used together and all isolates were identified by the molecular method, although some isolates were not identified by the classical method. For this reason, more safe and rapid studies have been conducted on *Rhizoctonia* with the use of DNA-based molecular methods. This positively affected the biological studies of *R. solani*. The importance of biological control against *R. solani* and other soil-borne pathogens and studies on this subject are continuously increasing. Numerous commercial bio-formulations are produced in the world and used in agriculture due to insufficient disease control by chemical and other control methods. However, these bio-formulations are not produced in some countries and are only obtained through imports. For example, it is understood that there are no licensed bio-formulations against *R. solani* in Turkey and some other countries (Table 1). The are many reasons for this situation. The main reasons of this is the inadequacy in legal legislation and licensing of commercial preparations, lack of specialized institutions and organizations for research, and the unwillingness of the pharmaceutical industry in those countries to start commercial production. Converting an antagonist to a bio-formulation and using it in the region where it is isolated increases the chances of success in the management of the disease since climatic conditions, especially temperature, have a great influence on the biocontrol of antagonist species, and production of antibiotics and enzyme activities. The presence of other microorganisms in the soil flora may also prevent the development and effectiveness of the biocontrol agent. This situation may not completely adversely affect the biocontrol activity of biological control agents. However, it can reduce its effectiveness over time. In this case, one of the best methods is obtaining a biocontrol agent with a certain potential is to give *Trichoderma* species the same temperature, humidity and nutritional value similar to the nature from where it was isolated and then wait for its function in disease control.

Funding

This research received no external funding.

Declaration of Conflicts of Interest

No conflict of interest has been declared by the author.

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CITATION: Aydın, M.H., 2022. *Rhizoctonia solani* and Its Biological Control. *Turkish Journal of Agricultural Research*, 9(1): 118-135.