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Factors affecting biocontrol of Rhizoctonia diseases and growth promotion of potato by *Trichoderma* species

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Dedication

I devote my thesis to

The spirit of my grandfather and grandmother

and

My father, mother, brothers, wife, daughters

And friends: my support in life

Abstract

Rhizoctonia solani AG-3PT causes stem canker and black scurf disease of potato which results in economic yield loss of up to 35-50% around the world. Until now, progress in the management of these diseases has been slow. This study looked at the effects of biological control strategies for stem canker and black scurf of potato caused by *R. solani* AG-3PT using soil organisms alone or in combination with resistant potato varieties or fertilizers or intercropping systems with *Brassica* plants.

Trichoderma species were studied because they have a known role in minimising the impacts of pathogens and improving plant growth. Eight isolates of *Trichoderma* obtained from healthy tubers and soil reduced the severity of disease and promoted growth of potato plants in laboratory and glasshouse experiments. *T. harzianum* strain T5 and *T. hamatum* strain T8 had the best performance in prevention of disease and enhancing the plant growth and potato yield.

The information on comparative sensitivity of varieties of potato will assist farmers to make informed decisions in terms of black scurf disease management. Six potato varieties utilized in this study showed a range of sensitivity reactions to *R. solani* AG-3PT, but none of these varieties were completely resistant to the disease. The Sapphire and Royal Blue varieties showed the lowest level of infection of tubers by sclerotia of *R. solani* AG-3PT at harvest, whereas Sebago and Desiree varieties showed the highest level of sclerotia on tubers.

Laboratory experiments showed that Sebago sprouts contained materials that reduced the pathogen growth and prevented the growth of biocontrol agents compared with other potato varieties. The interaction between two *Trichoderma* isolates (T5 and T8) and the pathogen in glasshouse experiments with three potato varieties (Sebago, Desiree and Sapphire) showed the same disease reduction and plant growth promotion for all varieties. There was therefore no interaction between plant resistance and biocontrol.

In culture experiments, there were small but significant effects of seven nutrients (N, P, K, Ca, Mg, Fe and Mn) on the interactions between *Trichoderma* species and the pathogen. One series of pot trials on the effects of N, K and Mn on biocontrol did not detect an interaction between the nutrients and disease control or growth promotion. However, a field trial did show that the effect of *T. hamatum* T8 on growth promotion of potato in the presence of the pathogen was

greater at low levels of NPK fertilizer. As a result, farmers may reduce fertilizer level when the biocontrol agent is applied.

Biofumigation has been used as an alternative method for controlling soilborne plant pathogens. Biocontrol agents T5 and T8 were not pathogenic to broccoli or cabbage and promoted their growth. *R. solani* was sensitive to compounds from the root tissues of broccoli and cabbage, whereas *Trichoderma* isolates, especially T8, were tolerant to compounds from root tissues. In a glasshouse experiment either intercropping with cabbage or broccoli, or inoculation with T8, prevented disease symptoms on potatoes. In a field trial, intercropping with cabbage or treatment with T8 controlled the stem canker and black scurf disease caused by *R. solani* AG-3PT. Isolate T8 also greatly increased the growth rate of the cabbages. The growth of potato plants was reduced by competition with the cabbage, indicating the need for further work on refining the intercropping system.

Overall this study provides insight on the efficacy of *Trichoderma* isolates for biological control, and the effect of *Trichoderma* isolates on potato varieties resistance, fertilizers and cropping system to control stem canker and black scurf of potato caused by *R. solani* AG-3PT.

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is currently not being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been fully acknowledged in this thesis.

Usamah A. Alkarim A. Almunam Alshimaysawe

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Chapter 1. General Introduction

1.1. Project overview

The tubers of potato plants (*Solanum tuberosum* L.) are a vital vegetable crop, and are known as a healthy food rich in vitamins A, B and C. Also, the potato is high in carbohydrates, primarily starch, minerals, fiber, and protein and is low in fat (Rodríguez-Falcón et al., 2006). Potato plants belong to the order Solanales and the family Solanaceae. Potato plants are the fourth most important world food crop after rice, maize and wheat in terms of cultivated area, production and consumption (Marfil et al., 2015; Rahman et al., 2012).

Many papers have shown that different microbes can cause diseases on potato plants. Several possible causes of potato diseases include viruses, bacteria, nematodes and fungi. There are many types of fungi that can cause diseases of potatoes, but *Rhizoctonia solani* Kuhn anastomosis group 3 (AG-3PT) is one of the most significant disease-causing organisms in potato (Demirci et al., 2009), responsible for both stem canker on the plant and black scurf on the tuber. This fungus has different types and strains, is prevalent world-wide, and is found in all kinds of soil (Bains et al., 2002). The loss of yield caused by stem canker and black scurf disease of potato can be approximately 35-50%, resulting in high economic loss for growers (Rahman et al., 2012; Virgen-Calleros et al., 2000; Zhang et al., 2014). In recent years, the potato sowing area has been increasing due to increasing population which leads to the spread of soilborne fungal diseases, including Rhizoctonia diseases of potato (Fiers, 2010). *Rhizoctonia solani* has become a serious problem in countries including China, India, Great Britain, France, Finland, Australia, New Zealand, South Africa, Mexico, Canada and Uruguay (Balali et al., 1995; Campion et al., 2003; Das et al., 2014; Lehtonen et al., 2008a; Platt et al., 1993; Truter and Wehner, 2004; Virgen-Calleros et al., 2000; Woodhall et al., 2007; Zhang et al., 2014). *Rhizoctonia solani* can increase to high levels as a result of poor management activities, such as not growing resistant varieties, not using crop-rotation for a long time and overuse of chemical control (Dekker, 1976; Lehtonen et al., 2008b; Tsror, 2010).

In Iraq, growers have utilized pesticides, fungicides and herbicides very intensively in agriculture, so there has been increased pollution of the soil and chemical resistance in pathogens, which leads to plant diseases and productivity losses. In addition, there has been a lack of interest in using biological control agents (Personal observations). There is a need to

develop new technologies which reduce *Rhizoctonia* inoculum in soils and crops through the using of crop-rotations with cereal crops and biologically safe options like biocontrol agents to diminish the harmful impacts on potato plants.

The use of *Trichoderma* strains as biocontrol agents to reduce or eliminate symptoms of Rhizoctonia stem canker and black scurf disease of potatoes is a possible alternative to conventional methods of management. Strains of *Trichoderma* are known for many effects on plants and soil organisms, including inducing root branching and increasing shoot biomass through the colonization of roots of plants, antibiotic activity, parasitizing other fungi, competition with harmful plant microorganisms, endophytic competence, and enhancing plant nutrient uptake (Contreras-Cornejo et al., 2016; Li et al., 2015). *Trichoderma* added to the soil can improve soil fertility and soil structure (Ellouze et al., 2014). There are several studies on *Trichoderma* as biological control agents that can reduce soilborne diseases, including stem canker and black scurf disease caused by *R. solani* AG-3PT. However, some researchers have suggested that the interactions between *Trichoderma* isolates with cropping system or fertilizer management or use of resistant potato varieties may have a role in increasing inhibition of soilborne plant pathogens and at the same time promoting plant growth and increasing yield (Beagle-Ristaino and Papavizas, 1985; Ellouze et al., 2014; Guchi, 2015; Naz et al., 2008; Rauf et al., 2015). For this reason, the interactions between *Trichoderma* isolates as biological control agents with variety resistance, fertilizers and intercropping with brassicas were examined in this study.

1.2. Objectives

The objective of this work was to examine the efficacy of *Trichoderma* isolates for biological control as well as the interactions between *Trichoderma* and different aspects of the cropping system in order to improve management of *R. solani* AG-3PT in potato. The aims were to answer the following questions:

Does resistance to disease alter the effect of biological control agents?

Do fertilizers influence the effect of biocontrol agents?

Do other plants in the cropping system have an effect on biological control?

1.3. Structure of the thesis

The Literature Review in Chapter 2 provides information on the disease, beneficial *Trichoderma* isolates in disease management and their mechanisms, resistance of potato varieties to the pathogen, application of fertilizers and their effects on microorganisms and impacts of biofumigation on soilborne fungal disease.

The trials in Chapter 3 isolated and identified *Trichoderma* fungi as biocontrol agents. These isolates were tested for antagonism in dual culture, antibiotic production and reduction of symptoms in infested soils. In addition, uniform potato seedlings were produced in tissue culture for use in experiments.

The trials in Chapter 4 evaluated six potato varieties for disease resistance against *Rhizoctonia solani* AG-3PT and the interaction of resistance with the effect of *Trichoderma* isolates on these varieties. Furthermore, it included a comparison of the effects of compounds in potato tissues on radial growth and dual culture interaction between pathogen and biocontrol agent, and tested induced resistance in a variety with moderate susceptibility.

Chapter 5 describes experiments on the effect of nutrients on antagonism by using dual culture and antibiotic production tests. The interaction between fertilizers and biocontrol agents on disease and plant growth was tested in pot trials with different nutrients. Finally, a field trial tested the interactions between different formulations of NPK fertilizer and the effects of *Trichoderma* species.

Chapter 6 describes the first part of a test of the hypothesis that biofumigant crops can alter the balance between pathogens and their antagonists. The effects of *R. solani* AG-3PT and *Trichoderma* isolates on *Brassica* species were examined. Growth and dual culture experiments tested the effects of volatile and non-volatile compounds of *Brassica* plants on growth and interaction between the pathogen and biocontrol agents. The effect of root exudates of potato, cabbage and broccoli on the interaction between pathogen and biocontrol agents was also tested.

The second part (Chapter 7) tested the effects of *Trichoderma* species in an intercropping system between potato and *Brassica* plants in a glasshouse trial and a field trial.

Chapter 8 (General Discussion) integrates the key discoveries for each chapter and specifies the gaps in knowledge with suggestions for future research arising from this study.

Chapter 2. Literature Review

2.1. Introduction

The soil fungus *Rhizoctonia solani* is widespread throughout the world, and consists of both saprophytic and plant-pathogenic strains. Specific strains or sub-groups can cause disease on a wide variety of different plant species and types, with some forms able to cause black scurf and stem canker disease of potato (Ritchie et al., 2013). Abundant experiments have utilized various techniques of control to mitigate the harmful effects of this pathogen. Biological control strategies are one of these processes which utilize soil microbes or natural materials (Hicks et al., 2014).

The interaction of biocontrol *Trichoderma* species with different aspects such as fertilizers, resistance of varieties and intercropping system (*Brassica* species) can reduce or eliminate Rhizoctonia diseases in potato plants. This chapter will review previous work on the pathogen, disease and biological control which is relevant to all aspects that are related to the research.

2.2. Description of disease

2.2.1. Pathogen

Stem canker of potato and black scurf diseases are caused by *Rhizoctonia solani* AG-3PT (synonym: *Thanatephorus cucumeris* (Frank) Donk), and can be found on almost all underground portions of plants at different times during the season (Kumar et al., 2017a). Generally, *Rhizoctonia solani* has been divided into subgroups called anastomosis groups (Chand and Logan, 1983), labelled from AG-1 to AG-13. Isolates of AG-2, AG-3, AG-4, AG-5, AG-7 and AG-8 have been reported as causal agents of potato diseases (Baijnath, 2012), as well as AG-9 that was identified by Ferrucho et al. (2012). However, AG-3 is the most common of these on potatoes (Carling et al., 2002). Two reproductively isolated groups have been found in AG-3, one of which is restricted to tobacco (Kuninaga et al., 2000). Consequently, the strains that are pathogenic to potato are referred to as AG-3PT. The fungus is a basidiomycete, but the basidiospore stage is extremely rare. *R. solani* is not known to produce asexual spores, but it

has an asexual life cycle, where it produces mycelium and black sclerotia on infected potato tubers (Kirk et al., 2007; Ogoshi, 1987).

2.2.2. Symptoms

Symptoms are responses expressed by the host plant as a result of injury by the pathogen, or exterior or interior changes that appear on the plant after an infection by any type of disease. Symptoms depend on the sort of pathogen and the type of plant. Potato plants are infected by *Rhizoctonia solani* AG-3PT on underground and aboveground parts of plants in two phases, infection of growing plants (stem canker) and infection of tubers with sclerotia (black scurf) (Atkinson et al., 2010; Kumar et al., 2017a; Tsror, 2010).

The disease affects the plant at all stages of growth and even small seedlings are infected by the pathogen. Tsror (2010) stated that symptoms appear in the form of stem canker (cankers on sprouts, underground stems and stolons which limit the plant growth). Also, brown cankers on sprouts that may become necrotic can prevent or postpone emergence. Furthermore, it causes desiccated, gummy and stunted parts of plants that include stems, stolons and roots. The disease causes chlorosis and purpling of leaves. Thus, it stops the flow of sucrose solution from the leaves to the tubers. As a result, potato plants produce a small amount and poor quality of tubers when attacked by the pathogen *R. solani* AG-3PT under suitable environmental conditions in early stages of plant development (Campion et al., 2003; Tsror, 2010).

Infection by the pathogen will cause cracks and distortions of tubers. Black scurf makes tubers distorted by forming sclerotia on different portions of tubers in the early season. Sclerotia are an irregularly shaped mass of hyphae with dark to black colours which serve as survival and reproductive bodies (Wharton et al., 2007).

2.2.3. Disease cycle

Rhizoctonia solani AG-3PT overwinters as sclerotia which can remain under environmental extremes in field soil for at least 18 months (Ritchie et al., 2013), and as mycelia on infected tubers in plant residue or in infected soil (Kirk et al., 2007). The fungus infects the roots, stolons, buds, tubers and stems of all sizes under suitable conditions, including cold weather when both the temperature of air and soil are between 5 and 25^{o}C, high soil moisture, high organic matter, and a neutral to acid soil pH 7 or less (Kirk et al., 2007).

Initially, symptoms in the early season appear in the crown zone of plant. Sometimes symptoms do not appear on stems, only on tubers. During the growing season stolons and roots can be attacked at any time. In addition, late-season damage may result in formation of aerial tubers. After that, sclerotia start to form on tubers while still attached to a mother plant, and formation of sclerotia continues into storage (Wharton et al., 2007).

2.2.4. Disease assessment

Disease assessment involves the measurement and quantification of plant disease, which is of essential importance in the study and analysis of plant disease epidemics which can lead to yield loss caused by pathogens (Cooke, 2006). Several different methods of assessment have been used for Rhizoctonia diseases on potatoes, depending on the growth stage, the symptoms being expressed, and the purpose for assessment. Emergence is the percentage of germinated tubers which is generally assessed from first emergence to 21 to 40 days after planting. The percentage of seed germination can be reduced by seed piece decay due to the pathogen (Larkin, 2016; Muzhinji et al., 2018). The pathogen can also affect the tips of sprouts and either kill the sprouts or delay their emergence (Larkin, 2016). In general, reduced emergence due to the effect of the pathogen can reduce the plant stand densities and yield. However, compensation by the plants may mean that lower density gives more stems and tubers per plant, so the relationship between plant number and yield can be weak (Bussan et al., 2007). However, emergence is still useful as an early indicator of disease.

Canker severity is the amount of disease which is expressed on the different parts of potato plants including stems, stolons and roots. In field trials, canker severity rating can be based on the disease incidence such as proportion of stems, stolons and roots with lesions (Atkinson et al., 2010; Tsror and Peretz-Alon, 2005). Most papers calculate the proportion of length or area of the tissue covered with lesions. This can be given as a mean percentage (Atkinson et al., 2010; Muzhinji et al., 2018). Commonly severity is evaluated by placing stems or stolons into a small number of categories with different disease levels. For example, a 0-4 scale can be used with categories of no lesions, < 10% area covered, 10-25% area covered, 26-50% area covered and > 50% area covered. The number of stems or stolons in each class is used to calculate a disease index, often on a 0-100 scale (Wilson et al., 2008a). When disease index is calculated out of 100, it usually gives a higher numerical score than the mean percent severity, because the categories usually give greater weight to low disease severities.

If canker severity is severe, the stem might be girdled, interfering with the normal movement of water and carbohydrates throughout the potato plants (Wharton et al., 2007). Canker severity is usually related to reduced marketable yields of tubers (Khandaker et al., 2011; Larkin, 2016). However Tsror (2010) claimed that disease severity is not always associated with yield reduction. It is difficult to find the evidence on which this claim is based, but Tsror and Peretz-Alon (2005) said that in areas with high temperatures the major damage is to quality of tubers, and rarely to the total yield. Because tuber quality is affected more by scurf than canker, assessing canker symptoms may be less useful in some situations. There is also a problem with using cankers because they do not always appear on the plant; however, the relationship between pathogen effects and plant growth is important.

Black scurf is caused by *R. solani* AG-3PT and can be found on all underground parts of plants, particularly progeny tubers (Tsror and Peretz-Alon, 2005). In field trials, black scurf rating can be based on the disease incidence such as proportion of roots and tubers with sclerotia (Tsror and Peretz-Alon, 2005). Most papers calculate the proportion of number or area of the tuber covered with sclerotia. This can be given as a mean percentage (Muzhinji et al., 2018). Generally, black scurf severity is evaluated by placing roots or tubers into a small number of categories with different disease levels. For example, a scale of 0-6 can be used with different categories of no sclerotia, < 5% are covered, 6-10% area covered, 11-25% covered, 26-50% area covered, 51-75% covered and 76-100% area covered (Atkinson et al., 2010). The number of roots or tubers in each class is used to calculate a disease index, often on a 0-100 scale (Atkinson et al., 2010; Ritchie et al., 2013).

Black scurf sclerotia causes economic loss and affects the quality of tubers (Kumar et al., 2017a). Sclerotia can survive in soil for many years under different conditions. Therefore, measuring scurf severity index helps to know the carryover of sclerotia inoculum in the field and predict future epidemic disease occurrence (Tsror and Peretz-Alon, 2005).

Rhizoctonia canker reduces the shoot and root dry weight, and the number of stolons and stems (Bienkowski, 2012; Hicks et al., 2014). The pathogen can penetrate the tips of sprouts before they emerge from the soil and then attacks the stolons after growing later. Cankers can stop or slow the growth of the infected stem or stolon. Cankers can sever the stolon or shoot from the plant or kill the growing point (Wharton et al., 2007). This research has shown that the pathogen can affect plant growth without obvious or severe canker symptoms. Plant growth parameters, such as shoot dry weight, can be used as a measure of the effect of

the pathogen on the plant. Canker has greatest effects on total yield and scurf has greatest effects on marketable yield (quality) (Hicks et al., 2014; Larkin, 2016). Maximising yield is the ultimate goal of disease management, but yield is affected by more than just disease.

Therefore, almost all papers give data on symptoms on potato plants, either stem canker or black scurf disease severity and incidence, but not on plant growth. They are based on the visual appearance of the plant, rather than the effects of disease on growth and yield. In field experiments for two years, Hide et al. (1992) used three varieties of potato (Desiree, Maris Piper and Pentland Squire) with and without inoculum of *Rhizoctonia solani* grown on wheat to assess the effect of *R. solani* on plant growth and yield at Rothamsted Experimental Station, UK. In all varieties, plant emergence was delayed by inoculating with *R. solani*, and also decreased significantly the plant growth parameters such as weights of shoot, number of tubers and yield, compared with the uninoculated treatments. Also, Daami-Remadi et al. (2008a) showed the effect of *Rhizoctonia solani* sclerotia on the growth of potato plants (cv. Spunta) and yield. They used five levels of infection by *R. solani* based on a scale (1-5) to measure the disease severity on potato plants that were grew into plastic pots. They found that high levels of infection reduced significantly the stem fresh weight, stem dry weight, root fresh weight, root dry weight, and tuber fresh weight, compared with the other levels.

There is a shortage of studies that have been done on the relationship between infection with *R. solani* AG-3PT and potato plant growth parameters such as shoot and root dry weight, plant height, number of stolons, number of tubers and tuber fresh weight. Diseases are considered important because of their effects on plant growth and yield. Therefore this study was focused on the relationship between pathogen and plant growth parameters in different areas.

2.2.5. Management

There are several methods to manage disease caused by the pathogen *Rhizoctonia solani* AG-3PT in potato. Cultural control which means alterations to cropping practices is very important to control soilborne diseases. Cultural control practices include use of disease-free propagation material, pathogen-free soil, crop-rotation, intercrops, tillage, timing of harvest and haulm and soil management and irrigation (Brierley et al., 2009; Tsror, 2010). For example, tillage management can change the availability of different nutrients in the soil, and affect pathogen survival, where tillage or burial systems can reduce pathogens directly through the

placement of plant residues in the soil (Ritchie et al., 2013), or indirectly by the intensity of the activity of soil microbial and competition effects (Bailey and Lazarovits, 2003). In addition, tillage management immediately affects the chemical and physical soil properties like soil temperature and humidity, root growth and nutrient absorption and populations of plant pathogens. These factors may affect the variability and viability of pathogens and the sensitivity or resistance of the plants (Blok et al., 2000).

Chemical control with fungicides have been used to control *R. solani* AG-3PT disease on potato by different methods like potato seed treatment and in-furrow fungicide application (Kirk et al., 2007; Tsror, 2010). In seed treatment, there are many types of chemical materials used to control the pathogen such as Tops MZ (thiophanate-methyl 2.5% and mancozeb 6%), Moncoat MZ (flutolanil 1.5% and mancozeb 6%), Maxim MZ (fluioxinil 0.5% and mancozeb 6%), Nubark Maxim (fluioxinil 0.5%) and Maxim 4FS (fluioxinil 40.3%), while Quadris (azoxystrobin 22.9%) and Moncut 70DF (flutolanil 70%) were utilized in-furrow (Kirk et al., 2007). Although chemical control might be efficient in the short term, the long term effects are less profitable. Fungicides can kill beneficial microorganisms along with the pathogens in the soil (Cook and Baker, 1983), which can diminish the productivity and quality of the soil, basically affecting plant health and productivity. Moreover, pathogens might develop resistance to the chemical substances utilized for their control (Dekker, 1976). Fungicides are extra inputs and expenses for the farmer, and may negatively impact on the environment and human health through surface flow, persistence, residues and leaching. Many pathogenic microbes in plants have developed resistance against fungicides. There is a need to find sustainable alternatives with low cost for the combat of plant pathogens. Biological control is a solution that works within the microbial ecology, and is likely to give long-term control of the pathogens, is environmentally friendly and does not have any type of pollution (Larkin et al., 1998).

The use of resistant varieties would obviously improve the control of *R. solani* AG-3PT disease in the field (Mohsan et al., 2016). Many studies have shown that different varieties of potato were more resistant or susceptible to the Rhizoctonia disease and other soilborne pathogens. Examples of resistance to disease are discussed in detail later in the literature review.

Biological control is the use of antagonistic organisms to inhibit the pathogen. An example is using seed treatment with the biocontrol agent *Trichoderma harzianum* which can

reduce stem canker and black scurf disease of potato caused by *R. solani* (Beagle-Ristaino and Papavizas, 1985). Interactions between microorganisms and plants are necessary for plant health; nevertheless, the effect of the interactions with plants range from positive (symbionts which support fertilizer acquisition), to negative (pathogens which cause plant diseases) (Cripps-Guazzone, 2014; Lal et al., 2016). Many previous studies have used *Trichoderma* strains for biological control because they are generally rhizosphere competent, have antibiotic and mycoparasitic effects, induce resistance, and show endophytic colonization and competition for nutrients (Cripps-Guazzone, 2014). Biocontrol could be used to control the Rhizoctonia disease in potato in combination with other methods as an integrated management process. This aims to emphasise involvement of different techniques (such as cultural control, more resistance of varieties, fertilizers and intercropping system) for managing the effect of Rhizoctonia disease and increasing yield. Biological control of Rhizoctonia on potatoes will now be discussed in detail.

2.3. Biological control of *Rhizoctonia* on potatoes

2.3.1. Definition

The main goal of the use of biological control is the reduction or mitigation of pathogens and the disease they cause by using other non-pathogenic organisms. Biocontrol organisms can be bacteria, fungi, nematodes or viruses (Lal et al., 2016; Pal and Gardener, 2006). Biological control mainly relies on the use of organisms which are antagonistic to the target plant pathogens (Arora and Dwivedi, 1980; Larkin, 2016).

2.3.2. Biological control of Rhizoctonia disease of potato

The effect of isolates of *Trichoderma* as biocontrol agents was recognized in the 1930s, and since then isolates of *Trichoderma* have been confirmed to control several plant diseases of vegetable crops and fruit (Lal et al., 2016; Weinding, 1932). Many attempts have been conducted to use different biocontrol agents to control stem canker and black scurf disease on potato. For instance, when seed tubers of potato were treated with *Trichoderma viride* and *Bacillus cereus* strain B4 to control disease, incidence of scurf was reduced by up to 42% (Somani and Arora, 2010).

Some bacteria strains (*Pseudomonas* spp.) associated with the plant rhizosphere have also been used to control black scurf disease and showed significant ability to reduce the incidence and severity of this important disease in addition to enhancing plant growth (Mrabet et al., 2013; Tariq et al., 2010). Tariq et al. (2010) tested 28 isolates of bacteria recovered from infected and healthy potato plants in Pakistan for their inhibition level and found nine of these isolates that reduced the fungal growth in vitro (Tariq et al., 2010). Grosch et al. (2005) also reported that three isolates of bacteria (*Pseudomonas fluorescens* B1, *Pseudomonas fluorescens* B2, and *Serratia plymuthica* B4) from potato-associated bacterial communities, were tested as biocontrol agents against *Rhizoctonia solani* in potato. In growth chamber trials, all BCAs significantly decreased potato disease severity caused by *R. solani*, but *Pseudomonas fluorescens* B1 showed the greatest effect. In field experiments, the disease severity was significantly reduced. The highest scurf-disease-reduction effect on potato was accomplished by strain B1, followed by B2 and then B4 at the rate of 37, 33 and 31%, respectively, while the potato tuber yield increased by 12% for strain B1, 6% for B2 and 17% for B4 compared with the control treatment (*R. solani*). Further, Berg et al. (2005) examined endophytic and ectophytic bacterial isolates from potato by using dual cultural method against soilborne diseases (potato early dying disease and stem canker) caused by *Verticillium dahliae* and *Rhizoctonia solani*, respectively. The isolates of *Pseudomonas putida* and *Serratia plymuthica* 3Re4-18 as biological control agents were effective to inhibit the plant pathogens, and at the same time were shown to improve plant growth.

El Khaldi et al. (2016) used two date palm composts with their associated fungi and found that the severity and incidence of black scurf and stem canker were significantly reduced in greenhouse trials, however, using the extract of date compost after heating or filtration had no effect on *R. solani* in vitro. The interaction type between *R. solani* hyphae and tested antagonistic fungi was compared by light microscopy and showed different mechanisms of actions such as mycoparasitism, mycelia lyses and formation of mycelia cords via anastomosis.

The use of some isolates of *Trichoderma* effectively diminished the viability of *R. solani* sclerotia in greenhouse tests (Beagle-Ristaino and Papavizas, 1985), when fermentor biomass containing chlamydospores of *T. harzianum* and *T. virens* was added to soil prior to planting. This resulted in an increase in the viable populations of antagonists in soil and on roots to control disease. *Trichoderma harzianum* (Tri4 fermentation biomass) and *Trichoderma virens* (GL-21) reduced germination of *R. solani* sclerotia by 83 and 86%, respectively. The

best inhibition percent of disease were 49 and 45%, respectively, for *T. viride* and *Trichoderma virens* (Beagle-Ristaino and Papavizas, 1985). Also, *Trichoderma viride* and *Trichoderma virens* reduced disease incidence significantly in the field between 50% for *T. viride* and 55% for *T. virens* compared with pentachloronitrobenzene (PCNB). The biocontrol agents were more effective than chemical treatment of PCNB fungicide (Beagle-Ristaino and Papavizas, 1985). Moreover, stem canker disease incidence and severity were reduced by 37-75% using four different biocontrol agents including *Trichoderma virens* Gl-21, and *Trichoderma harzianum* strain T-22 was applied to the soil in two seasons in a field trial (Larkin, 2016). In this trial, biocontrol formulations of two fungi and two bacteria were separately applied in the field with a combination chemical/ biological treatment and chemical seed treatment. The biological treatment controlled the disease and increased the productivity and quality of potato in the two studied seasons.

The use of *Trichoderma harzianum*, non-pathogenic *R. solani* and cattle manure compost amendment (CMC-H) in field trials diminished black scurf disease under organic cultivation of potatoes (Barak and Sneh, 2001). The application of non-pathogenic binucleate *R. solani* (RS 521 and RU 56-8-AG-P) significantly diminished disease incidence and severity, while *T. harzianum* reduced disease incidence by 97% in furrow application, and also diminished the severity of stem canker disease in potato plants (Barak and Sneh, 2001; Wilson et al., 2008b). Also, Wilson et al. (2008a) showed that the combination of flutolanil fungicide as a seed treatment and biocontrol agent *T. harzianum* can protect potato plants from stem canker and black scurf diseases caused by *R. solani* AG-3PT. It reduced the disease level from 31 to 11%, and at the same time increased the size of potato tubers from 35 to 60%, while *Streptomyces griseoviridis* and *Gliocladium catenulatum* did not give any protection against the pathogen (*R. solani*) in potato plants. Bernard et al. (2014) claimed that soil management practices may promote potato production and disease control. Three different methods to suppress soilborne diseases, (rapeseed rotation crop, compost amendment, biological control amendments *Trichoderma virens, Bacillus subtilis*, and *Rhizoctonia solani* hypovirulent isolate Rhs1A1), when applied in a rapeseed rotation crop diminished the incidence and severity of stem canker, black scurf, common scab, and silver scurf, while compost amendment increased disease incidence and severity. The combination of biocontrol agents and soil managements significantly reduced potato diseases, while the combination between compost and hypovirulent isolate *R. solani* 1A1 led to reduce black scurf of potato incidence.

Three isolates of *Trichoderma* were obtained from *Rhizoctonia solani* sclerotia (*Trichoderma reesei* G1/8 which was reclassified recently as *Trichoderma parareesei* (Atanasova et al., 2010), and *T. viride* G3/2 and G1/9). They were identified by using PCR fingerprints, and tested as biological control agents against *R. solani* AG-3PT (Grosch et al., 2006). In this study, the ability to parasitise and reduce sclerotia germination was tested at different temperatures between 12 and 20^{o}C in a growth chamber and on PDA medium. *Trichoderma parareesei* and *T. viride* significantly reduced the germination of *Rhizoctonia* sclerotia at the rate of 10.4% at 20^{o}C and 21.9% at 12^{o}C. Consequently, the strains of *Trichoderma* were able to suppress the pathogen (*R. solani*) under different conditions.

In New Zealand, the applications of *Trichoderma* strains (*T. virens*, *T. harzianum*, *T. barbatum*, *T. harzianum*, *T. rossicum* and *T. crassum*) significantly reduced the *Rhizoctonia* stem canker disease of potato plants and increased plant yield and biomass production under glasshouse conditions. They used a combination of conidial suspensions of biocontrol agents against *R. solani* AG-1, 2 in a glasshouse trial and AG-3PT in a field trial, with inoculum on barley seeds in pot trials (Hicks et al., 2014).

Morris et al. (1995) reported that *Verticillium biguttatum* produced antifungal hydroxymethyl-phenols as metabolites with strong antibiotic activity during biocontrol in addition to enzymes which dissolve and penetrate *R. solani* AG-3PT cell walls. Likewise, Van den Boogert and Luttikholt (2004) tested the interaction between non-pathogenic *Verticillium biguttatum* and synthetic fungicides to control black scurf disease caused by *R. solani* AG-3PT and other potato diseases. They found that some fungicides such as azoxystrobin, chlorothalonil and thiabendazole were fungitoxic to the biocontrol agent *V. biguttatum* in vitro and in field trials. The combinations between the biological control agent *V. biguttatum* and cymoxanil or propamocarb were examined with unripe potato tubers, where these combinations diminished black scurf disease and tuber rot disease caused by *Pythium* sp. and *Phytophthora* sp.

Demirci et al. (2009) found that the hyphae of *V. biguttatum* coiled around *R. solani* hyphae (AG-3PT), with the hyphae penetrating the fungal host cell walls. According to McQuilken and Gemmell (2004) *V. biguttatum* produced chitinase, β-1, 3-glucanase and protease. So, these enzymes may play a role in dissolving and penetrating *R. solani* hyphae (Demirci et al., 2009). In addition, *V. biguttatum* isolates decreased the viability of *R. solani* hyphae on PDA, where all treatments of *V. biguttatum* penetrated *R. solani* hyphae and infected

the cells of host, resulting in death. Also, the isolates of *V. biguttatum* decreased germination of *R. solani* sclerotia. The percentages of germination with *V. biguttatum* (ME-3 and MC-7) were 18 and 2%, respectively, whereas the viability of non-inoculated sclerotia was 83.5% (control) (Demirci et al., 2009).

Some microbial endophytes are also reported to have significant antibiotic activity against the pathogen (*R. solani*). This was tested in the laboratory and glasshouse (Lahlali and Hijri, 2010), when six microbial endophytes were isolated and identified by morphology and PCR as *Alternaria longipes*, *Epicoccum nigrum*, *Phomopsis* sp., and *Trichoderma harzianum*. The ability of these microbial endophytes to suppress the pathogen was tested and the biggest inhibition zone against *R. solani* was 82% for biocontrol agent *T. harzianum*, followed by 39% for *Phomopsis* sp., 21% for *A. longipes*, 21% for *E. nigrum* E18, 11% for *E. nigrum* E1 and 10% for *E. nigrum* E8. Also, they found that biological control agent *T. harzianum* works as a mycoparasite and competitor to penetrate hyphae and obtain nutrients from it. However, *E. nigrum* and *A. longipes* create secondary metabolites to inhibit *R. solani*, whereas *Phomospsis* sp. compete for nutrients with the pathogen.

Carisse et al. (2001) showed that strain P130A of *Microsphaeropsis* sp. was used to treat potato tubers to control Rhizoctonia stem canker and black scurf diseases. *Microsphaeropsis* sp. significantly decreased the percentage of sclerotia germination from 82 to 5.8%. The fungal antagonist colonized hyphae of *R. solani* AG-3PT and inhibited mycelial growth through the induction of toxins and a rupture of the pathogen plasma membrane and then the penetration of pathogen. Therefore, it caused cytoplasmic disruption and collapse of plasma membranes of the pathogen (Carisse et al., 2001; Jager and Velvis, 1988; Wicks et al., 1995).

Yao et al. (2003) demonstrated that the use of mycorrhization (*Glomus etunicatum*) at a low level from 5 to 7% decreased the infection of *R. solani* in potato plantlets (Yao et al., 2002). The mycorrhizal fungus encouraged phytoalexins in the potato roots to inhibit the growth of *Botrytis cinerea*, *Pectobacterium atrosepticum* and *Phytophthora infestans*, while the accumulation of solavetivone or rishitin was not detected in the potato shoots (Morandi, 1996; Wyss et al., 1991). However, in vitro, the bioassays of rishitin and solavetivone reduced *R. solani* growth at high concentrations (Engström et al., 1999; Glazener and Wouters, 1981; Yao et al., 2003).

Brewer and Larkin (2005) used biocontrol agents (*Bacillus subtilis, Cladorrhinum foecundissimum, Laetisaria arvalis, Paenibacillus polymyxa, Pseudomonas fluorescens, Penicillium* sp., *Rhizoctonia zeae, Stilbella aciculosa, Verticillium biguttatum, Trichoderma virens, T. harzianum* and *Trichoderma* sp.) in glasshouse pot trials with field soil (silt loam) to inhibit *R. solani* AG-3PT. This study showed that the black scurf severity was reduced by 40-49% by *Rhizoctonia zeae, Stilbella aciculosa,* chemical control and *Bacillus subtilis*, whereas the lowest percentage of inhibition was just over 20% for *Pseudomonas fluorescens*. In this experiment, all the fungi and bacteria were used in a suspension of spores or in a suspension of bacteria cells. This resulted in inhibition of potato stem canker and black scurf disease in the greenhouse (Brewer and Larkin, 2005).

Ultimately, biological control is one of the most important components of integrated pest management (IPM) programs which concentrate strongly on the protection of plant pathogens. So, the present study will work on *Trichoderma* species as biocontrol agents for stem canker of potato disease. There are several ways for biocontrol agents to work, but this study will focus on *Trichoderma* isolates ability to protect potato plants from stem canker disease and the integration of these isolates with other factors to reduce the pathogen and enhance plant growth.

2.4. Modes of action of biocontrol agents

2.4.1. Antibiosis and secondary metabolites

Antibiosis is inhibition of one organism by metabolites of another. The metabolites which have the ability to inhibit or kill another organism are called toxins. Most *Trichoderma* species produce volatile and non-volatile toxic compounds such as harzianic acid, tricholin, peptaibols, alamethicins, antibiotics, 6-penthyl 1-a-pyrone, viridian, gliovirin, massoilactone, glisoprenins, heptelidic acid, koningic acid and others (Benitez et al., 2004; Howell, 2003; Kumar et al., 2016). Benitez et al. (2004) reported that *Trichoderma* has the ability to secrete metabolites which either hinder germination of spores (fungistasis) or adjust the rhizosphere by acidifying the soil or kill the pathogens by damaging cells, so that soilborne fungi cannot grow. These authors found that *T. harzianum* strains were the most effective at inhibiting *R. solani* growth compared with other *Trichoderma* strains. *Trichoderma* species produce secondary metabolites with antifungal or antibacterial activity when the interaction occurs with

other organisms which are causing plant disease (Benítez et al., 2004; Sivasithamparam and Ghisalberti, 1998).

The production of volatile and non-volatile compounds by *Trichoderma* isolates which were isolated from chickpea suppressed the growth of soilborne pathogens such as *Fusarium oxysporum* f. sp. *cicerci, Rhizoctonia solani* and *Sclerotium rolfsii* on PDA plates (Nagamani et al., 2017). Howell (2003) mentioned that *Trichoderma virens* as a biocontrol agent produces antibiotics such as gliovirin and gliotoxin which strongly inhibited the growth of *Pythium ultimum, Phytophthora* species and *Rhizoctonia solani*. Also, Tariq et al. (2010) demonstrated for a *Pseudomonas* strain (StS3) that the effect of diffusible antibiotic and volatile antibiotics as fungistatic metabolites were similar in inhibiting *R. solani* AG-3PT growth. The isolates of *Pseudomonas* StT2 and StS3 had biocontrol efficacy against *R. solani* growth of 65 and 74%, respectively, and they gave a significant increase in the yield of tubers. In addition, the mycelium growth of *R. solani* was inhibited by antifungal activity of *Trichoderma harzianum* through the enhancing of enzyme production (protease, β-1,3-glucanase and chitinase), where the rate of disease incidence was one third of the control of *R. solani*, while the percentage of yield increase of potatoes was 95.2% (Tariq et al., 2010). *Trichoderma* species produce antibiotics that play a role in inhibiting the pathogen growth on different culture media. While the effect of *Trichoderma* and their secondary metabolites are not visible on roots in the soil, they have been demonstrated in vitro (Contreras-Cornejo et al., 2016).

2.4.2. Mycoparasitism

Trichoderma can parasitise other fungi. It has been shown to include four steps: chemotropism, recognition, attachment and coiling, penetration of cell wall and digestion of host cell content (Steyaert et al., 2003). Chemotropism of an antagonists means direct growth towards the host, commonly along a chemical gradient of sugars and/or amino acids. It commonly precedes recognition and is possibly non-host specific, however, the two processes might be related. Recognition is being mediated by lectin-carbohydrate binding between *Trichoderma* strains and host. Then *Trichoderma* isolates coils around the host hyphae and penetrate the hyphae by appressoria (Barak et al., 1985; Steyaert et al., 2003). There has been a wealth of protein and molecular studies on genes that are involved in mycoparasitism by *Trichoderma* species, including nitrogen and carbon. *Trichoderma* strains produce cell wall lytic enzymes. The cell wall enzymes may also have an effect without necessarily involving

parasitism. The fungal cell wall contains proteins (3-20%), polysaccharides (80%), with pigments, lipids and inorganic salts in lower amounts. The cell wall lysis of the host is performed by the action of glucanases, chitinases, and proteinases which are produced by *Trichoderma*, and might be combined with antibiotics (Schirmböck et al., 1994; Steyaert et al., 2003). For example, the first apparent physical contact between *T. harzianum* and its host, *R. solani*, occurrs from 2 to 3 days after inoculation, followed by growth inhibition. After that, *T. harzianum* hyphae coil around *R. solani* hyphae and penetrate the host to use the nutrients. The host hypha then dissolves and dies (Cumagun, 2012; dos Reis Almeida et al., 2007).

A previous study has shown three *Trichoderma harzianum* isolates differed in their ability to attack *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium aphanidermatum*, based on β-1, 3-glucanase, cellulase and chitinase production (Elad et al., 1982). Also, parasitism of *R. solani* sclerotia was proved in culture, but this situation has been in doubt in natural environments in soil (Cumagun and Ilag, 1997). Rudakov (1978) mentioned that mycoparasitic activity by *Trichoderma* spp. often occurs in nutrient-rich media; however, species of *Trichoderma* are perhaps not parasitic in nature. *T. hamatum* as a parasite on *Pythium* spp. or *R. solani* AG-3PT grew directly on the host hypha by producing appressoria on PDA medium in culture. After contact by the biocontrol agent, the hypha coiled around the pathogen and eventually collapsed the hyphae (Chet and Baker, 1981; Chet et al., 1981). A previous study found that the genes of endochitinase of *Trichoderma* spp. when cloned into potato plants resulted in an augmentation of their property of resistance against pathogenic fungal growth (Kumar et al., 2016).

2.4.3. Induced resistance

The meaning of induced resistance is stimulating the plant to produce compounds or form barriers locally or systemically that can protect the plant against pathogens, by using organisms or chemicals that are applied to the plants (Bailey and Lumsden, 1998). When *Trichoderma* species colonize the plant this can lead to lower reduction in disease efficiency which is caused by various plant pathogens and induced systemic resistance at the site of inoculation (Kumar et al., 2016). *Trichoderma* strains protect plants against root soilborne pathogens including viral, fungal and bacterial pathogens through the colonization on the roots and also stimulate plant defense mechanisms, which indicates the induction of resistance similar to hypersensitive response (HR), induced systemic resistance (ISR) and systemic acquired resistance (SAR) in plants (Benitez et al., 2004). The plant resistance can result from

the increasing concentration of enzymes and metabolites such as chalcone synthase (CHS) and phenyl-alanine ammonio-lyase (PAL), involved in the biosynthesis of phytoalexins (HR response), chitinases and glucanases. These comprise pathogenesis-related proteins (PR) and enzymes involved in the response to oxidative stress (Benitez et al., 2004). For example, Harman et al. (2004) claimed that there are three ways to recognize induced resistance. The first includes pathogenesis-related proteins produced due to attack by other phytopathogenic organisms. The second is when necrosis or wounding of the plant induces production of pathogenesis-related proteins. The third is when beneficial microbes induce systemic resistance in plants. A previous research illustrated that the efficacy of *T. harzianum* to protect potato plants from infection by *R. solani* at the first stages of growth (7 days of growth). *T. harzianum* colonies induced plant defense responses against the pathogen (Gallou et al., 2009).

Gallou et al. (2009) used qRT-PCR to compare the expression of defense response genes in potato roots in laboratory plants inoculated with *R. solani* AG-3PT and/or *T. harzianum*. The effect of *T. harzianum* against *R. solani* was illustrated by the induction of lipoxygenase expression at 24 hours post-inoculation (hpi) and glutathione-S-transferase 1 expression at 72 hpi in plantlets inoculated with *T. harzianum* and *R. solani* at the same time. The studies showed that the addition of metabolites of *T. harzianum* can work as elicitors to induce plant resistance against the pathogens. Thus, *T. harzianum* works on defense responses of potato plants against *R. solani*, and is dependent on signalling by the molecules salicylic acid and jasmonic acid (Gallou et al., 2009; Viterbo et al., 2002). A previous study indicated that treating plants with *Trichoderma* spp. stimulated systemic resistance through production of jasmonic acid and ethylene which are effective against pathogens (Yadav et al., 2015).

2.4.4. Competition for nutrients

Competition for nutrients can be defined as an indirect interaction between organisms. This could be particularly important, where a resource is in very limited supply, and one fungus has a high demand for the resource such as nitrogen, carbon, potassium or iron. One of the competitors may be more efficient than others in sequestering the nutrients, without apparent interaction. Competition for nutrients is a mechanism used by *Trichoderma* species to control or suppress soilborne plant pathogens (Cripps-Guazzone, 2014; Vinale et al., 2008). *Trichoderma* species have a high ability to mobilize and take up soil nutrients in comparison to other organisms (Chet et al., 1997).

A previous study indicated that competition for nutrients could play an important role in the biological control of *Rhizoctonia solani* by *Trichoderma lignorum* in the culture media (Howell, 2003). Populations of microbes compete for nutritional elements and other environmental resources. Therefore, some microbes exude compounds into the environment that are toxic or inhibitory to their rivals in order to eliminate other species (Fredrickson and Stephanopoulos, 1981). Nelson (1991); Kloepper et al. (1980) reported that biological control agents absorb micro nutrients from the soil more effectively than the pathogens, such as through the production of siderophores as in the interaction between *Pectobacterium atrosepticum* and *Pseudomonas fluorescens*. Almost all filamentous fungi need essential elements like iron for survival. Most fungi secrete low-molecular weight ferric iron chelators, called siderophores to acquire environmental iron (Eisendle et al., 2004). Chet and Inbar (1994) illustrated that some strains of *Trichoderma* spp. produce effective siderophores which chelate iron and prevent other fungal infections.

2.4.5. Competition for space

One of the biological control methods depends on the ability of *Trichoderma* as a biocontrol agent to colonize the root system and prevent soilborne pathogens from infecting the plant. Colonization by *Trichoderma* strains on the plant roots can be affected by various factors such as nutrients, plant species, environment and others. *Trichoderma* species can be established easily in any rhizosphere soils and also can persist for a long time on plants up to many months (Kumar et al., 2016). *Trichoderma* strains are able to colonize plant roots and stimulate plant growth in addition to protection against infectious plant diseases. There are some strains of *Trichoderma* that can establish for a long time through plant root colonization and penetrate into the epidermis, and then they release or produce compounds which induce localized and systemic acquired resistance and plant defense against pathogens (Benitez et al., 2004).

Rhizosphere competence means the efficiency of microorganism to colonise, grow and develop in the rhizosphere soil under different conditions as symbiotic associations with plant roots. *Trichoderma* strains have shown rhizosphere competence and colonization of various plant species (Cripps-Guazzone, 2014). *T. hamatum* LU593 isolate had greater colonization than other isolates in the rhizosphere of lettuce plants in potting mix and protected the roots from infection by *Sclerotinia minor* (Rabeendran et al., 2006). A previous study indicated that two isolates of *Trichoderma* (*T. atroviride* LU132 and *T. hamatum* LU592) colonized pine roots grown in potting mix. They found that *T. hamatum* was a better colonizer than *T.*

atroviride which was reclassified recently as *Trichoderma harzianum* (Mach et al., 1999), and had a strong rhizosphere competence and root penetration. *T. hamatum* was shown to improve plant health and increase growth parameters (shoots and roots) (Hohmann et al., 2011). Also, *T. hamatum* LU592 was shown to increase the activity zone beyond the rhizosphere up to 1 cm away from pine roots grown in unsterile potting mix (Hohmann et al., 2012).

Endophytic colonization is the ability of organisms to inhabit plant organs and colonize internal plant tissues without causing any visible effects or apparent damage to the plant tissues (Bailey and Melnick, 2013). *Trichoderma* species are known to show endophytic colonization for several plant species roots (Harman et al., 2004). Also, Chow et al. (2018) tested the interaction between endophytic biocontrol agent (*Trichoderma asperellum*) and pathogen (*Ganoderma boninense*), with and without interaction in oil palm plants to determine their co-existence in root, stem and leaf tissues by quantitative PCR. Inoculation of oil palm plants was by soil drenching using 100 mL (6 $\log_{10}$ cfuml^{-1}) of fungal inoculum. The trial was done under semi controlled glasshouse conditions for 7 weeks and samples sterilized carefully. The results showed that *T. asperellum* reduced the amount of pathogen in all tissues (Chow et al., 2018). The endophytic colonization of cucumber plants (roots and stems tissues by two isolates of *Trichoderma harzianum* (T-E5 and SQR-T037) enhanced the indole acetic acid production and increased the plant growth parameters in glasshouse soil and hydroponic trials (Zhang et al., 2013). They found that colonization numbers of T-E5 and SQR-T037 in the rhizosphere soil were nearly similar at the beginning of sampling (about 10^6 ITS copies g^{-1} soil) by using quantitative real-time PCR (Zhang et al., 2013).

2.4.6. Growth promotion

Trichoderma isolates play an important role to increase plant growth parameters. *Trichoderma* spp. can even exert positive effects on plants with an enhance in plant growth (biofertilization) and the stimulation of plant-defence mechanisms (Benitez et al., 2004). Also, strains of *Trichoderma* species such as *T. viride*, *T. harzianum*, *T. koningii*, *T. hamatum*, *T. reesi* are being utilized as biofertilizers, but *T. viride* and *T. harzianum* are most commonly used biofertilizer. These *Trichoderma* strains increased the plant growth promotion when applied as seed treatment or spraying through the use of spore suspensions or grown on bran to treat the soil (Kamal et al., 2018).

A previous study indicated that some *Trichoderma* species can interact directly with plant roots, augmenting plant growth potential, tolerance to abiotic stresses and resistance to disease (Hermosa et al., 2012). Lo and Lin (2002) tested some *Trichoderma* species which were isolated from rhizosphere soil and root zone in Taiwan. In a glasshouse trial, *Trichoderma* species increased significantly the bitter gourd seedling height from 26 to 61%, root exploration of 85-209%, leaf area of 27 to 38%, and root dry weight of 38-62%. Similarly, they also found that *Trichoderma* species increased significantly the growth of loofah and cucumber plants. Furthermore, the dry root weights and height for tomato plants were enhanced when isolate of *Trichoderma harzianum* P52 was applied compared with the control treatment (Mwangi et al., 2011). *T. harzianum* has the ability to solubilize insoluble or sparingly soluble minerals (Altomare et al., 1999). Applying *Trichoderma* strain N47 significantly increased the pea plant growth by 15% in wet shoot weight, 8% in root weight, and 14.66 cm in root length, compared with the untreated control plants (Naseby et al., 2000).

In plant growth promotion pot experiments, *Trichoderma harzianum* LU1491 increased number of potato tubers, *T. barbatum* LU1489 increased total tuber weight, and *Trichoderma* sp. 792 LU1483 increased average tuber weight, compared with the untreated plants (Hicks et al., 2014). They also found that *Trichoderma virens* LU544 increased the tuber weight by 210%, and *T. harzianum* LU144 by 146%, compared with the *Rhizoctonia solani* control. Moreover, in vitro, Murali et al. (2012) demonstrated that seed treatment with spore suspensions of *Trichoderma* sp. as plant growth promoting fungi gave maximum germination of pearl millet of 91% in the Petri-dishes, compared with the untreated control seeds. Benitez et al. (2004) showed that crop productivity can be promoted up to 300% after the addition of biocontrol agents *Trichoderma hamatum* or *T. koningii* in the field trials.

Trichoderma viride VKF3 isolate promoted the growth of *Vigna radiata*, *Vigna mungo* and *Sesamum indicum* in vitro conditions by produced a high indole acetic acid (IAA), that increased the fresh and dry weights of three plants compared with the untreated control plants (Kumar et al., 2017b).

In addition, in hydroponic trials, the antagonism of *Trichoderma harzianum* SQR-T037 has been shown to improve the nutrients uptake (Mn, Fe, Zn, Cu and P) by tomato plantlets in the field, which led to an increase in the biomass of tomato plantlets grown in a nutrient-limiting soil (Li et al., 2015). Also, applying *Trichoderma asperellum* strain T34, in hydroponic

grown tomato plants, allowed the use of ammonium sources at different ratios without an excessive risk of increased severity of Fusarium wilt disease (Borrero et al., 2012).

Generally, *Trichoderma* strains are well known to increase plant growth promotion and decrease pathogens, as well as create positive environment with symbiotic relationship with plants by releasing growth hormones, proteolytic enzymes and endochitinase.

2.5. Susceptibility of potato varieties to *Rhizoctonia solani* AG-3PT

There are many ways to determine the resistance or susceptibility to Rhizoctonia disease in potatoes either in glasshouse or field trials. Advantages of glasshouse trials can be controlled conditions, especially temperature and soil moisture. For example, Carling and Leiner (1990) showed that *R. solani* AG-3PT caused more damage at 10°C than at 15.5 or 21°C on sprouts and roots of emerging potato plants. On the other hand, there is some disadvantages of glasshouse trials. There may be less opportunity to get symptoms due to pot size limiting the growth, so it is difficult to grow to full maturity to get scurf. On the other hand, field trials will show what happens in actual production. However disadvantages with the field are that it needs the space and time to do this, and also requires infested soil. Field trials can also be affected by other pathogens present in the soil.

Previous studies showed that there was a different effect between sterilized potting mix and unsterilized potting mix, and also between sterilized soil and unsterilized soil on the pathogenic severity on the plants. Sterilized potting mix or soil can show the pathogen effect without other organisms/pathogens (El-Aziz et al., 2013), while unsterile soil has many organisms such as bacteria, fungi, mycorrhiza and others (Olanya et al., 2009). Therefore, the disease is more severe in sterilized soil or potting mix and may over-estimate virulence compared with unsterile soil or potting mix.

Different methods of inoculation by pathogen have been studied. The inoculum of pathogen grown on grain or a similar substrate, and added to soil media can obtain uniform inoculation at a known rate which is similar to sowing into infested soil (El-Aziz et al., 2013). When natural infestation of soil by pathogen is used as inoculum method, the amount of pathogen inoculum cannot be controlled (Mohsan et al., 2016). Coating tubers by slurry of sclerotia is also one of the inoculum methods which is not commonly used, but it is easy to

control, and has little environmental influence (Olanya et al., 2009). Furthermore, the inoculation of tubers with mycelium of *R. solani* after sterilizing the tubers can be used to assess sclerotial production in the laboratory trials. It leads to the formation of sclerotia only without the presence of other pathogens (Buskila et al., 2011).

Most papers measure the black scurf to determine the resistance to disease (Atiq et al., 2013; Djébali and Belhassen, 2010; Mohsan et al., 2016; Zhang et al., 2014). However, a few researchers measure both the stem canker and black scurf to determine the resistance to disease (El-Aziz et al., 2013; Olanya et al., 2009). The correlation between severity of stem canker and scurf between varieties may be low (Olanya et al., 2009). The use of one method either stem canker or black scurf severity is not enough to give a reliable indicator to determine the resistance to Rhizoctonia disease in potatoes.

There have been many papers on testing of resistance of potato varieties to *R. solani* (El-Aziz et al., 2013; El-Naggar et al., 2013; Khandaker et al., 2011). For example, Pietkiewicz and Chorozewski (1983) tested the sensitivity of 44 potato varieties and discovered that seven varieties were more resistant to formation of sclerotia of *Rhizoctonia solani* than other varieties, but Odra, Poprad and Reda had the highest resistance to the pathogen. Zhang et al. (2014) studied a simple method to evaluate potato resistance to black scurf caused by *Rhizoctonia solani* AG-3PT, by inoculated tuber pieces with *R. solani* AG-3PT in the laboratory. Potatoes susceptible to *R. solani* AG-3PT were removed quickly while potato varieties resistant to *R. solani* AG-3PT were used in the field for screening which led to savings in money and time. Also, in the field condition, Mohsan et al. (2016) evaluated eighteen potato varieties against black scurf disease. Four varieties were found to be resistant and one of them showed moderate resistance while six varieties were susceptible and eight varieties were moderately susceptible to *R. solani* AG-3PT (Mohsan et al., 2016). The reaction of nine potato varieties against *R. solani* AG-3PT was tested under greenhouse conditions. Potato varieties were divided into three groups and each group was different in their susceptibility to disease caused by *R. solani* AG-3PT. The highest amount of free, conjugated and total phenols, fraction of phenolic compound, oxidative enzymes and the amino acid were found in the resistant variety group compared with the highly susceptible group (El-Naggar et al., 2013).

Yanar et al. (2005) tested the sensitivity of 22 local and commercial varieties of potato against *Rhizoctonia solani* AG-3PT under glasshouse conditions in Turkey. They found that five varieties of potato (Alleddian-sarisi, Victoria, Aybasti-beyazi, Romanya-beyazi and

Golkoy) had significantly higher resistance to Rhizoctonia stem canker disease than other varieties which showed the same susceptibility to the pathogen.

Daami-Remadi et al. (2008b) assessed the reaction of nine potato varieties under artificial and natural infection by *Rhizoctonia solani* by the quantification of symptoms (black scurf and stem canker), parameters of plant growth and expected effect on yield. The data showed that Spunta and to a lower degree Elodie were more sensitive to the pathogen than other varieties, while Daisy and Alaska were the most resistant or tolerant to disease. In spite of its high sensitivity to the pathogen and lower growth rate in comparison to the other potato varieties examined, Spunta variety showed the largest tuber fresh weight, but with an obvious black scurf incidence.

Previous studies have tested 20 isolates of *Rhizoctonia solani* AG-3PT on nine varieties of potato under glasshouse conditions in pot trials to assess their pathogenicity. The data indicated that there were significant interactions between the *R. solani* AG-3PT isolates and potato varieties. Therefore, this study proposed the occurrence of physiological specialization among the isolates of *R. solani* AG-3PT for pathogenicity on potato plants. Hence, potato varieties must be checked by utilizing many *R. solani* AG-3PT isolates, which will increase the opportunity of identifying varieties of potato that have tolerance or resistance to many *R. solani* AG-3PT isolates (El-Aziz et al., 2013).

Ozgonen and Erkilic (2013) examined the susceptibility of 11 varieties of potato against *Rhizoctonia solani*, *Fusarium solani*, *Phytophthora erythroseptica* and *Pythium deliense* inoculated artificially into sterilized soil in polyethylene bags under glasshouse conditions. More sensitive varieties were Jearla and Satina, while Agria and Hermes varieties were highly resistant to all potato diseases.

In conclusion, there are levels of resistance available to *R. solani* in potato, but it is not known how resistance interacts with biological control either in the laboratory or glasshouse trials or field trials.

2.6. Effects of fertilizers on control of Rhizoctonia diseases in potato

2.6.1. The vital importance of nutrients to the potato plants

The nutrients in fertilizers play a vital role in increasing plant resistance to some pathogens in addition to promote the non-pathogenic organism's activity. The target of using plant nutrients is keeping high potato yields with low nutrient losses to the environment in the potato fields (Huber, 1980).

Previous study showed that adding moderate levels of nitrate nutrition (NO^{-3}) to the potato plants promoted plant growth (shoot and root), but at the same time the high supply of nitrate decreased plant growth due to increased nitrate toxicity in the leaf and root tissues (Ulrich and Fong, 1973). Also, Jönsson and Asp (2013) showed that the nutrients Mg, Ca, Cd and K increased significantly in the potato plants (shoot and root tissues) when potato plants were grown in nitrate fertilization (NO^{-3}), while NH_4^+ decreased the above nutrients in the potato plants. In addition, Ros et al. (2008) demonstrated that the addition of nitrogen fertilization can induce resistance in potato plants against late blight disease caused by *Phytophthora infestans*. A previous study indicated that adding Ca and N to the soil according to recommended rates for potatoes, will increase resistance to soft rot disease and blackleg disease which are caused by *Pectobacterium* spp. (Czajkowski et al., 2011; Ngadze et al., 2014). Moreover, McGuire and Kelman (1986) demonstrated that the addition of high calcium fertilization (Ca) to potato plants diminished soft rot disease caused by *Pectobacterium atrosepticum*, and at the same time, it improved root and foliage growth, and reduced the susceptibility to diseases.

The use of different nutrients (Ca, Fe, K, N, Mg and S) with two kinds of soilborne pathogens (*Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*) led to changes in the dry weight for both fungi growing in nutrient solution. The elements Mg, S, and K significantly increased *Fusarium* dry weight. The elements Fe, K and Mg significantly increased dry weights of *Rhizoctonia* (Cruz and Russo, 1985).

Klikocka et al. (2005) reported that sulfur nutrition (S and K_2SO_4) significantly reduced common scab and stem canker diseases on potato plants caused by *Streptomyces scabies* and *R. solani*, respectively. In addition, elemental sulfur fertilization can reduce soil

pH which is more effective at controlling infections with *Streptomyces scabies*. Sulfur nutrition decreased the infection rate by *R. solani*. Thus, sulfur will promote plant growth and induce resistance against the pathogens (Klikocka et al., 2005).

A previous study has stated that maintaining healthy potato roots is a requirement for high yields in potato crops. For example, in China, high yields for long periods of crop planting were obtained with the use of organic sources of nutrients or waste products (Cook, 1986). Also, Davis et al. (2001) demonstrated that the factors most relevant to soil integrity were organic nitrogen, organic matter and available nutrients, and were accompanied by reduced risk of Verticillium wilt and higher potato tuber yield.

Panique et al. (1997) claimed that there is no effect on the pathogen *Rhizoctonia solani* by using different concentrations of potassium (K) rates in potato fields, but at the same time the addition of potassium significantly increased tuber size from 170 to 370 g. While Chaudhary et al. (2015) indicated that application of a proprietary biological potassium fertilizer significantly reduced stem canker of Desiree potato caused by *R. solani* AG-3PT but had no significant effect on scurf or yield loss in a glasshouse experiment. Also, Klikocka et al. (2005) reported that the application of sulfur (S) and potassium sulfate (K_2SO_4) significantly increased tuber yield and decreased the severity of infection of *R. solani* which causes stem canker of potato in a field experiment by adding 0, 25 and 50 kg/ha of each element to the soil.

2.6.2. Combination of fertilizers with biocontrol agents

The interaction of different concentrations of fertilizers with antagonistic microbes can control many plant diseases and promote plant growth characteristics. Some biological control agents such as *Pseudomonas* spp., *T. hamatum* and *T. harzianum* play a crucial role in competition for important micronutrients, where they absorb Fe to increase their growth, and they deprive the pathogen (*R. solani*) of this vital element; therefore, the pathogen will be inhibited (Tariq et al., 2010).

Bio-fertilization with biocontrol agent *Trichoderma* could improve the root colonization to enhance root development and growth, production, resistance to the stress and nutrients use. Hence, the addition of *Trichoderma* to the soil with nutrients has been reported to increase crop production and shoot and root growth in plants (Benitez et al., 2004; Kavoo-Mwangi et al., 2013).

Cohen et al. (2005) showed that calcium fertilization (Ca) plays an important role in the production of extracellular proteases of the biological control agent *Trichoderma viride*; therefore, it may increase the production of fungistatic metabolites to inhibit pathogens.

Previous study reported that in presence of nitrogen sources, the antagonism of *Trichoderma* species was stimulated on solid culture media through the release of enzymes against soilborne pathogens (Khattabi et al., 2004; Šimkovič et al., 2008). Production of enzymes by *Trichoderma* species was affected by using different nitrogen sources such as potassium nitrate, urea and ammonium sulfate (Donzelli and Harman, 2001). Lakhesar et al. (2010) showed that adding nitrogen fertilization (N) with *Trichoderma harzianum* as biocontrol agent to straw medium inhibited a pathogenic fungus (*Fusarium pseudograminearum*), while carbon nutrition had no effect to reduce the pathogen on straw medium.

In antagonism trial, Ordóñez-Valencia et al. (2009) tested eight concentrations of $KHCO_3$ (0, 2, 4, 6, 8, 10, 25 and 50 mM) in PDA medium. The outcomes demonstrated that $KHCO_3$ concentrations higher than 8 mM inhibited significantly the growth of *Trichoderma* sp. as antagonistic fungus and *Sclerotinia sclerotiorum* in the Petri-dishes. Also, in radial growth experiment, Erper et al. (2011) used ten concentrations of $KHCO_3$ (0, 2, 4, 6, 8, 10, 25, 50, 75 and 100 mM) in PDA medium. The results showed that the use of high concentrations of $KHCO_3$ in amended PDA effectively suppressed the mycelial growth of *R. solani* AG-4 and *Sclerotinia sclerotiorum*, and also have negative effect on the *Trichoderma* sp. as a biocontrol agent which dramatically decreased the growth with increasing concentration of potassium bicarbonate. Moreover, adding fertilizer sources to the soil such as ammonium sulphate, potassium, nitrate, urea or manure showed increased antagonistic activity of *Trichoderma harzianum* against *Sclerotium rolfsii* (Khattabi et al., 2004). Therefore, all nutrients have different effects on biological control. For example, competition for potassium in culture media and in the nutrient solution between *Trichoderma* sp. and *Sclerotium cepivorum* was studied by Ortega-Aguilar et al. (2011), as one of the mechanisms of biological control.

Huang et al. (2011) demonstrated that utilizing individual application of composts decreased the pathogen growth (*Rhizoctonia solani*) by 20%, which causes damping off disease on cucumber, while the combination between biological control agent *Trichoderma harzianum* SQR-T37 and organic nutrition was more effective to inhibit the pathogen than utilizing the biocontrol agent alone.

To summarize, fertilizers have a crucial role in inhibition of the pathogen, and promotion of biocontrol agents. Therefore, fertilizers and non-pathogenic isolates may reduce the severity of a number of vital potato diseases such as stem canker and black scurf disease.

2.7. Effects of *Brassica* plants on control of soilborne potato diseases

Potato grown continuously increases the pests and diseases, and as a result of this decreases the potato yields and potato quality. Therefore, rotation crops can improve soil structure and fertility and reduce soilborne pathogens. For example, potatoes can be rotated every year with *Brassica* green manure crops to decrease soilborne diseases and production costs and increase yields and quality in the markets. The big challenge for potato growers is soilborne disease control so that for some of these diseases, seed treatments and bio-fumigation can inhibit or reduce the pathogens, but they are not consistently practical or effective, and integrated management for disease control is in demand (Larkin and Griffin, 2007).

2.7.1. Soil biofumigation

Biofumigation is defined as suppression of soilborne pests and pathogens by the use of plants that contain inhibitory chemicals (Fan et al., 2008). It is well known as an environment-friendly alternative to methyl bromide to improve sustainable agriculture production systems (Mihajlović et al., 2017). Glucosinolates and biofumigation utilizing *Brassica* plants are promising alternative to chemical materials for controlling potato plant disease caused by *R. solani*. Aparna and Girija (2018) showed that some plant extracts including cabbage, cassava, garlic creeper, radish, moringa, neem and mustard to be highly effective biofumigants on *Rhizoctonia solani* that causes collar rot and web blight disease on cowpea in vitro by suppressing the growth of the mycelium and sclerotial germination. Complete suppression of the pathogen on PDA was obtained on treatment with cabbage, cassava, garlic creeper and mustard (Aparna and Girija, 2018).

Brassica crops are any plant of the genus *Brassica* such as turnip, cabbage, mustard, broccoli, cauliflower, kale and rape, in the Brassicaceae family. Almost all *Brassica* plants produce glucosinolates and release isothiocyanate (ITC) to different degrees to inhibit pathogens of potatoes. For example, mustard plants produce high concentrations of isothiocyanate that work as fungistatic volatiles to inhibit pathogens such as *Helminthosporium*

solani and *Verticillium dahliae* on potato plants (Olivier et al., 1999). *Brassica* crops have been used as green manures to inhibit soilborne pathogens including *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium sambucinam* and *Sclerotinia sclerotiorum* on potato plants (Larkin and Griffin, 2007).

The plants can be used as rotation crops or for intercropping or ploughed back into the soil as green manure (Bernard et al., 2014; Thornton et al., 1993). For instance, Larkin et al. (2017) studied the effect of long-term specific cropping system by establishing a rotation of barley with some red cover followed by two years of growing potato and found that this system significantly promoted the production of potato. Larkin and Halloran (2014) tested four different kinds of production management which are cover crops through the use of *Brassica* spp. (mustard and rapeseed), as green manure, incorporated of harvested crop-residue, and harvested crop-residue not incorporated, in rotation of potato crop, and their effects on disease and yield. They found that combination of mustard blend managed as a green manure was most effective, decreasing black scurf disease by 54% and increasing yield by 25% (Larkin and Halloran, 2014). They also found that only mustard blend consistently decreased common scab by 11%. All studied rotation crops managed as green manures lowered disease by 15-26% and increased yield by 6-13% compared with other management practices.

The use of different concentrations of isothiocyanates (methyl, propenyl, butenyl, pentenyl, benzyl and phenylethyl ITC) released by *Brassica* crops inhibited soilborne pathogens like *Gaeumannomyces*, *Rhizoctonia*, *Fusarium*, *Pythium* and *Bipolaris* by 50% of radial growth (Morra and Kirkegaard, 2002; Sarwar et al., 1998; Villalta et al., 2016).

Also, soil fumigation by using mustard green manure, metam sodium and 1,3 dichloropropene in different soil types increased the population of the biocontrol agent *Pseudomonas* spp. effectively, and at the same time decreased the fungal populations in five soil types significantly for *Pythium* spp. by 97%, *Fusarium* spp. by 84%, and *V. dahliae* by 56%. However, soil fumigation with metam sodium significantly decreased soilborne pathogens and vital soil processes such as C and N mineralization (Collins et al., 2006).

Brassica crops contain different levels of glucosinolates that differ in their biocidal activity on soilborne diseases. Some oilseed Brassicas including turnip, swede, white mustard and brown mustard have been shown in the laboratory to suppress *Rhizoctonia* stem canker disease of potato. The activity of Brassica shoot and root materials significantly reduced the

radial growth of *R. solani* that was grown in Petri-dishes and exposed to biofumigation from the various Brassica dried materials. Root from turnip oilseed rape had the biggest impact, depressing the *Rhizoctonia* colonies by 32% followed by 47% for swede oilseed rape and brown mustard (Booth et al., 2002). Bhandari et al. (2015) determined the glucosinolates GSL contents in different tissues (shoot, root, sprouts and seeds) of *Brassica* crops such as cabbage, broccoli, cauliflower, radish, pak choi, leaf mustard, kale and Chinese cabbage. They found that glucosinolates varied significantly among plants, tissues and stages of growth, where GSL concentrations were the highest in seeds for most plants, and were the lowest in the shoots of most plants.

In a field trial for two years, Cochran and Rothrock (2015) studied the applications of *Brassica napus* 'Jetton' and *Brassica juncea* 'Fumus' and 'Bionute' at the levels of 700, 1400, and 4200 g/m^2 fresh biomass weight as a green manure cover crops for management of *R. solani* on petunia and impatiens. The results showed that a high level of *Brassica* green manure crops decreased root discoloration by 7% and 9%, crown lesions by 24% and 21%, and *R. solani* isolation by 8% and 15% for petunia and impatiens, respectively, through the releasing high rates of glucosinolates into soil.

Handiseni et al. (2016) showed that nine *Brassica* plants significantly inhibited *R. solani* AG-1 mycelium growth which causes sheath blight disease on rice through the growth with PDA in vitro and also when used as soil amendment. The results indicate that mustard (*B. juncea*) had the highest mycelium inhibition by 90% compared with other *Brassica* plants due to release of high levels of glucosinolates.

Yasumoto et al. (2011) reported that the effect of rapeseed root exudates at 48 hr after sowing greatly reduced the percentage of germination of rapeseed by approximately 57% in the river sand when supplied with root exudates of rapeseed compared without root exudates by the stair-step method (a type of sand culture with a Hoagland hydroponic medium recirculating through a staircase bed). When applying rapeseed root exudates, root length and plant length of rapeseed had also decreased by 8.5 and 5.6 cm in comparison to the control without root exudates by 11.8 and 7.8 cm, respectively (Yasumoto et al., 2011).

Therefore, when using living Brassicas with potato crop as an intercropping system, it is more likely to be root exudates than ITCs from tissue breakdown that have an effect either on potato growth or on fungi growth.

2.7.2. Combination of *Brassica* crops with biocontrol agent

The purpose of this section is to provide a general overview of the important concepts that are fundamental for understanding the interaction between *Brassica* crops and biological control agents.

Trichoderma species can promote growth of *Brassica* species. Rabeendran et al. (2000) found that the applications of *Trichoderma tomentosum* (5Sr2-2) and *T. longipile* (6Sr4 and 3Sr4-2) significantly increased leaf area, shoot and root dry weight of cabbage (*Brassica oleracea* L.) by dipping transplant roots in conidial suspension in a glasshouse trial.

A previous study showed that biofumigation volatiles may be lethal for a few days only and need to be combined with fumigation, heating and biocontrol agents to prevent pathogens effectively in the soil (Larkin and Griffin, 2007; Stapleton and Duncan, 1998). Also, Cohen et al. (2005) found that adding the residues of *Brassica* green manures or growing it as a mixed crops with potato plants, increased the biocontrol agent (*Streptomyces* spp.) population in the soil which led to increase nitric oxide-producing bacteria and the induction of plant defense responses.

Biocontrol *Trichoderma* showed less sensitivity to ITCs than pathogens, including *R. solani* AG-3PT. Smith and Kirkegaard (2002) demonstrated that biological control agents (*Trichoderma* spp. and *Penicillium chrysogenum*) were less sensitive to inhibitory compounds from Indian mustard such as isothiocyanate than any of the potato pathogens. Similarly, Larkin and Griffin (2007) also reported that the combination between non-pathogenic fungi as biological control agents (*Trichoderma virens* and *Penicillium chrysogenum*) and *Brassica* crops significantly diminished some plant pathogens, where they isolated biocontrol agents from soil. They showed that growth inhibition zone by leaf tissue of Indian mustard was between 73 and 100% for *F. oxysporum* and *R. solani* on potatoes, respectively, while root tissue of canola inhibited *R. solani* growth by 26%.

Galletti et al. (2008) claimed that the combination of *Trichoderma* and biofumigation by *Brassica* crops seed meal (BCSM) was more effective to suppress soilborne pathogens (*Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*), where BCSM contains glucosinolates which break-down to release isothiocyanates. They examined forty *Trichoderma* spp. isolates in the laboratory with toxic volatile organic compounds released

from *Brassica* crops for their tolerance to volatile and non-volatile compounds. Generally, the *Trichoderma* isolates were more tolerant than the pathogens.

Montfort et al. (2011) discovered that using Indian mustard as a biofumigation crop incorporated into the soil with the biocontrol agent *Trichoderma harzianum* prevented or highly inhibited the pathogens (*Pythium sulcatum* and *Rhizoctonia solani*) on carrot crops and reduced the incidence of brown rot disease.

Two isolates of *Trichoderma* (*T. harzianum* and *T. viride*) were selected from thirty isolates of *Trichoderma* based on their high reduction of pathogen growth of *Sclerotinia sclerotiorum* (Alkooranee et al., 2015). 100 mL of spore suspensions of each *Trichoderma* was sprayed on leaves and stems of *Brassica* plants (*Brassica napus* and *Raphanus oleracea*), and the outcomes revealed that biocontrol agents cause induced acquired resistance in the plants and reduced disease incidence of Sclerotinia stem rot disease.

To sum up, *Brassica* plants can be used as a crop-rotation or intercropping or mixture crops to reduce potato diseases. Biocontrol *Trichoderma* is less sensitive to ITCs than *R. solani*. The effect of *Trichoderma* in combination with cover crops or green manures of *Brassica* species has been tested against Rhizoctonia diseases of potatoes. However, the combination between *Brassica* plants and *Trichoderma* has not been tested against *R. solani* in potato by intercropping between *Brassica* crops and potato plants inoculated with *Trichoderma* sp. at the same time as mature plants for each crop in the field.

2.8. Conclusion

Rhizoctonia solani remains a big problem in potato production in most countries. It affects many growth stages and can cause different diseases and yield and quality losses. Biological control is one way to make management more environmentally safe and clean. To utilize this kind of biological control there is a need to find better management and secure material with low cost to reduce the pathogen effects on the plant. Many studies show biocontrol is feasible. *Trichoderma* spp. are commonly found to be the best BCAs. Potatoes differ in resistance to the pathogen, but it is not known how these potato varieties interact with biocontrol agents. Different tests reported an influence of nutrients (fertilizers) to promote plant growth and reduce pathogen growth, but they are also very likely to be important in effects on biocontrol agents. Numerous papers show that *Brassica* green manures reduced soilborne

pathogens when it used as residues, incorporated or rotation crop with other plants. *Brassica* could also be used in combination with biocontrol agents, but *Brassica* crops have not been tested yet in intercropping with potatoes to alleviate or diminish the activity of *R. solani* in soil.

Chapter 3. Isolation and screening of antagonists

3.1. Introduction

Potato losses have been nearly 22% per year due to bacteria, fungi, viruses and pests, which attack potato tubers and potato plants. Estimated losses annually of potato are more than 65 million tons and stem canker disease of potato alone accounts for 35-50% of this (Rahman et al., 2012; Virgen-Calleros et al., 2000; Zhang et al., 2014). *Rhizoctonia solani* AG-3PT is a fungal pathogen which causes stem canker and black scurf of potato. *R. solani* causes brown cankers on underground stolons and stems in addition to black sclerotia on the tubers. Infected potato seeds and soilborne inoculum are the initial sources for stem canker and black scurf of potato (Bains et al., 2002). The pathogen is carried over to the next season as black sclerotia on the potato tubers and in the soil. It is prevalent in all potato growing areas of the world (Demirci et al., 2009).

Trichoderma species have been shown to reduce disease in potato caused by *R. solani* in pot and field trials. A large number of *Trichoderma* species have been shown to be effective on pathogen growth and increased plant growth (Hicks et al., 2014; Larkin, 2016; Somani and Arora, 2010). In order to do trials that may improve the efficiency of this method, it is necessary to obtain suitable non-pathogen isolates. Although many strains of *Trichoderma* and other biocontrol agents have shown an effect against stem canker and black scurf disease, it is likely that non-pathogens isolated from soil or root zone of potato plants will be well adapted to potato cropping systems.

Trichoderma species are saprotrophic fungi that are well known to be one of the best candidates of biological control agents to manage soilborne pathogens. Modes of action of this fungus include antibiosis, mycoparasitism, competition for nutrients and space, tolerance to stress through enhanced root and plant development, and induced systemic resistance (Devi et al., 2017). Likewise *Trichoderma* isolates produce volatile and non-volatile compounds that inhibited the percentage of mycelial growth of pathogens (*Rhizoctonia bataticola*, *Fusarium oxysporum ciceri* and *Sclerotium rolfsii*) in Petri-dishes (Nagamani et al., 2017). Traditionally, fungal biocontrol agents have first been tested in dual culture assays. However, as pointed out by Waghunde et al. (2016), the best and most reliable method for testing antagonists of Rhizoctonia disease is in bioassays with the plant and pathogen. *Trichoderma* strains stimulate

growth of plant and increase yield by inhibiting soilborne fungal plant pathogens (Devi et al., 2017; Hermosa et al., 2012; Van Wees et al., 2008; Waghunde et al., 2016).

Currently, there have been increasing numbers of biological control case studies of *Rhizoctonia solani* to protect the potatoes from the infections. Some organisms have been proven to diminish or inhibit the disease severity and incidence of *R. solani*, including *Trichoderma viride, T. harzianum, T. hamatum, Verticillium biguttatum, Trichoderma virens* and *Bacillus subtilis* (Mayo et al., 2015; Virgen-Calleros et al., 2000). In most published papers numerous strains and isolates of *Trichoderma* have been confirmed to be rhizosphere competent on various plants and under various biotic and abiotic conditions, an additional feature that contributes to biological control potential for many plant diseases (Devi et al., 2017; Harman et al., 2004; Nawrocka et al., 2018; Yadav et al., 2015).

The objective of this study was to isolate the strains of *Trichoderma* that were suitable for more detailed trials on antagonism of stem canker and black scurf of potato caused by *R. solani* AG-3PT. Fungi were isolated from potato soil, identified using morphological and molecular methods, screened for dual culture, antibiosis tests and antagonistic ability in bioassays, and tested for their effect on growth.

3.2. Materials and Methods

3.2.1. Inoculum preparation of the pathogen and pathogenicity test

Rhizoctonia solani AG-3PT isolate DAR 40050 from potato was supplied by the Agricultural Scientific Collections Trust, Orange Agricultural Institute, NSW Australia. It was maintained on slant medium which contained potato dextrose agar (PDA). Inoculum was prepared by adding 100 g of wheat grain to 200 mL of distilled water which were mixed well in 500 mL flasks and left overnight. The grain was then autoclaved for 1 hr at 121°C on each of two successive days. The flasks were cooled and inoculated with 12 discs of 0.5 cm diameter from cultures of the pathogen grown for 5 days on PDA at 25°C. The fungus was allowed to colonize the wheat grain for 3 weeks at 20°C in the dark. The contents of the flasks were mixed every 2 days by shaking to enhance the colonization of the seeds.

Pathogenicity tests were performed to confirm the virulence of the isolate. Potato cv. Desiree seed tubers were sterilized by using 1% sodium hypochlorite (NaOCl) with two drops of Triton X-100 detergent (Sigma-Aldrich) as a mix for 2 minutes, and then washing with sterilized distilled water. They were planted in 2:1 sand:potting mix (Searles Premium potting mix, Searles, Kilcoy QLD) in 20 cm diameter plastic pots. There were four replicates for each treatment. The pathogenicity of the isolate was confirmed in glasshouse tests by inoculating potato tubers with the pathogen (*R. solani* AG-3PT) grown on wheat bran and also *R. solani* was grown on sterilized toothpicks for 7 days on PDA at 25°C to wound the stem tissue with the toothpicks to inoculate the pathogen inside the stem of potato plants, and comparing with untreated control (sterilized toothpicks only) for visible symptoms of stem canker on stems and black scurf on tubers after 12 weeks of growth. The isolate of *R. solani* AG-3PT caused stem canker and black scurf diseases of potato.

3.2.2. Isolation and identification of *Trichoderma* isolates

Tubers and soil containing roots were collected from two potato farms at Dorrigo, New South Wales, Australia in February 2015. At Beaumont Farm it was just before harvest, and at Plateau Farm just after harvest but with some roots and tubers still in the soil. Around 2 kg soil and 5-8 tubers with roots were collected from each farm. One *Trichoderma* isolate was collected from soil in which potatoes were growing at Armidale Community Garden, New South Wales, Australia in February 2015.

All strains of *Trichoderma* were isolated on modified potato dextrose agar (mPDA) and Trichoderma selective media (TSM). mPDA medium pH 6 was prepared by adding 19.5 g of powdered PDA to 500 mL of distilled water. After autoclaving for 25 minutes at 121°C, 0.015 g Rose Bengal in 10 mL sterilized distilled water was added to the medium before pouring into Petri-dishes (Dhingra and Sinclair, 1985). TSM medium was prepared by adding $MgSO_4.7H_2O$, 0.1 g; K_2HPO_4, 0.45 g; KCL, 0.075 g; NH_4NO_3, 0.5 g; glucose, 1.5 g and agar, 10 g in 500 mL of distilled water and autoclaving for 25 minutes at 121°C. The medium was allowed to cool to 45°C. After that, chloramphenicol, 0.125 g; captan, 0.1 g; Fongarid, 0.16 g and Terraclor, 0.1 g in 25 mL sterilized distilled water were added to the medium, and then poured into Petri-dishes (Martin, 1950).

Trichoderma strains were isolated from the tubers after washing in tap-water and sterilizing with 1% NaOCl for 30 seconds and washing three times with sterilized distilled

water. Then four pieces of skin of tubers were placed on mPDA and TSM, and incubated at 25°C. *Trichoderma* strains were isolated from soil by serial dilution with distilled water and 1 mL from a 10^{-4} dilution was poured onto TSM and mPDA media. The root zone was washed in tap water to remove all visible soil and then surface sterilized with 1% NaOCl for 30 seconds and washed three times with sterilized distilled water. The roots were cut into pieces that were 1 cm long using a sterile blade. The pieces were then placed on the TSM and mPDA media. Then emerging colonies were transferred onto fresh potato dextrose agar (PDA). Cultures were incubated for 7 days at 25°C, and then colonies were purified and determined to be *Trichoderma* species by morphological characters. The isolates of *Trichoderma* were isolated from different parts (soil, root zone and skin of tubers) (Table 3-1).

Identities of *Trichoderma* isolates were confirmed by ITS sequencing (Shahid et al., 2014). The fungi were grown for 3 to 4 days on PDA. Approximately 100 mg of mycelium was scraped off the plates with a sterile scalpel blade and placed into a microcentrifuge tube containing a small quantity of 0.5 mm diameter glass beads. DNA was extracted using the Isolate II Plant DNA Kit (Bioline). Lysis buffer PA1 was added to the tubes and then they were shaken for 30 seconds at 30 Hz on a Retsch MM300 Mixer Mill. The manufacturer's protocol was then followed for the remainder of the extraction procedure.

PCR was done using ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) primers with MyTaq master mix (Bioline) (White et al., 1990). The PCR conditions were an initial denaturation at 95°C for 1 min, 30 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 10 s, and a final extension step at 72°C for 5 min. PCR products were sequenced by the Australian Genome Research Facility using the same primers as for PCR. Sequences were edited in MEGA 6 and submitted to TrichoKEY (http://www.isth.info/tools/molkey/) for identification.

Table 3-1 *Trichoderma* isolates collected from different areas and plant parts.

Isolate	Farm	Isolated part
T1	Plateau	Soil
T2	Beaumont	Soil
T3	Plateau	Root zone
T4	Community garden	Skin of tubers
T5	Beaumont	Soil
T6	Beaumont	Soil
T7	Plateau	Skin of tubers
T8	Plateau	Root zone

3.2.3. Antagonistic activity of *Trichoderma* spp. against *R. solani* in dual culture

Eight isolates of *Trichoderma* spp. were tested in a dual culture assay against *R. solani* AG-3PT on PDA medium in 9 cm Petri-dishes. The medium was inoculated with a 5 mm diameter disc of antagonist positioned diametrically opposite a 5 mm diameter disc of the pathogen. In controls the pathogen was inoculated alone near one edge of the Petri-dishes. The distance between the discs was 7 cm. All pairings were inoculated on the same day and each combination was replicated three times. The plates were incubated at 22°C for 7 days. The growth of the pathogen was measured from 4 to 7 days. The inhibition was calculated on the day before the colonies came into contact. The growth in control was the furthest radial distance grown by the pathogen in the absence of the antagonist and growth in treatment was the distance grown on a line between inoculation positions of the pathogen and antagonist (Chand and Logan, 1984; Sivakumar et al., 2000). The percentage inhibition of growth was calculated by using the following equation:

$$Inhibition = 100 \times \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control}$$

Dual culture Petri-dishes were visually inspected to determine the presence or absence of brown colour after 7 days of incubation at 22°C (brown colour indicates lysis of pathogen hyphae by the antagonist) (Shalini and Kotasthane, 2007; Sivakumar et al., 2000).

3.2.4. Antagonistic activity of culture filtrates of *Trichoderma* spp. against *R. solani*

Trichoderma isolates were grown in 250 mL plastic tubes, each containing 50 mL of sterilized liquid potato dextrose broth (PDB), in a rotary shaker at 150 rpm and 22°C for 7 days. Cultures of the pathogen in PDB were used as the control. The supernatants were sterilized with 0.45 µm filters. After that, 5 mL of culture filtrate was placed in sterile Petri-dishes and then 15 mL of PDA with low sugar (2 g/L of glucose) was cooled and poured into the plates with gentle mixing. The plates were inoculated with 5 mm diameter agar plugs from a 5 day old culture of *R. solani* AG-3PT at the centre of the Petri-dishes. There were three replicates for each treatment. These plates were incubated at 22°C for 5 days. Radial growth of the pathogen was measured and compared to control growth (Elad et al., 1983; Tariq et al.,

2010; Whipps, 1987). This test was used as an indicator of potential antibiotic production by the *Trichoderma* spp.

3.2.5. Screening isolates in pot trials

There were 10 treatments in this trial: no inoculation as the negative control, *R. solani* alone as the positive control and *R. solani* AG-3PT plus each of the eight isolates of *Trichoderma*. Two experiments were done separately, in vermiculite and also 2:1 sand:potting mix (Searles Premium potting mix, Searles, Kilcoy QLD) in 20 cm diameter plastic pots (Hicks et al., 2014). Inoculum of the pathogen was prepared as in section 3.2.1, and 16 g of inoculum was mixed in the growth medium in each pot (Abd-El-Khair et al., 2010; Singleton et al., 1992). Potato cv. Desiree seed tubers were sterilized by using 1% sodium hypochlorite (NaOCl) with two drops of Triton X-100 detergent (Sigma-Aldrich) as a mix for 2 minutes, and then washing with sterilized distilled water. Conidial suspensions of each *Trichoderma* strain (10^6 conidia/mL water) were used to treat the potato tubers. The suspensions of *Trichoderma* conidia were prepared from 7-day-old cultures on PDA in 9-cm Petri-dishes which were covered with 10 mL of sterilized distilled water and shaken for a few minutes. The resulting suspension was filtered through sterilized Miracloth. The concentration of spores was determined with a haemocytometer, and was adjusted to 10^6 conidia/mL water by using sterilized distilled water (Jegathambigai et al., 2009). The tubers were soaked in conidial suspensions for 1 h, and dried for 3 h at room temperature (Sivan and Chet, 1989) before sowing one tuber in each pot. The plants were irrigated with 300 mL of water every second day. There were 3 replicates for each treatment laid out in a completely randomized design. This experiment was done under controlled conditions in a glasshouse at 20°C (Ramsay and Bawden, 1983). Potato plants were harvested 7 weeks after emergence. The stem canker disease severity was measured at the time of harvest by measuring the length of lesion on stems based on a scale (0-4): 0 = no disease, 1 = less than 10% of stem area covered with lesions, 2 = 10-25% of stem area covered with lesions, 3 = 26-50% of stem area covered with lesions and 4 = stem girdled with lesions (Atkinson et al., 2010). Plant growth was measured by assaying the number of stolons, the number of tubers, tuber fresh weight and the shoot and root dry weight. The shoots and roots were dried in an oven at 60°C until constant weight (Hicks et al., 2014).

3.2.6. Tissue culture propagation

Certified seed tubers of potato cv. Desiree were washed under tap-water to remove soil, and surface-sterilized with a solution of 1% NaOCl in 10% ethanol for 5 min (Saker et al., 2012a; Zhang et al., 2005a; Zhang et al., 2005b). Small pieces were taken carefully from inside the tuber with a sterile scalpel after peeling the surface and placed on 20 mL of M3 medium (Saker et al., 2012b) in Petri-dishes. M3 contained vitamins (inositol 0.1 g, glycine 0.02 g, thiamine HCl 0.01 g, pyridoxine HCl 0.05 g, nicotinic acid 0.05 g, D-biotin 0.005 g and folic acid 0.05 g), and growth regulators (1-naphthalene acetic acid 0.003 g, gibberellic acid 0.05 g and N6-benzyladenine 0.03 g), and other supplements (casein hydrolysate 1 g, sucrose 25 g and agar 7 g) per litre added to MS Basal Salts (Sigma-Aldrich) (Murashige and Skoog, 1962). The Petri-dishes were incubated in a growth chamber at 25 ± 2°C and after four weeks shoots grew from the tuber pieces. They were used as a source of nodal cuttings for micro-propagation. The nodes were cut under aseptic conditions and subcultured onto modified MS medium B to multiply the plantlets (Espinoza et al., 1984). The culture medium contained basal MS salt mixture (Sigma-Aldrich), sucrose 30 g, agar 8 g, gibberellic acid 0.25 ppm, thiamine 1 mL of 40 g/100 mL solution and Ca pantothenate (vitamin B_5) 2.00 ppm, per litre. The cultures were incubated at 25°C with 16 h day length under white fluorescent lamps. Shoots were used as cuttings for transfer to MS solid basal medium without growth regulators for root production and complete plantlet development at 25°C and 16 h photoperiod to use as a source of seedlings for different experiments (Esna-Ashari and Villiers, 1998).

3.2.7. Effect of *Trichoderma* on stem canker in plantlets from tissue culture

The effect of selected *Trichoderma* isolates on stem canker in tissue culture plantlets was assessed in glasshouse trials. Potato plantlets (cv. Desiree) were obtained from the tissue culture system after 8 weeks after plating nodal cuttings. Potato plantlets were sterilized with sodium hypochlorite 1% for 30 second and then washed with sterilized distilled water for four times and then placed, 1 plantlet per pot, in plastic pots 10 x 10 cm diameter with sand-potting mix for this test. The treatments were: no inoculation as the control, *R. solani* as the control and *R. solani* AG-3PT plus each of 2 isolates of *Trichoderma* (*T. harzianum* T5 and *T. hamatum* T8). Inoculum of *R. solani* prepared as described in section 3.2.1 was added into the pots by mixing thoroughly about 8 g around the plantlets (Singleton et al., 1992). *T. harzianum* (T5) and *T. hamatum* (T8) were grown on PDA and incubated for 7 days at 25°C. Spore suspensions

(100 mL, 10^6 conidia/mL water) of each isolate were added around the seedlings into pots immediately after pathogen inoculation. 100 mL of sterilized distilled water was used to irrigate the control treatments. There were three replicates for each treatment. This test was carried out under controlled conditions in a glasshouse at 20°C for 3 weeks. Lesion length of stem canker was measured in centimetres. Plant growth was measured by assaying the shoot and root dry weight. The shoots and roots were dried in an oven at 60°C until constant weight.

3.2.8. Effect of *Trichoderma* on growth of plantlets from tissue culture

T. harzianum T5 and *T. hamatum* T8 were grown on PDA in Petri-dishes and incubated for 7 days at 25°C. Conidial suspensions (100 mL) of each isolate were gained by flooding plates with sterilized distilled water and conidial concentration was adjusted to 10^6 conidia/mL water based on haemocytometer counts (Jegathambigai et al., 2009). Potato plantlets (cv. Desiree) with 7-10 leaves were obtained from tissue culture. They were sterilized with 1% NaOCl for 30 second and then washed with sterile distilled water for four times, and dipped in conidial suspensions of each isolate (100 mL) for 30 minutes before planting, 1 plantlet per pot, into 15 x 15 cm diameter pots containing sand-potting mix (2:1). Three seedlings used as control plants were dipped in 100 mL SDW for 30 min. There were three replicates for each treatment. Measurements were taken after growth for 4 weeks. Roots and shoots were washed and then dried in an oven at 60°C until constant weight (Rabeendran et al., 2000; Windham et al., 1986).

3.2.9. Induction of systemic resistance

Potato seedlings (cv. Desiree) were obtained from tissue culture as in section 3.2.6. The treatments were uninoculated as the control, *R. solani* only as the control, spore suspension of *T. harzianum* T5 + plus stem inoculation of *R. solani* and spore suspension of *T. hamatum* T8 + plus stem inoculation of *R. solani.* The plantlets were transferred, 1 plantlet per jar, to 100 mL Hoagland solution in glass jars. The lid was pierced in the centre to allow the shoot to grow outside the jar. A small amount of sterilized cotton wool was put around the stem to hold the plantlet and prevent contamination. The spore suspension of T5 or T8 was added as 1 mL to each glass jar in order to allow successful root colonization (Chinta et al., 2015). *Rhizoctonia solani* was grown on sterilized toothpicks for 7 days on PDA at 25°C, and then the plantlets were inoculated 2 days after adding the spore suspensions of biocontrol agents by wounding

the stem tissue with the toothpicks to penetrate the pathogen inside the stem of potato plantlets. 1 mL sterilized distilled water was added to the Hoagland solution and one sterilized toothpick to the stem as the control treatment. This test was carried out under controlled conditions in a glasshouse at 20°C for 16 days. There were three replicates for each treatment. The potato seedlings were assessed for length lesion on stems by centimetre. The effect of the treatments was estimated by determining shoot and root dry weight. The shoots and roots were dried in an oven at 60°C until constant weight.

3.2.10. Data analysis

All results were expressed as means and treatment effects were tested by one way ANOVA (analysis of variance) with statistical program SPSS version 22. Log_{10} transformation was used when necessary to correct for non-homogeneity of variance. Tukey's multiple range test was used for mean separation at the 5% level. ANOVA tables are presented in the Appendix.

3.3. Results

3.3.1. *Trichoderma* isolates

Eight isolates of *Trichoderma* spp. were obtained from healthy potato tubers and soil containing roots. ITS sequences from DNA extracts of 7 isolates were obtained for identification of *Trichoderma* isolates. The isolates were identified as *Trichoderma harzianum* (T1, T2, T4 and T5) and *Trichoderma hamatum* (T3, T6 and T8). The sequence obtained for T7 was too low in quality for identification, but because this isolate did not give strong biocontrol activity in subsequent experiments it was not further identified. The GenBank accession numbers for nucleotide sequences is T5 MH675469 and T8 MH675470. Sequences obtained for other isolates of *T. harzianum* and *T. hamatum* were identical to those of T5 and T8 respectively.

3.3.2. Dual culture

All fungal antagonists significantly inhibited the growth of the pathogen (*Rhizoctonia solani*) compared to the control. The highest percentages of growth inhibition were 51% and 48% by T8 and T2; respectively, whereas T4 and T5 gave the lowest inhibition of 43% and 38%, respectively (Table 3-2).

The initial physical contact between *Trichoderma* spp. and *R. solani* occurred within 4 days after inoculation, followed by the inhibition of pathogen growth. By 7 days after inoculation in dual cultures, there was parasitism of hyphae of *R. solani* by the *Trichoderma* hyphae, including overgrowth of hyphae of the pathogen and brown colour at the contact zone. All *Trichoderma* isolates caused brown colour of the colonies of *R. solani* at the contact zone except T3, T6 and T8 (Table 3-2; Figure 3-1). The *Trichoderma* species overgrew the pathogen to varying degrees. All isolates of *Trichoderma harzianum* overgrew the *R. solani* AG-3PT with high degree, whereas *T. hamatum* overgrowth on the edge of the *R. solani* AG-3PT colony was low, especially T8 and T6.

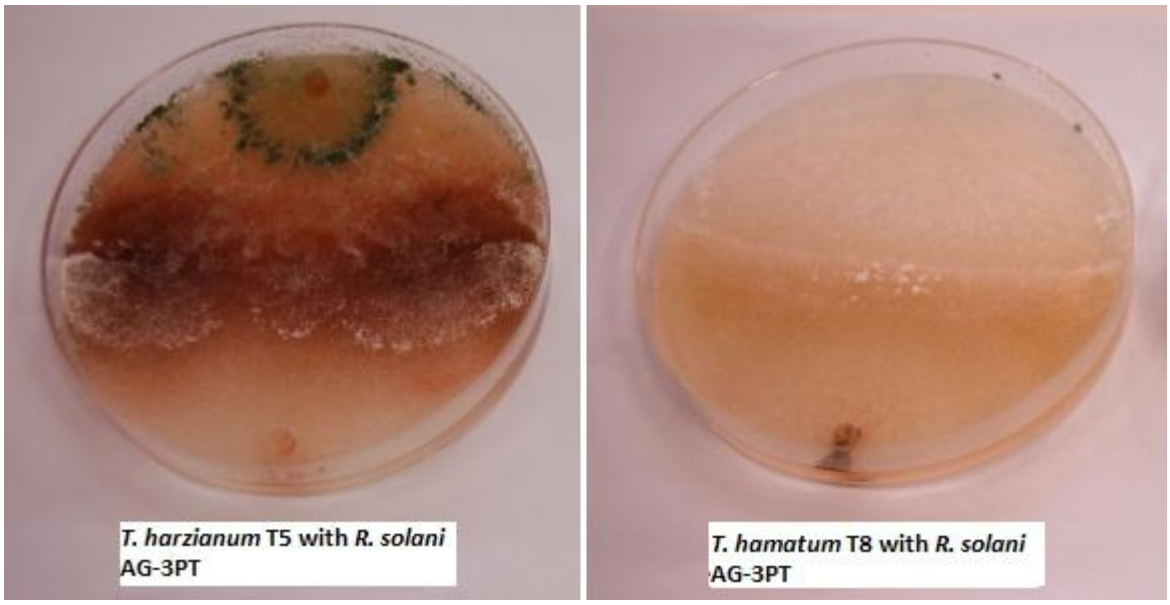

Figure 3-1 Dual culture between two isolates of *Trichoderma* (T5 and T8) against *Rhizoctonia solani* AG-3PT showing brown colour and overgrowth of the pathogen by *Trichoderma* isolates, especially T5.

Table 3-2 Colony interactions between *Rhizoctonia solani* and isolates of *Trichoderma* in dual culture method on potato dextrose agar

Antagonists	Growth inhibition (%)[A]	Brown colour[B]
T1 *T. harzianum*	45.82^{abc}	+
T2 *T. harzianum*	48.35^{ab}	+
T3 *T. hamatum*	46.86^{ab}	-
T4 *T. harzianum*	43.28^{bc}	+
T5 *T. harzianum*	37.91^{c}	+
T6 *T. hamatum*	45.37^{abc}	-
T7 *Trichoderma* sp.	47.76^{ab}	+
T8 *T. hamatum*	51.34^{a}	-

[A]Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD). [B] + Brown colour of *R. solani* AG-3PT, - No brown colour.

3.3.3. Antagonistic activity of culture filtrates

Culture filtrates of T5, T1, T7 and T8 significantly inhibited the growth of the pathogen compared with the controls (Figure 3-2). There were no significant differences in inhibition of pathogen growth between culture filtrates of T2, T3, T4 and T6, and the culture filtrate of *R. solani* (Figure 3-2).

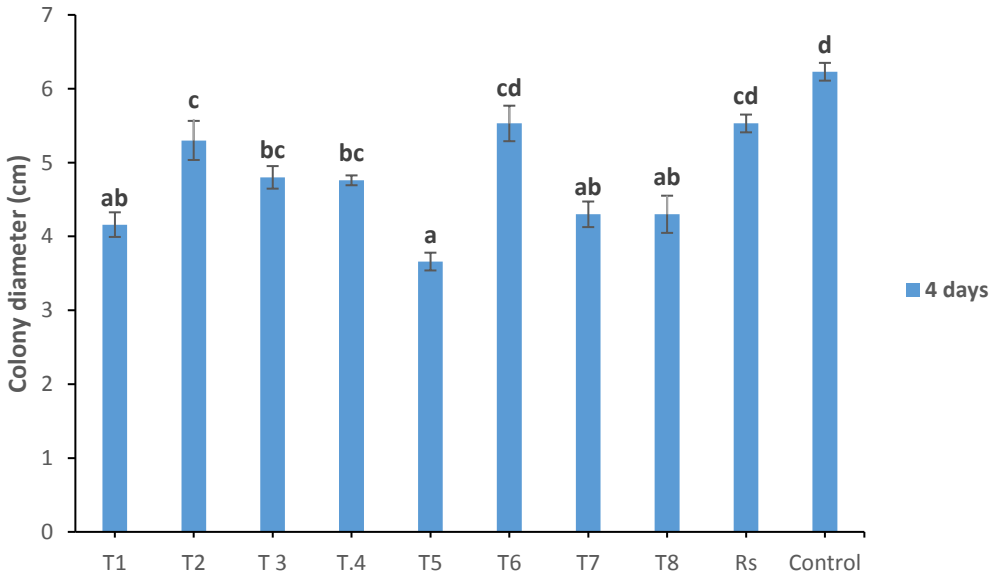

Figure 3-2 Growth of *R. solani* hyphae (Rs) in response to culture filtrate produced by *Trichoderma* spp. isolates (T1-T8) and *R. solani* on potato dextrose agar at 20°C. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P=0.05 (Tukey test).

3.3.4. Screening isolates in pot trials

The effect of *R. solani* on potato plants significantly reduced biomass and yield parameters in sand-potting mix and vermiculite under glasshouse conditions (Table 3-3 and Table 3-4; Figure 3-3). Stem canker symptoms occurred in sand-potting mix in *R. solani* only treatment at 0-10% severity with just a small number of sclerotia on tubers. There were no stem canker symptoms or sclerotia in *Trichoderma* treatments in sand-potting mix, or in any treatment in vermiculite.

In sand-potting mix, *R. solani* significantly reduced shoot dry weight by half compared with uninoculated plants (Table 3-3). Although all other growth and yield parameters were low in the *R. solani* treatment, the differences from the uninoculated control were not significant. There were no symptoms of disease in plants treated with any of the *Trichoderma* isolates. All isolates of *Trichoderma* gave significantly greater shoot dry weights than the *R. solani* treatment, and isolates T6, T7 and T8 gave significantly greater shoot dry weight than the uninoculated control (Table 3-3).

Most isolates of *Trichoderma* gave significantly greater shoot dry weight, number of stolons and tuber fresh weight than the plants inoculated with *R. solani* only when grown in sand-potting mix (Table 3-3).

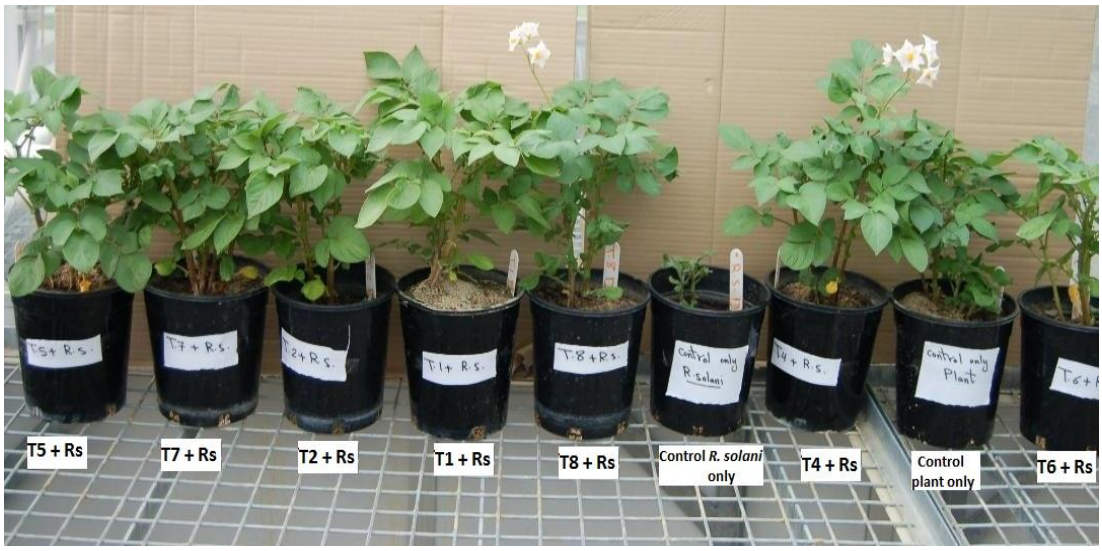

Figure 3-3 Potato plant growth inoculated with isolates of *Trichoderma* on Desiree variety and *R. solani* AG-3PT in sand-potting mix in a glasshouse experiment at 20°C.

Table 3-3 Effects of eight *Trichoderma* isolates (T1-T8) for suppression of Rhizoctonia disease (Rs) on Desiree tubers grown in sand-potting mix in a glasshouse experiment at 20°C

Treatment	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)
Control	3.4^{b}	1.76^{ab}	11.0^{ab}	6.0^{ab}	179.7^{ab}
Rs	1.7^{a}	1.4^{a}	5.7^{a}	3.0^{a}	110.6^{a}
T1 + Rs	5.5^{bc}	2.8^{bc}	14.0^{b}	8.7^{b}	214.1^{b}
T2 + Rs	4.1^{bc}	2.5^{abc}	14.7^{b}	7.33^{ab}	201.1^{b}
T3 + Rs	5.44^{bc}	3.2^{c}	11.0^{ab}	7.0^{ab}	220.96^{b}
T4 + Rs	4.9^{bc}	2.6^{bc}	11.7^{b}	6.7^{ab}	171.8^{ab}
T5 + Rs	5.44^{bc}	3.3^{c}	12.3^{b}	7.7^{ab}	228.9^{b}
T6 + Rs	6.3^{c}	2.95^{bc}	14.0^{b}	8.7^{b}	236.2^{b}
T7 + Rs	6.3^{c}	3.3^{c}	14.0^{b}	7.0^{ab}	190.1^{b}
T8 + Rs	6.2^{c}	3.6^{c}	16.3^{b}	9.7^{b}	247.2^{b}

Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD).

In vermiculite, inoculation with *R. solani* significantly reduced root dry weight, number of stolons and tuber fresh weight compared with the uninoculated control (Table 3-4). In vermiculite, only T7 and T8 significantly increased shoot dry weight compared with the *R. solani* control (Table 3-4). T1, T2, T3, T7 and T8 significantly increased the root dry weight compared with the pathogen-only control (Table 3-4). All isolates of *Trichoderma* significantly increased the number of stolons, number of tubers and tuber fresh weight compared with *R. solani* alone (Table 3-4).

Table 3-4 Effects of eight *Trichoderma* isolates (T1-T8) for suppression of Rhizoctonia disease (Rs) on Desiree tubers grown in vermiculite in a glasshouse experiment at 20°C

Treatment	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)
Control	1.9^{ab}	1.59^{b}	6.3^{b}	2.7^{ab}	122.2^{b}
Rs	0.95^{a}	0.58^{a}	2.0^{a}	1.3^{a}	43.4^{a}
T1 + Rs	2.40^{ab}	2.1^{b}	11.7^{b}	4.7^{bc}	131.03^{b}
T2 + Rs	2.24^{ab}	1.5^{b}	13.3^{b}	4.0^{bc}	135.15^{b}
T3 + Rs	2.06^{ab}	1.5^{b}	7.3^{b}	3.7^{bc}	116.65^{b}
T4 + Rs	1.97^{a}	1.4^{ab}	7.3^{b}	3.3^{bc}	114.34^{b}
T5 + Rs	2.08^{ab}	1.4^{ab}	9.3^{b}	5.0^{bc}	128.57^{b}
T6 + Rs	1.98^{ab}	1.5^{ab}	7.7^{b}	3.3^{bc}	107.6^{b}
T7 + Rs	2.73^{b}	1.6^{b}	12.7^{b}	6.7^{c}	146.33^{b}
T8 + Rs	2.80^{b}	1.7^{b}	13.7^{b}	6.7^{c}	210.3^{b}

Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD).

3.3.5. Effect of *Trichoderma* on stem canker in plantlets from tissue culture

The pathogen significantly reduced the shoot and root dry weight compared with other treatments. lesions were present on the stems of potato plantlets in the *R. solani* only treatment. The area of stem covered by lesions in this treatment was 0.17 cm. There were no symptoms of disease in plants treated with any of the *Trichoderma* isolates or untreated control.

T. hamatum T8 was significantly more effective than *T. harzianum* T5 in the growth promoting activity which included shoot and root dry weight in comparison to the control. Plants inoculated with *R. solani* and either T5 or T8 had significantly greater shoot dry weight than the uninoculated control (Figure 3-4).

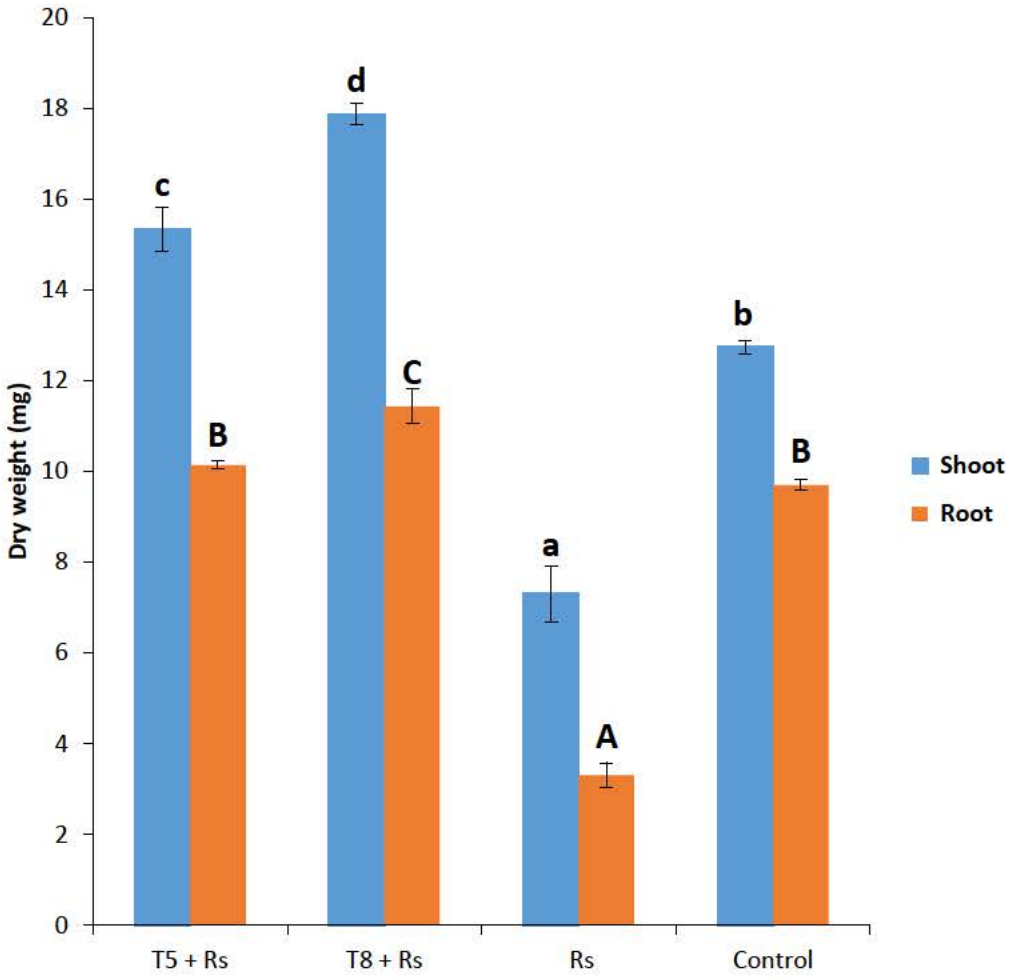

Figure 3-4 . Mean dry weight of potato plants (cv. Desiree) inoculated with *Trichoderma* strains (T5 + T8) and *R. solani* (Rs) in the sand-potting mix in a glasshouse trial at 20°C. T5: *T. harzianum*; T8: *T. hamatum*; Rs: *R. solani* only; control: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

3.3.6. Effect of *Trichoderma* on growth of plantlets from tissue culture

This experiment was done to test for growth promotion effects in the absence of pathogen. Application of conidial suspensions of *Trichoderma* through the colonization of roots significantly increased biomass production in comparison to the untreated control in pots with sand-potting mix under glasshouse conditions (Figure 3-5). Compared with untreated control treatment, *T. hamatum* T8 and *T. harzianum* T5 increased shoot dry weight by 4.4 and 2.5 times; respectively, and for root dry weight, by 3.1 and 2.5 times, respectively (Figure 3-5).

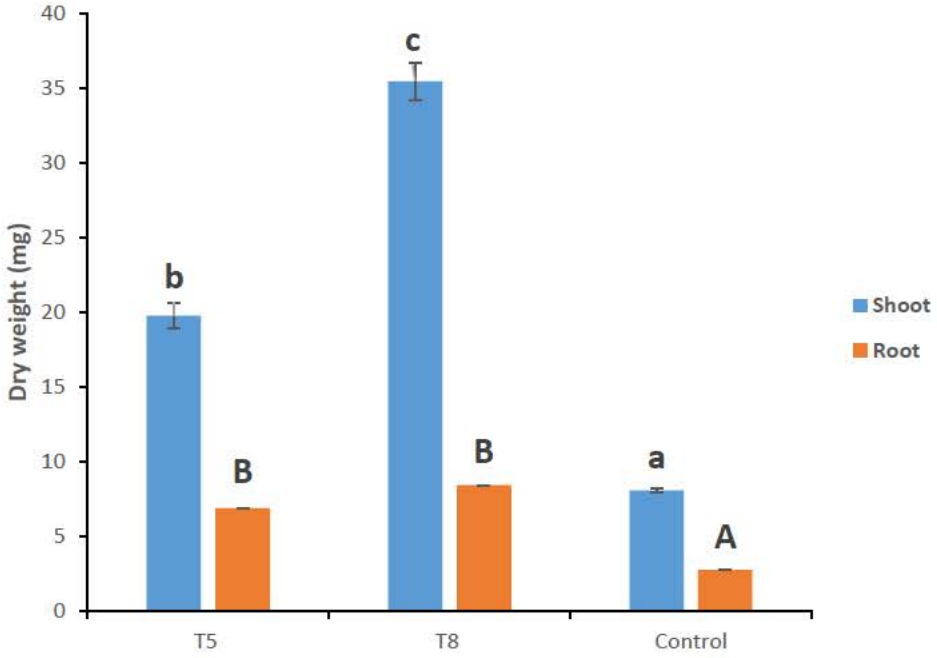

Figure 3-5 Dry weight of potato plantlets inoculated with *Trichoderma* strains (T5 + T8) at 20°C. T5: *T. harzianum*; T8: *T. hamatum*; control: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

3.3.7. Induction of systemic resistance

The experiment was carried out to evaluate the systemic protection against *R. solani* induced by *T. harzianum* T5 and *T. hamatum* T8 in cv. Desiree, by inoculating the roots with the antagonists and the stem with the pathogen. At 7 days after inoculation, lesions had appeared on the stems treated with the pathogen only by 0.43 cm covered area with lesions, and potato plantlets growth was significantly reduced (Figure 3-6).

Treatment of plants with *Trichoderma* strains prevented the appearance of symptoms and at the same time significantly promoted plant growth (Figure 3-6). *T. hamatum* strain T8 provided significantly higher biomass production of potato (shoot and root dry weights) than *T. harzianum* strain T5. Compared with the *R. solani* only treatment, T8 and T5 increased shoot dry weight by 6.2 and 3.3 times; respectively, and for root dry weight, by 4.2 and 3.2 times, respectively (Figure 3-6). While relative to untreated control, T8 and T5 increased shoot dry weight by 2.8 and 1.5 times; respectively, and for root dry weight, by 1.8 and 1.3 times, respectively (Figure 3-6).

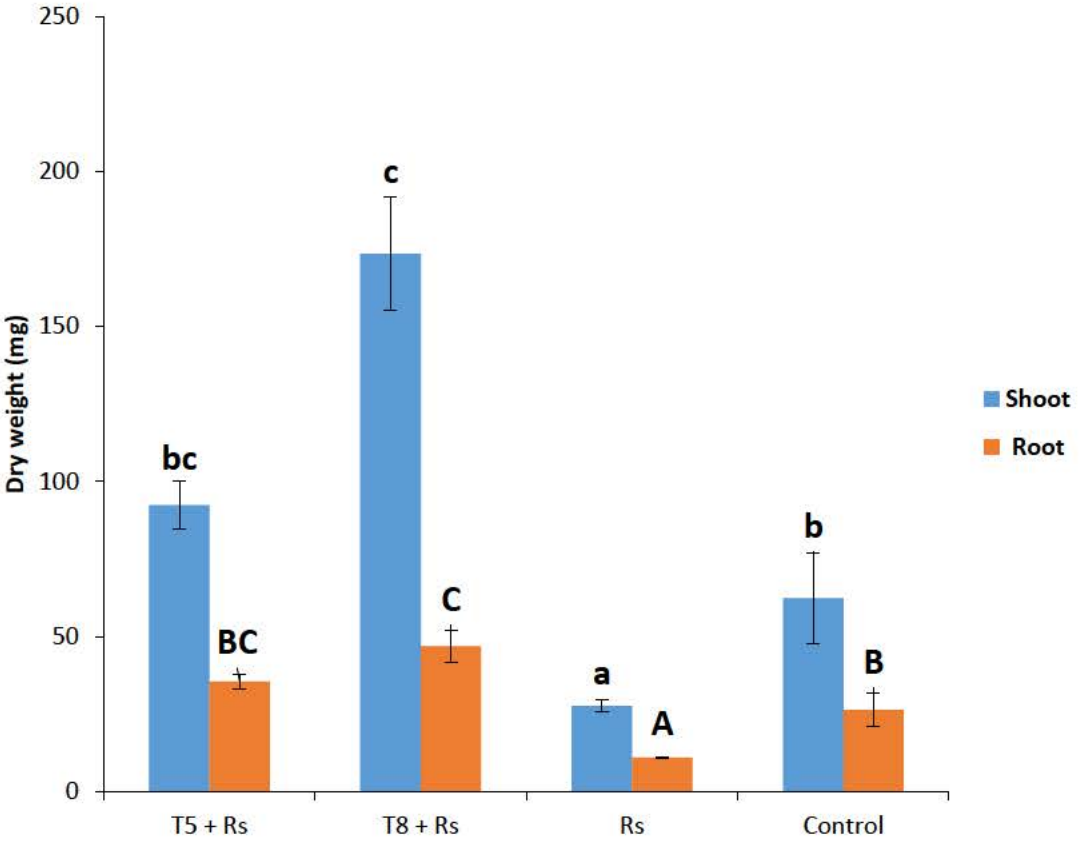

Figure 3-6 Dry weight of potato (cv. Desiree) after root inoculation with two isolates of *Trichoderma* (T5 + T8) and stem inoculation with *R. solani* (Rs) in the hydroponic growth system under glasshouse conditions at 20°C. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

3.4. Discussion

The aim of this chapter was to isolate *Trichoderma* strains that are suitable for more experiments on antagonism of stem canker and black scurf of potato caused by *R. solani* AG-3PT. The study resulted in the selection of two antagonistic fungi, which belonged to the species *T. harzianum* and *T. hamatum*. These isolates were chosen for further experiments. *T. hamatum* T8 was the most effective in dual culture and increased growth parameters, while T5

gave the highest inhibition of pathogen growth in the antibiosis test and also increased potato plant growth. Most studies have screened larger numbers of isolates. For example, Hicks et al. (2014) tested 53 isolates of *Trichoderma* as biocontrol agents to reduce stem canker disease of potato caused by *R. solani* under glasshouse and field trials. They found that some isolates significantly reduced the stem canker disease and increased the plant growth. Durak (2016) tested 81 *Trichoderma* strains which were isolated from soil samples as biocontrol agents to reduce *R. solani* on potato plants in vitro and in vivo trials. Also, Brewer and Larkin (2005) tested 28 isolates of fungi as biocontrol agents to inhibit stem canker disease caused by *R. solani* AG-3PT in a glasshouse and field trials. They found that some isolates inhibited the pathogen growth, and one isolate (*Rhizoctonia zeae*) prevented the pathogen from causing more severe disease. It was originally intended to screen a larger number of isolates in this study. But because isolates with high biocontrol effect and strong growth promotion were found among the first eight isolates tested, no further screening was conducted, with one representative of each of *T. harzianum* T5 and *T. hamatum* T8 chosen for further experiments.

All *Trichoderma* isolates inhibited the growth of *R. solani* AG-3PT, both in dual culture and by culture filtrates. Isolates of both species *T. hamatum* and *T. harzianum* have been shown to produce toxic secondary metabolites, and secreted cell wall degrading enzymes, which could affect growth of *R. solani* AG-3PT. *Trichoderma* strains are well known to produce many enzymes that lead to cell-wall dissolution of pathogens such as protease, β-1,3-glucanase and chitinase, compete positively with plant pathogens for nutrients and space (Whipps, 2001), and also produce appressoria in contact with the host then coil around the hyphae and attack them leading to the death of the parasitized fungus. The ability of *Trichoderma* isolates to suppress *R. solani* makes them potential biocontrol agents (Benítez et al., 2004; Chet et al., 1981; Kumar et al., 2016; Mihuta-Grimm and Rowe, 1986; Mishra et al., 2011; Papavizas, 1985; Sivakumar et al., 2000).

In the glasshouse, *R. solani* AG-3PT decreased shoot and root growth, reduced the number of stolons and tubers, and reduced tuber fresh weight. It also caused canker lesions on stems and stolons, and sclerotia (scurf) on tubers. Canker disease on potato plants was obviously occurring because of reduction of stolon number through killing growing tips, but the appearance of lesions on surviving stems was inconsistent. Treatment with *Trichoderma* spp. prevented the appearance of symptoms and also increased tuber fresh weight and biomass production in comparison to the pathogen control treatment in both sand-potting mix and

vermiculite in the pot assays. *Trichoderma* spore suspensions prevented sclerotium formation on the plants in all glasshouse experiments, and the treatments with *Trichoderma* isolates significantly increased potato biomass production, number of stolons and tuber yield. The results indicated that *T. hamatum* T8 had the best effect on growth against *R. solani* stem canker disease compared to the other isolates. So, this isolate should be considered for further assessment as a biocontrol agent.

In tissue culture plantlets, *T. hamatum* T8 and *T. harzianum* T5 significantly increased the biomass weight of Desiree potato plantlets inoculated with *R. solani* AG-3PT compared with control treatments in sand-potting mix. Stem canker was obviously occurring in the *R. solani* only treatment, but no canker was seen with *Trichoderma* treatments or untreated control. Some isolates of *Trichoderma* can reduce or eliminate the growth of *R. solani* and promote plant growth by different mechanisms such as antagonism, hyperparasitism, antibiotics, competition for nutrients and space, induced systemic resistance, endophytic competence, and biofertilization and stimulation of plant defence (Kumar et al., 2016).

Trichoderma isolates also promoted the growth of potato plantlets in the absence of disease. The literature suggests that *Trichoderma* species play a crucial role in plant growth through the colonization of root systems which changes plant metabolism, alters the content of phenolic compounds, hormones, amino acids and soluble sugars; and also rate of photosynthesis; transpiration and water content (Contreras-Cornejo et al., 2016; Martínez-Medina et al., 2014). At the same time, they have been reported to release secondary metabolites in the rhizosphere area, which can enhance shoot and root dry weight (Kavoo-Mwangi et al., 2013; Rubio et al., 2012). The increase in plant growth by inoculation with *Trichoderma* may be due to the absorption of organic and inorganic substrates from the soil, which support the plant roots and increase biocontrol activity (Benitez et al., 2004). Similarly, Hicks et al. (2014) reported that strains of *Trichoderma* spp. individually or in combination increased plant growth parameters and suppressed Rhizoctonia disease of tissue culture potato plantlets. An important factor is that *Trichoderma* isolates have been proven to enhance the development of plant roots, which is often accompanied by augmented yield of plant and biomass (Harman et al., 2004; Rubio et al., 2012). (Kumar et al., 2016) The plant yield increase measured in the present study, was probably due to the interactions between biocontrol agent and potato tubers directly before sowing, for instance from acquisition of nutrients such as P, Fe, Cu, Mn, and Zn or hormone regulations, most notably auxins, cytokinins, gibberellins,

ethylene and abscisic acid or perhaps from a collection of both these impacts (Contreras-Cornejo et al., 2016; Hermosa et al., 2012; Hicks et al., 2014).

In a hydroponics system, induced systemic protection of potato was seen in plantlets whose roots were treated with biocontrol agents. *T. hamatum* T8 gave significantly higher biomass production of potato than *T. harzianum* T5 compared with the control treatment. Canker was clearly appearing on stems in the *R. solani* only treatment, but no canker was seen with other treatments. Canker was probably due to toothpicks inoculated with pathogen directly affecting the stems. *Trichoderma* isolates were inoculated onto roots, with the *R. solani* was inoculated on stems two days later. Although this is an indication of potential induced systemic resistance in plants, or there is also a possibility that the *Trichoderma* colonised the tissue endophytically and interacted directly with the pathogen (Contreras-Cornejo et al., 2016), so it is not definitively due to induced resistance. Also, *Trichoderma* strains have been reported to stimulate growth of plants and defences against plant pathogens, and elicit the induced systemic resistance by the expression of defence-related genes of the jasmonic acid or salicylic acid pathways (Hermosa et al., 2012). A report by Shoresh and Harman (2008) who studied the root colonization of maize by a biocontrol agent of *Trichoderma* in relation to systemic induced resistance and growth response. They found that root colonization by *Trichoderma* induced changes in the proteome in the shoot and root of maize seedlings which included both in defence related proteins and those associated with increased photosynthetic and respiratory rates which enhance plant growth.

In summary, the disease level was rated as mild or inconsistent, but the effect of *R. solani* AG-3PT on potato plant growth and yield was high. Therefore, the emphasis of this study will be on plant growth parameters to compare between treatments and control (pathogen only). The expectation was that *Trichoderma* would reduce the severity of canker and scurf symptoms (Brewer and Larkin, 2005; Durak, 2016; Hicks et al., 2014; Mrabet et al., 2013). The results illustrated that treatments with T5 and T8 eliminated stem canker and black scurf symptoms, so it was difficult to use symptoms (disease level) to measure the effects of the interaction with other factors. But there was a reduction in growth and tuber yield due to the pathogen which was quantitative, so it is an alternative strategy to study the *Trichoderma*-pathogen-plant interaction. Growth parameters are often easier to work with because they are continuous variables that have normal distributions and meet the assumptions of most statistical tests. One complication is that there is a growth promotion effect of the *Trichoderma* by itself,

which is difficult to separate from the effect of *Trichoderma* on disease. *T. harzianum* strain T5 and *T. hamatum* strain T8 were chosen for further experiments in the next chapters.

Chapter 4. Effect of potato varieties on antagonism and growth promotion

4.1. Introduction

In recent years, the use of fungicides for controlling soilborne fungal diseases like *Rhizoctonia* has become undesirable due to environmental pollution. Hence, the utilization of plant resistance to pathogens in addition to using crop rotation may be the most efficient way to control the pathogen (Scholten et al., 2001). According to Naz et al. (2008), selecting and planting of resistant potato varieties is becoming an economic and efficient manner to combat stem canker and tuber black scurf disease. In recent years, collaborative research efforts have been focused on the identification, selection and integration of potato resistance genes into varieties with tolerable horticultural characteristics in order to manage disease (Gudmestad et al., 2007).

Many papers tested different varieties of potato for their resistance to *Rhizoctonia solani*. For example, El-Naggar et al. (2013) tested nine potato varieties for their susceptibility to stem canker and black scurf disease. The results showed there was difference in their susceptibility which was divided into three categories based on proportion of infection of stem canker and black scurf. These ranged between the most susceptible variety (8-10% covered area with sclerotia, and 16-19% stem canker), moderately susceptible (5-7% covered area with sclerotia, and 8-14% stem canker) and the most resistant (3-5% covered area with sclerotia, and 5-7% stem canker). In another study, 20 isolates of *R. solani* AG-3PT were tested on nine potato varieties for their resistance to the stem canker and black scurf disease. Isolate, variety and interaction between isolate and variety had highly significant effects on the stem canker and black scurf parameters (El-Aziz et al., 2013). Zhang et al. (2014) tested 20 potato varieties with different amounts of wheat bran inoculum of *R. solani* for their resistance and susceptibility to black scurf disease. The varieties were divided into five categories from immune to highly susceptible.

Potato varieties vary in their resistance or susceptibility to Rhizoctonia disease, and this could be attributed to differences in the plant anatomy, morphology and biochemical components of either plants or tubers (El-Naggar et al., 2013; Mohsan et al., 2016). A previous study showed that coloured potatoes, rich in anthocyanins are receiving increased interest on

the markets. Coloured potatoes had better resistance to soft rot disease caused by *Pectobacterium carotovorum* (Wegener and Jansen, 2007). The greater resistance of coloured potatoes was related to total soluble phenols and anthocyanins which contributed crucially to resistance expression in tuber tissue.

The effect of resistance may play an important role in the interaction between biocontrol agents and varieties to improve plant health by reducing disease. Therefore, the study of the host effect on disease suppression, on mechanisms important in biocontrol, and on growth is very important (Smith and Goodman, 1999). A previous study showed that *Bacillus mycoides* as biocontrol agent was more effective on a variety resistant against Cercospora leaf spot of sugar beet caused by *Cercospora beticola* than in a susceptible variety (Jacobsen et al., 2004). Similarly, Banani et al. (2014) indicated that the highest level of *T. harzianum* efficacy was observed in the varieties of grapevine that were least susceptible to the downy mildew caused by *Plasmopara viticola*, which led to enhanced plant growth. On the other hand, Kraft and Papavizas (1983) showed that *Trichoderma* species as biocontrol agents were less effective on resistant varieties against damping-off of tomato caused by *Pythium torulosum*, and Fusarium root rot in peas caused by *Fusarium solani* f. sp. *pisi*. However, *Fusarium oxysporum* as a biocontrol was equally effective on resistant and susceptible varieties against root-knot nematode *Meloidogyne incognita* (Dababat et al., 2008). There seems to be no general principle to predict what the effect of resistance on biocontrol will be.

There have been no papers that compare the effectiveness of biocontrol of Rhizoctonia diseases on different varieties of potato. Biocontrol *Trichoderma* isolates could be less effective on more resistant potato varieties. Thus, the interaction between *Trichoderma* as a biocontrol agent and resistant varieties of potato can be used as an ideal control to manage Rhizoctonia diseases and plant growth promotion.

The aim of the present work was to test whether potato variety affects the interaction between *Trichoderma* isolates against *Rhizoctonia solani* AG-3PT. Because there is evidence that pigmentation can affect resistance to some potato diseases, varieties with a range of colours were chosen. The possibility that chemistry of tissue could affect interaction was tested by effects of tissue type on growth and antagonism.

4.2. Materials and Methods

4.2.1. Effect of *R. solani* AG-3PT on potato varieties in a glasshouse trial

The effect of *R. solani* AG-3PT was examined on six varieties of potato plants, with contrasting coloured tubers. The selection of varieties was based on coloured potatoes which contain different amounts of secondary metabolites which may give better resistance to diseases (Wegener and Jansen, 2007). The varieties were Desiree (pink skin, yellow flesh), Sebago (white skin, white flesh), Dutch Cream (white skin, yellow flesh), Royal Blue (purple skin, yellow flesh), Ruby Lou (pink skin, white flesh) and Sapphire (purple skin, purple flesh). Certified seed tubers were bought from Diggers Club, Dromana, Victoria for Sapphire and from Goodman Seeds, Bairnsdale, Victoria for the other varieties. Sand with potting mix 2:1 was used in this experiment. Potato seeds were sterilized by using 1% NaOCl with two drops of Triton X-100 detergent (Sigma-Aldrich) for 2 minutes, washed with sterilized distilled water, and then dried for 3 hr at room temperature. Pathogen inoculum on wheat seeds as in Chapter 3 was mixed in potting media at the rate of 16 g around potato tubers in plastic pots (20 x 20 cm). One tuber was sown in each pot. The control for each variety (uninfested soil) used autoclaved wheat seeds that had been soaked in water but not inoculated. There were 3 replicates for each treatment. This test was carried out under controlled conditions in a glasshouse at 20^{o}C (8 weeks). The total number of sclerotia, number of tubers and number of sclerotia per tuber were measured per plant. It was planned to rate scurf on a scale (0-5): 0 =no sclerotia present, 1 =less than 1% of tuber area covered, 2 =2-10% of tuber area covered, 3 =11-20% of tuber area covered, 4 =21-50% of tuber area covered and 5 =51% or more of tuber area covered (Zhang et al., 2014). Because the density of sclerotia on tubers was less than 2%, therefore, the use of the scale was not helpful. The whole experiment was repeated, with 3 replicates for each treatment to confirm the results. The measurements recorded in the repeat experiment were number of stolons, number of tubers, sclerotia per tuber, shoot and root dry weight and tuber fresh weight. The shoots and roots were dried in an oven at 60°C until constant weight (Campion et al., 2003).

4.2.2. Effects of powdered plant tissue on the growth and interaction of *R. solani* AG-3PT and *Trichoderma* sp.

The differences in chemical composition and nutrient content between potato varieties could affect the growth and interaction between *R. solani* and *Trichoderma* sp. A system was developed to study this in Petri-dishes, using powdered tissue from the peel of tubers and sprouts. The varieties used were from tubers and sprouts of potatoes Sebago, Desiree, Sapphire, Royal Blue, Ruby Lou, and Dutch Cream. The tubers were left for three weeks at room temperature at 22-25°C to grow sprouts. The tubers were washed under tap water and sterilized by using 1% NaOCl with two drops of Triton X-100 detergent (Sigma-Aldrich) per litre for 2 minutes, then washed with sterilized distilled water. The sprouts were removed from the tubers, and then a vegetable peeler was used to peel the skins off the tubers. Sprouts and peelings were placed into sterilized plastic containers separately, frozen at -18°C for one week, and then transferred to a freeze drier machine for 5 days. The samples were then ground in a coffee grinder inside the laminar flow cabinet to obtain the powder of each plant tissue. Water agar (WA) was prepared by adding 500 mL of distilled water to 8.5 g of agar in 1000 mL flask and shaken carefully. The medium was sterilized by autoclaving, and then left to cool then added 4 g of each powder to the medium before pouring into Petri-dishes. Agar plugs 5-mm-diameter containing hyphae of *R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8 which had been transferred from the margins of 5-day-old actively growing cultures on PDA were inoculated separately in the centre of Petri-dishes, in order to measure the radial growth 48 and 72 hours after incubation. Water agar was used as controls for each fungus (*R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8) which were inoculated separately at the centre of the Petri-dishes. There were three replicates for each treatment.

In dual culture assay, there was a similar trial which utilized a 5-mm- diameter disc of antagonist (T5 and T8) diametrically opposite a 5 mm diameter disc of pathogen (*R. solani* AG-3PT) to measure the inhibition zone. In controls the pathogen was plated alone on one side of the plate at the periphery. The plates were incubated at 25°C for both trials. The growth of the pathogen was measured on the day before contact. The experiment was replicated three times for each treatment. The percentage inhibition of growth was calculated by using the same equation as in Chapter 3.

4.2.3. Effect of *Trichoderma* and *R. solani* AG-3PT on three potato varieties under glasshouse conditions

The effect of *Trichoderma* isolates on disease caused by *R. solani* was assessed for three potato varieties (cvs. Sebago, Desiree and Sapphire). These varieties were chosen based on variations in resistance to the effect of pathogen from most susceptible to more resistant, respectively, and as representative of all varieties. There were four treatments for each variety: no inoculation control, *R. solani* only control, T5 plus *R. solani* or T8 plus *R. solani*. Plastic pots (20 X 20 cm) with Trevenna soil (Chromosol, loamy sand) were utilized in this experiment. Potato seed tubers were sterilized by using 1% NaOCl for two minutes, washed with sterilized distilled water, and then coated with spore suspensions of T5 or T8 (the same way as in Chapter 3). Pathogen inoculum on wheat seeds was prepared as in Chapter 3 and artificially mixed into soil at the rate of 16 g per pot, five days before sowing. Conidial suspension of two isolates of *Trichoderma* (*T. harzianum*, T5 and *T. hamatum*, T8) were prepared as in Chapter 3 for coating tubers. The control treatment (uninfested soil) used autoclaved wheat seeds that had been soaked in water but not inoculated. There were 3 replicates for each treatment with one tuber per pot. The experiment was carried out under natural light at 20^{o}C for 7 weeks. Plant growth parameters and black scurf disease were measured by examining the number of sclerotia, shoot and root dry weight, the number of stolons and tubers and tuber fresh weight (Hicks et al., 2014; Ramsay and Bawden, 1983).

4.2.4. Statistical analysis

Data were analysed using one-way or two-way ANOVA with statistical program SPSS version 22. Data were log-transformed when necessary to ensure homogeneity of variance. Tukey's multiple range test was used for mean separation at the 5% level. ANOVA tables are presented in Appendix 1.

4.3. Results

4.3.1. Effect of *R. solani* AG-3PT on potato varieties in a glasshouse trial

Sclerotia were observed on the progeny tubers after inoculation with *R. solani* AG-3PT, but all were low infection from 1 to 2% of tuber surface covered. Because the scale did not reflect the obvious differences between varieties; therefore, the sclerotia were counted. The infection noted after eight weeks post-planting, varied significantly depending on the variety of potato utilized. The number of sclerotia was significantly higher in the Sebago variety compared with Royal Blue, Sapphire and Ruby Lou. There were no significant differences in the number of tubers for all varieties which were used in this study (Table 4-1).

Table 4-1.Effect of *Rhizoctonia solani* AG-3PT on total number of sclerotia and the average number of tubers per plant of six varieties of potato. Data followed by the same letter are not significantly different at P = 0.05 (Tukey test).

Varieties	No. of sclerotia	No. of tubers
Sebago	25.7[b]	4.0[a]
Desiree	16.7[ab]	5.7[a]
Dutch Cream	13.0[ab]	7.0[a]
Royal Blue	5.3[a]	4.7[a]
Sapphire	4.3[a]	5.0[a]
Ruby Lou	9.0[a]	4.3[a]
Probability	0.002	ns

ns means not significant.

The number of sclerotia per tuber differed significantly depending on the potato variety (Figure 4-1). Variety Sebago had significantly more sclerotia per tuber than all other varieties, while Desiree potatoes had significantly more sclerotia per tuber than varieties Royal Blue and Sapphire (Figure 4-1).

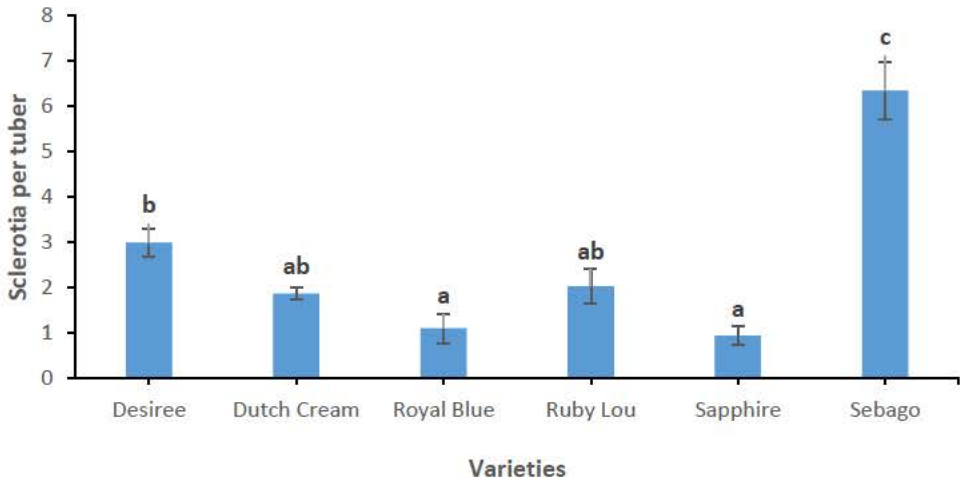

Figure 4-1. Number of sclerotia per tuber of six potato varieties infected with *Rhizoctonia solani* AG-3PT. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The experiment was repeated, with more growth parameters measured. There were significant effects of variety (P <0.001), pathogen (P <0.001), and interaction between variety and pathogen (P =0.001), on shoot dry weight. All varieties of potato had lower shoot dry weight in inoculated plants (Figure 4-2). The greatest relative effect of disease on shoot dry weight was seen in Royal Blue, Dutch Cream and Sapphire, and the least in Desiree (Table 4-2).

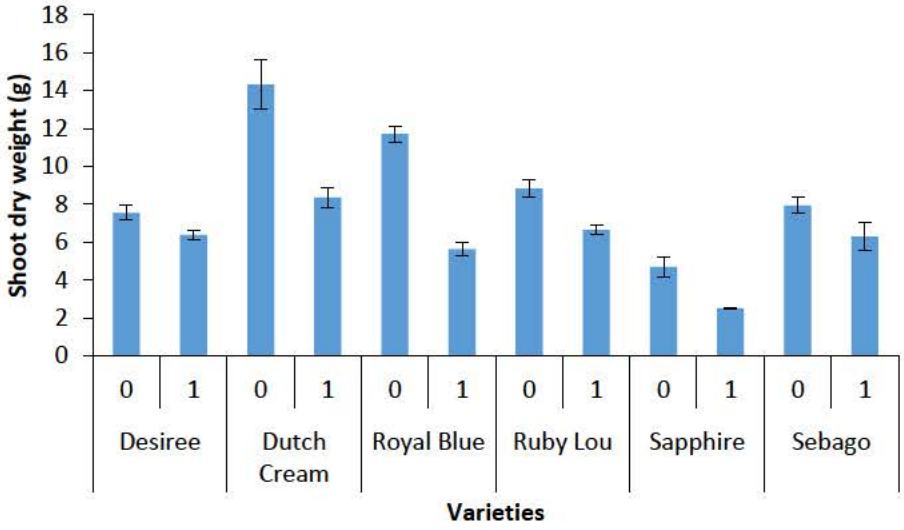

Figure 4-2. Effect of *Rhizoctonia solani* AG-3PT on shoot dry weight of six potato varieties in a repeat assay. 0 uninfested soil, 1 soil infested with *R. solani* AG-3PT. Error bars show standard errors (n=3).

The results showed that there were differences between the six potato varieties in the effects of *R. solani* AG-3PT, on different growth parameters (Table 4-2). Desiree variety had low effect on shoot dry weight, intermediate effect on tuber fresh weight but high effect on

stolon number. Dutch Cream variety had high effect on shoot dry weight and tuber fresh weight but intermediate effect on stolon number. Royal Blue variety had high effect on shoot dry weight, low effect on tuber fresh weight, high effect on stolon number and just few sclerotia. Ruby Lou variety had low effect on shoot dry weight, low effect on tuber fresh weight and low effect on stolon number. Sapphire variety had high effect on shoot dry weight, low effect on tuber fresh weight, low effect on stolon number and just few sclerotia. Finally, Sebago variety had low effect on shoot dry weight, high effect on tuber fresh weight but intermediate effect on stolon number (Table 4-2).

Table 4-2 Effect of *Rhizoctonia solani* AG-3PT on the growth parameters of six varieties of potato. Numbers show percentage decrease (or increase) in inoculated plants compared with controls.

	Decrease (%)				
Varieties	Shoot dry weight	Root dry weight	No. of Tubers	Tuber fresh weight	No. of stolons
Sebago	20	47	(129)[a]	45	20
Desiree	16	64	19	37	38
Dutch Cream	42	35	(60)	58	26
Royal Blue	52	35	(28)	21	31
Sapphire	46	44	24	26	11
Ruby Lou	25	32	23	27	13

[a] Values in parentheses represent an increase in number of tubers.

There were significant effects of variety ($P < 0.001$), pathogen ($P < 0.001$), and interaction between variety and pathogen ($P = 0.01$), on root dry weight. The root dry weight was reduced because of infection by *R. solani* (Figure 4-3). The largest reduction in root dry weight was seen in cv. Desiree, followed by Sebago and Sapphire (Table 4-2).

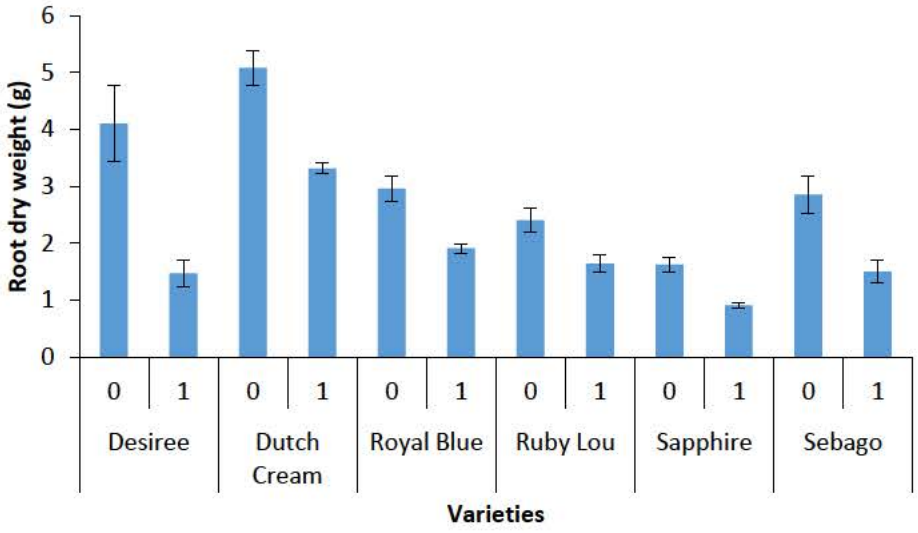

Figure 4-3. Effect of *Rhizoctonia solani* AG-3PT on root dry weight of six potato varieties in a repeat assay. 0 uninfested soil, 1 soil infested with *R. solani* AG-3PT. Error bars show standard errors (n=3).

The number of tubers differed significantly between varieties ($P < 0.001$), but there was no significant effect of pathogen on number of tubers (Figure 4-4). There was a significant interaction between variety and inoculation with the pathogen ($P = 0.02$). In three varieties (Sapphire, Ruby Lou and Desiree) the number of tubers was reduced by the pathogen while in the other varieties (Sebago, Royal Blue and Dutch-Cream) (Table 4-2), the number of tubers was increased by infection (Figure 4-4).

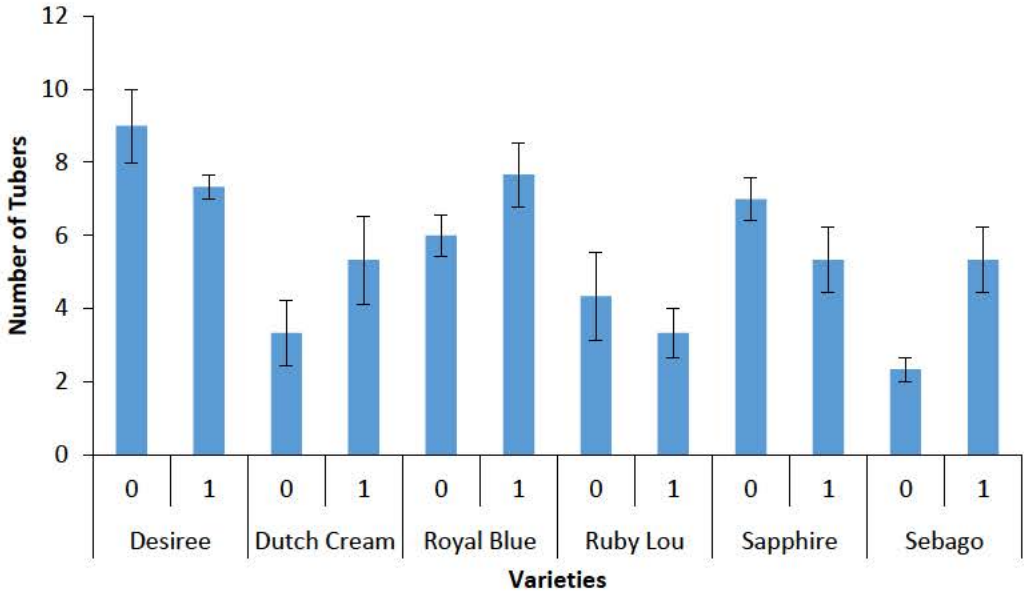

Figure 4-4. Effect of *Rhizoctonia solani* AG-3PT on number of tubers of six potato varieties in a repeat assay. 0 uninfested soil, 1 soil infested with *R. solani* AG-3PT. Error bars show standard errors (n=3).

The tuber fresh weight was affected significantly by variety (P <0.001), pathogen (P <0.001), and the interaction between variety and pathogen (P =0.01) (Figure 4-5). Dutch Cream plants produced significantly higher tuber fresh weight in the controls than all others, and Sapphire the lowest. Tuber fresh weight was reduced by infection with the pathogen, with the greatest relative reduction in Dutch Cream and Sebago and the least in Royal Blue and Sapphire (Table 4-2).

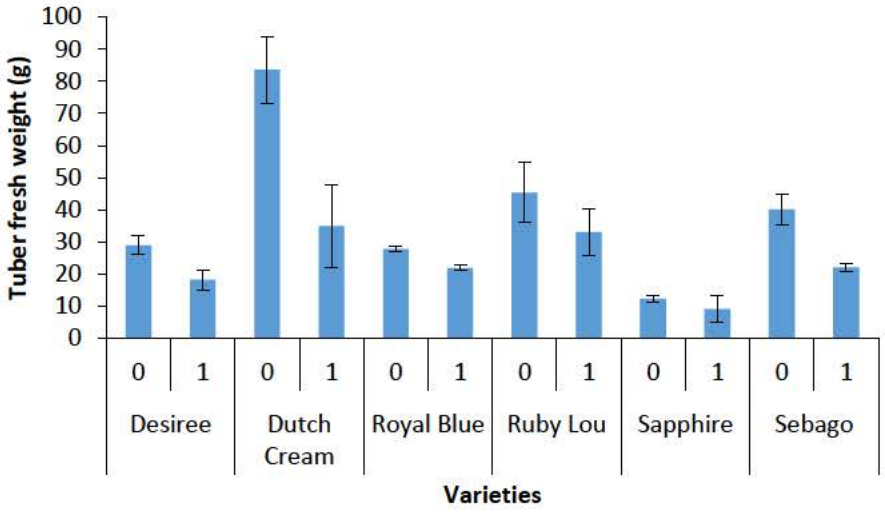

Figure 4-5. Effect of *Rhizoctonia solani* AG-3PT on fresh weight of tubers of six potato varieties in a repeat assay. 0 uninfested soil, 1 soil infested with *R. solani* AG-3PT. Error bars show standard errors (n=3).

The number of stolons was significantly affected by variety (P <0.001) and by pathogen (P <0.001), and by their interaction (P =0.001) (Figure 4-6). The highest number of stolons developed per plant, from 6 to 8, was noted for Dutch Cream and Royal Blue with Ruby Lou and Sebago having the lowest stolon number, from 4 to 5. The number of stolons was reduced by infection with the pathogen, with the greatest reduction in Desiree and Royal Blue, and the least in Sapphire and Ruby Lou (Table 4-2).

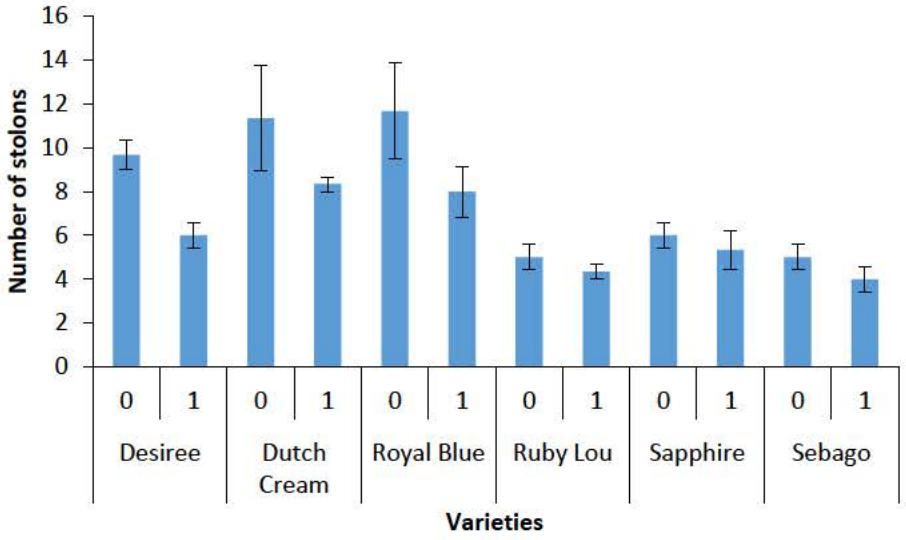

Figure 4-6. Effect of *Rhizoctonia solani* AG-3PT on the number of stolons of six potato varieties in a repeat assay. 0 uninfested soil, 1 soil infested with *R. solani* AG-3PT. Error bars show standard errors (n=3).

The number of sclerotia per plant differed significantly depending on the variety (P <0.001) (Figure 4-7). The number of sclerotia was significantly lower for Sapphire and Royal Blue than for all the other varieties.

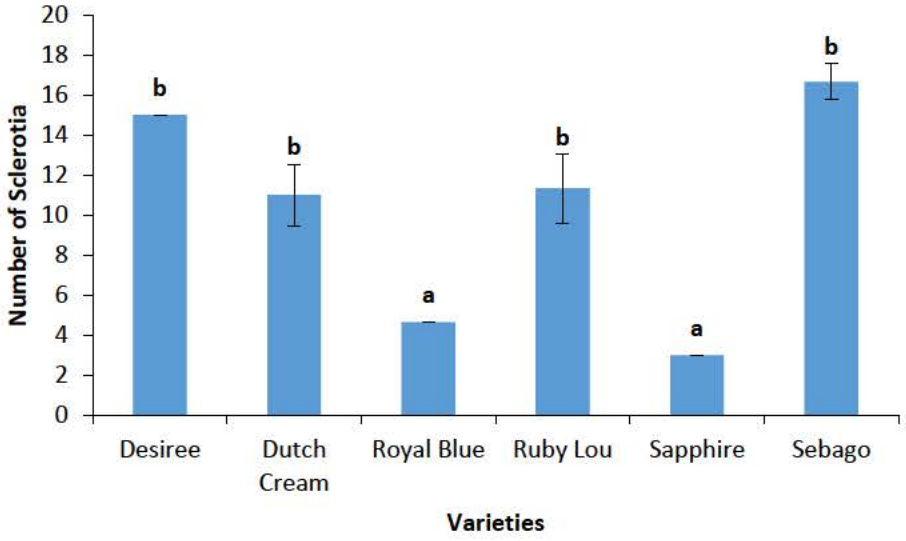

Figure 4-7. The number of sclerotia on tubers of six potato varieties inoculated with *Rhizoctonia solani* AG-3PT in a repeat assay. There were no sclerotia on uninoculated controls. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

4.3.2. Effects of powdered plant tissue on the growth and interaction of *R. solani* AG-3PT and *Trichoderma* sp.

The effects of plant tissue from peel and sprouts on the growth of *R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8 were determined. There were significant effects of variety ($P < 0.001$), tissue ($P < 0.001$), and interaction between variety and tissue ($P < 0.001$), on the growth of all fungi. The growth of *R. solani* was significantly increased by the plant tissues from peel and sprouts of different varieties of potato, but not of sprouts of Sebago in comparison to the control treatment (Figure 4-8). The overall differences between varieties were small. Growth of *R. solani* was significantly less on powdered sprouts of Sebago than on the other varieties.

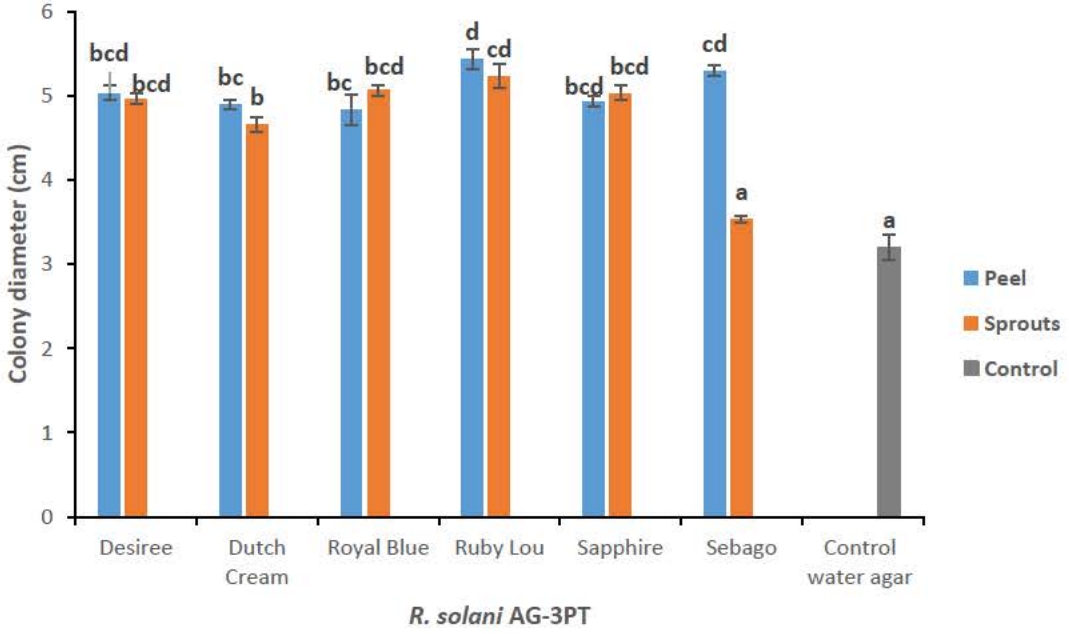

Figure 4-8. Growth rate of *Rhizoctonia solani* AG-3PT on water agar medium incorporating peel and sprout powder of six varieties of potatoes. The measurement was taken after 72 hours of growth. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The growth rate of *T. harzianum* T5 was significantly reduced by medium containing peel of Desiree in comparison to the control treatment, while growth was significantly greater on medium containing peel of Royal Blue and Sapphire compared with the other varieties or control treatment (Figure 4-9). Powdered material from potato sprouts caused a severe reduction in growth compared with peel, and T5 did not grow on medium made from sprouts of Sebago, compared with control treatment or other varieties.

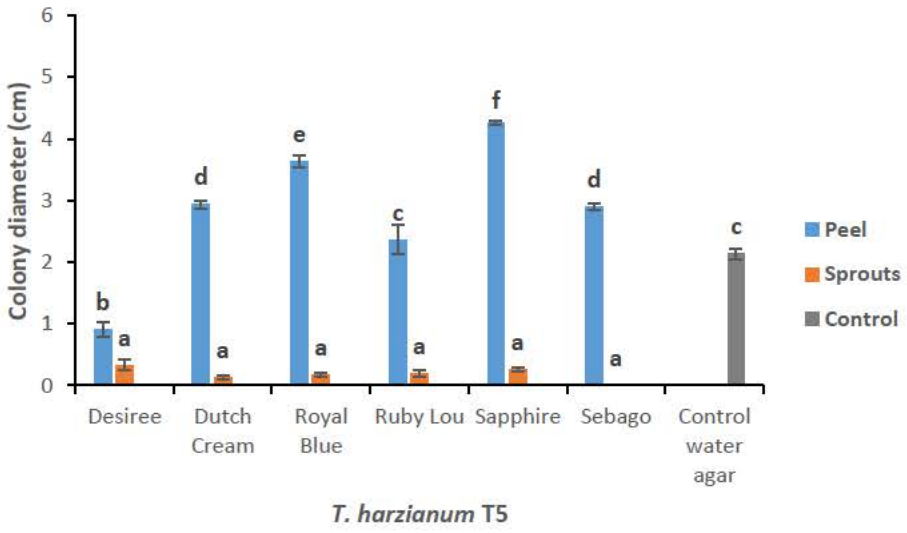

Figure 4-9. Growth rate of *Trichoderma harzianum* T5 on water agar medium incorporating peel and sprout powder of six varieties of potatoes. The measurement was taken after 72 hours of growth. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The growth rate of *T. hamatum* T8 was significantly and greatly reduced on medium containing peel of Desiree compared with the other varieties or control treatment, which differed only slightly (Figure 4-10). The growth of *T. hamatum* T8 was significantly increased by the medium containing peel of Dutch Cream, Royal Blue, Ruby Lou, Sapphire and Sebago, compared with control treatment. Growth of T8 on medium containing sprouts was much less than on medium containing peel ($P < 0.001$). Growth of T8 was significantly greater on medium containing sprouts of Dutch Cream and Royal Blue than the other varieties, and it did not grow on medium containing sprouts of Sebago at all in comparison to the control treatment or the other varieties.

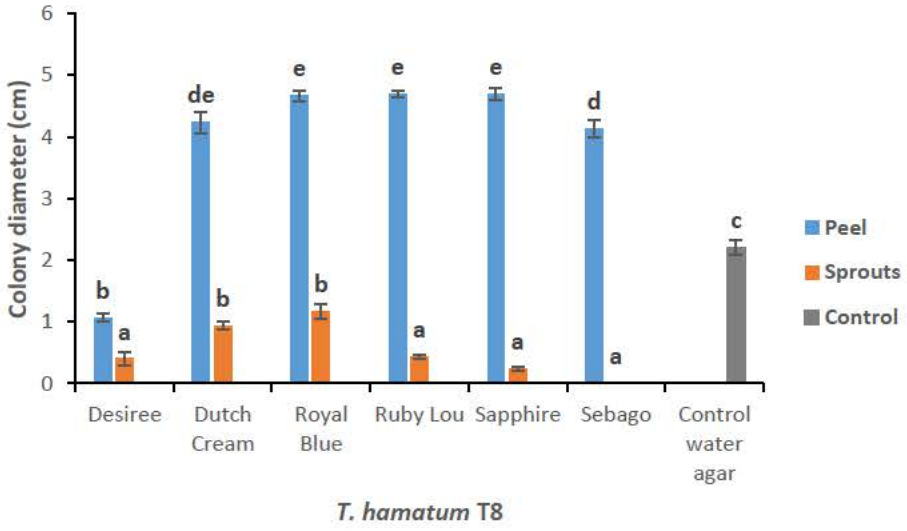

Figure 4-10. Growth rate of *Trichoderma hamatum* T8 on water agar medium incorporating peel and sprout powder of six varieties of potatoes. The measurement was taken after 72 hours of growth. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The inhibition of *R. solani* by *T. harzianum* T5 on different media from six potato varieties is shown in Figure 4-11. Growth inhibition of the pathogen was significantly higher when grown on potato peel medium than potato sprout medium for all varieties. In potato peel medium, the highest percentage inhibition of pathogen was with Dutch Cream, although the differences between most varieties were small. The lowest inhibition of growth on peel medium was observed with Desiree. In potato sprout media, T5 was significantly more effective in reducing the pathogen growth on Royal Blue than the other potato varieties.

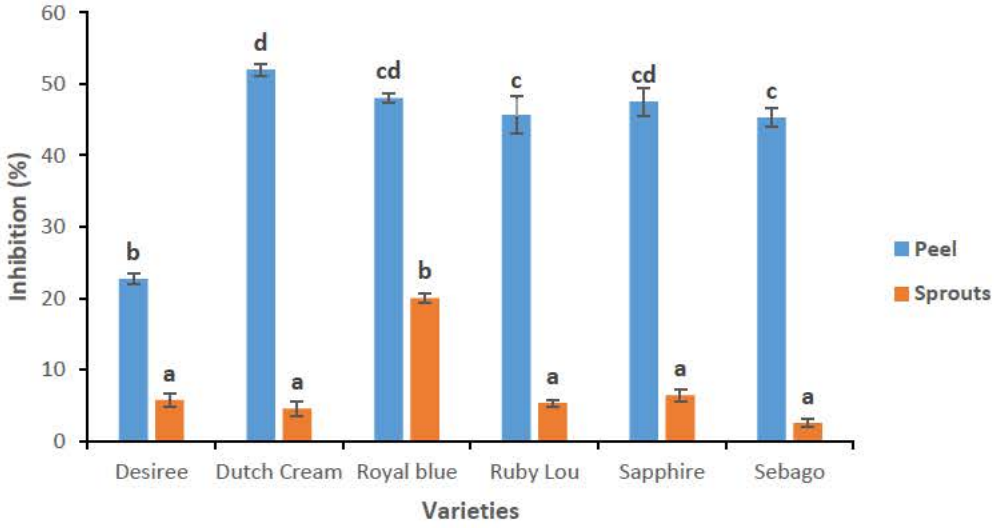

Figure 4-11. Inhibition of growth of *Rhizoctonia solani* AG-3PT by *Trichoderma harzianum* T5 in dual-culture on water agar medium incorporating peel and sprout powder of six varieties of potatoes. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The inhibition of *R. solani* by *T. hamatum* T8 on different media from six potato varieties is shown in Figure 4-12. Growth inhibition of the pathogen was significantly higher on potato peel medium than potato sprout medium for all varieties except Desiree. In potato peel media, Ruby Lou, Sapphire and Sebago gave the largest percentages of growth inhibition of the pathogen, while Desiree had the lowest percentage of pathogen inhibition. The sprout media had less effect on inhibition of *R. solani* by T8 than they did for T5. In potato sprout media, Desiree, Royal Blue and Dutch Cream had the largest percentages of growth inhibition of pathogen, whereas Sebago had the least inhibition of pathogen growth.

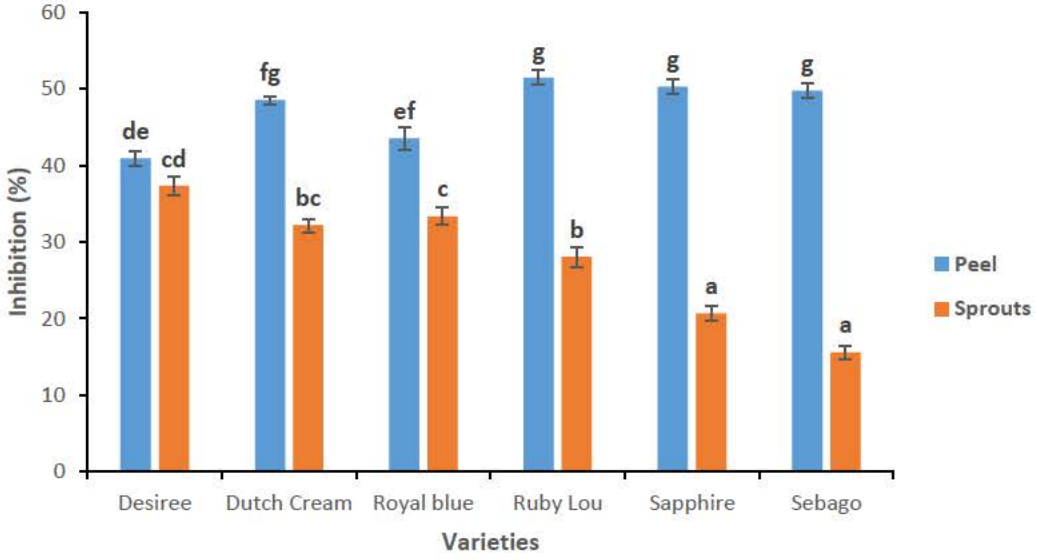

Figure 4-12. Inhibition of growth of *Rhizoctonia solani* AG-3PT by *Trichoderma hamatum* T8 in dual-culture on water agar medium incorporating peel and sprout powder of six varieties of potatoes. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

4.3.3. Effect of *Trichoderma* and *R. solani* AG-3PT on three potato varieties under glasshouse conditions

The number of sclerotia of *R. solani* was significantly higher in both Sebago and Desiree compared to the Sapphire variety (Figure 4-13). There were no sclerotia on uninoculated controls or plants treated with *Trichoderma* species.

When the experiment was analysed as a whole, there were significant effects of inoculation and variety on all parameters that were measured. The interaction between potato

varieties and fungal inoculation treatment was not statistically significant for any parameter, indicating that the effects of the two *Trichoderma* isolates were the same for all varieties. For clarity, data and analyses are presented separately for each variety in Tables 4-3 to 4-5.

When analysed separately, the reduction in growth due to the pathogen was not significant for any of the parameters measured for any variety (Table 4-3, Table 4-4 and Table 4-5). However, both T5 and T8 gave significant increases in shoot dry weight, root dry weight, number of stolons, and tuber fresh weight compared with the pathogen only control for all varieties (Table 4-3, Table 4-4 and Table 4-5). The growth promotion effect of T8 was generally greater than for T5. For instance, *T. hamatum* T8 had greater growth promotion than for *T. harzianum* T5 for all varieties in shoot and root dry weight, number of stolons, tuber fresh weight, and number of tubers except for Sebago variety.

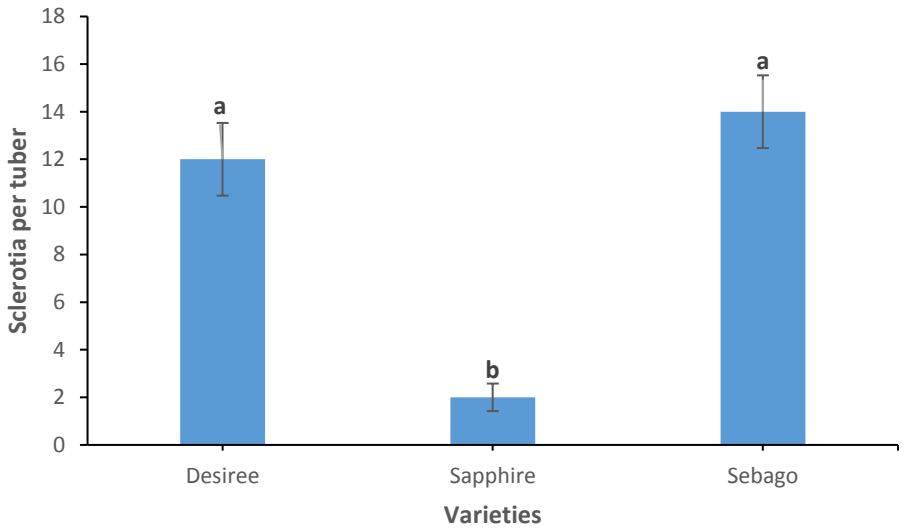

Figure 4-13. Number of sclerotia per tuber of three potato varieties infected with *Rhizoctonia solani* AG-3PT. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Table 4-3 Effect of *Rhizoctonia solani* and two isolates of *Trichoderma* on growth and yield of potato cv. Sebago grown in loamy sand in a glasshouse trial.

Treatment	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)
Control	6.01^{ab}	1.93^{a}	7.00^{ab}	5.67^{a}	129.75^{a}
R. solani	4.39^{a}	1.62^{a}	5.33^{a}	4.33^{a}	97.29^{a}
T5 + *R. solani*	7.78^{ab}	2.86^{a}	9.00^{bc}	8.00^{a}	169.08^{ab}
T8 + *R. solani*	8.57^{b}	3.10^{a}	10.00^{c}	5.67^{a}	228.04^{b}

Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD).

Table 4-4 Effect of *Rhizoctonia solani* and two isolates of *Trichoderma* on growth and yield of potato cv. Desiree grown in loamy sand in a glasshouse trial.

Treatment	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)
Control	5.09[a]	1.14[ab]	4.33[ab]	5.00[ab]	79.84[a]
R. solani	3.06[a]	0.71[a]	2.67[a]	3.00[a]	62.44[a]
T5 + *R. solani*	6.81[ab]	2.12[ab]	7.67[ab]	7.67[ab]	169.73[b]
T8 + *R. solani*	9.47[b]	2.53[b]	9.00[b]	8.50[b]	215.52[b]

Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD).

Table 4-5 Effect of *Rhizoctonia solani* and two isolates of *Trichoderma* on growth and yield of potato cv. Sapphire grown in loamy sand in a glasshouse trial.

Treatment	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)
Control	2.57[a]	1.06[a]	4.00[a]	4.67[a]	117.28[ab]
R. solani	3.83[a]	0.91[a]	3.33[a]	3.00[a]	111.41[a]
T5 + *R. solani*	6.17[b]	1.37[ab]	6.67[b]	5.00[a]	159.64[bc]
T8 + *R. solani*	6.82[b]	1.78[b]	8.00[b]	6.00[a]	185.32[c]

Numbers followed by the same letter within a column are not significantly different at P=0.05 (Tukey HSD).

4.4. Discussion

The goal of this chapter was to test whether potato variety interacts with the effect of *Trichoderma* isolates on *Rhizoctonia solani* AG-3PT. It was found that there were significant differences between varieties in the effect of *R. solani* AG-3PT on growth and yield. There were significant differences between varieties in the effects of tissue preparations on growth of *R. solani*, *T. harzianum* T5 and *T. hamatum* T8, and on antagonism between the *Trichoderma* isolates and *R. solani* AG-3PT. However, there was no significant interaction between variety and the effects of T5 and T8 in a pot trial.

The results indicated that some varieties of potato could reduce the formation of sclerotia of *R. solani* AG-3PT in glasshouse trials. In pot tests, there was significant variation in susceptibility among potato varieties when inoculated with *R. solani*. The results of this study has shown that Sebago and Desiree were highly sensitive to Rhizoctonia disease of potato, but varieties Sapphire and Royal Blue had higher levels of resistance to *R. solani* AG-3PT than the others. Ruby Lou and Dutch Cream varieties showed moderate susceptibility. The

number of sclerotia on tubers differed between varieties of potato but infection levels were low. Sclerotium formation depends on tuber maturity and it is difficult to grow mature tubers in pots (Dijst, 1989).

The differences in number of sclerotia on the potato tubers could be due to differences between varieties in resistance of the tissue to *R. solani* AG-3PT. However, it is also possible that differences between varieties in maturity of tubers at the end of the experiment may also have affected sclerotial production. Sclerotial production increases when the tubers mature (Dijst, 1989). This could complicate comparisons between varieties when looking at scurf symptoms in pot trials.

There were no significant effects of the pathogen on any growth parameters, unlike in the previous chapter. Daami-Remadi et al. (2008b) found that artificial infection with *R. solani* decreased the plant growth parameters of some potato varieties compared with non-inoculated control plants. The black scurf and stem canker diseases were shown to delay shoot emergence, reduce the number of stolons and root dry weight and yield, particularly with susceptible commercial varieties of potato. These growth parameters depend on the level of susceptibility to disease of the varieties (Daami-Remadi et al., 2008b; Tsror and Peretz-Alon, 2005). However, in Dutch Cream, Royal Blue and Sebago, *R. solani* inoculation increased the number of tubers in comparison to the non-inoculated control plants, although total tuber fresh weight was higher in control plants compared with those artificially inoculated by the pathogen. Similar results were obtained from a previous study, which showed that severity of black scurf and stem canker disease had no impact on total potato yield, but did change the tuber quality and distributions of tuber sizes (Simons and Gilligan, 1997). Moreover, they found that the size of tubers decreased in the artificial infection of *R. solani* treatment, compared with the control treatment. The results showed that Sapphire and Royal Blue had the greatest effect of *R. solani* on shoot dry weight, and least effect on yield. This means that effects on resource allocation were not the same for each variety. Khandaker et al. (2011) reported that the effect of *R. solani* with potato varieties have different effects on growth. For example, the highest disease incidence of potato was 42% observed in highly susceptible varieties reduced the yield to 10.91 tonnes/hectare, whereas the lowest disease incidence 22% in highly resistant varieties gave the highest yield of 18.51 tonnes/hectare. So, the variation on yield depends on relative differences in susceptibility of varieties to Rhizoctonia disease.

It is noteworthy that differences in resistance of potato varieties to *R. solani* AG-3PT were not very great. The difference between potato varieties in term of resistance to *R. solani* AG-3PT have been reported previously by (El-Naggar et al., 2013; Mohsan et al., 2016; Zhang et al., 2014). This could contribute to the lack of interaction between resistance and *Trichoderma*.

The hyphal growth rate of *R. solani* was significantly affected when the fungal media were supplemented with powder from peel and sprouts of the six potato varieties. Results showed that powdered peel of Ruby Lou, Sebago and Desiree varieties increased in vitro radial colony diameter of the fungus, while the highest reduction in colony growth was on powdered sprouts of Sebago variety. This suggested that if chemical compounds were involved, they play a role as toxins to many fungal plant pathogens, and are known be components of resistance against fungal diseases in plants (El-Naggar et al., 2013). There was an apparent correlation between tuber colour and resistance with the blue-skinned varieties Sapphire and Royal Blue having the fewest sclerotia. Similar work showed differences among the flower colours of legumes in the susceptibility to plant disease caused by *Phytophthora cinnamomi* (Panjehkeh et al., 2010). However, the powdered peel of Sapphire and Royal Blue did not inhibit growth of the pathogen in culture relative to the other varieties.

It was found that the in vitro hyphal growth of *T. harzianum* T5 and *T. hamatum* T8 was significantly higher on peel media in comparison with sprout media of all potato varieties which were used in this study, but the highest mycelial growth was obtained with Sapphire peel. From the results it was clear that biocontrol agents had no mycelial growth on Sebago sprouts medium. This strict inhibition may be due to release of some chemical compounds such as peptides, terpenoids, phenols, glycosides, polysaccharides, etc. that have high toxicity to negatively effect the growth of the biocontrol agents (Nedelnik and Repkova, 1998). Most likely, the growth of *Trichoderma* isolates was impacted by compounds in the growth medium of the peel or sprouts, as well as by potato variety. Consequently, this work is the first examination showing the role of peel and sprouts of potato on the growth of *Trichoderma* isolates.

The findings demonstrated that biocontrol agents have significant antagonistic efficacy against the pathogen (*R. solani* AG-3PT) when tested by dual culture in vitro. The percentage inhibition varied significantly according to the kind of potato variety, as well as between peel and sprouts. The percentage inhibition was higher in the potato peel amended media than

sprouts for all potato varieties for both *Trichoderma* isolates. This is consistent with sprouts reducing the radial growth of the *Trichoderma* isolates, but not *R. solani*, compared with peel. This might be related to the markedly higher contents of carbohydrates and phenolic compounds in peel tissue which may increase the activity of *Trichoderma* isolates. The highest percentage of inhibition against *R. solani* was recorded utilizing *T. harzianum* T5 on Dutch Cream peel, followed by *T. hamatum* T8 on Ruby Lou peel. The lowest inhibition was recorded using Sebago sprouts medium in both *Trichoderma* isolates, however, *T. hamatum* T8 gave greater inhibition on potato sprouts medium than *T. harzianum* T5. Several studies confirmed that *Trichoderma* spp. produce antibiotics, secrete enzymes and cell-wall degrading substance against various plant pathogens in addition to competition for nutrients and space to reduce or inhibit pathogen infections (Kim et al., 2002; Whipps, 2001). It was suggested that some phytotoxic substances which were released from peel or sprouts tissue of varieties of potato may have affected *Trichoderma* isolates growth. This work is the first to test the effect of peel and sprouts compounds for six potato varieties on their effect to control *R. solani* AG-3PT. More studies need to be done to identify the compounds responsible for the effects of the powdered material.

In the previous chapter, it was demonstrated that isolates of *Trichoderma* have various ability to reduce the activity of the *R. solani* AG-3PT, and also promote plant growth. The aim of this chapter was to identify the resistance among varieties, and the interaction between varieties and *Trichoderma* isolates against the pathogen. The results of the study are essential because they determined the behaviour of potato varieties by many assessment parameters against *R. solani* AG-3PT in vitro and under glasshouse conditions. The study showed that Sapphire and Royal Blue varieties were most resistant among other potato varieties to the disease. However, the effects of *Trichoderma* on disease and growth promotion were the same for all varieties, so there was no interaction between biocontrol and potato variety resistance. There were some weaknesses with the method, particularly the problem of looking at the disease in pots which restricts tuber formation. However, the overwhelming effect of the *Trichoderma* isolates occurred through growth promotion, and it did show that this occurred in varieties that differed in their growth responses to *R. solani* AG-3PT. Overall, the effect of *Trichoderma* isolates on pathogen (*R. solani* AG-3PT) and growth promotion were similar on all tested potato varieties.

Chapter 5. Effect of nutrients on antagonism and growth promotion

5.1. Introduction

Fertilizers are simply plant nutrients applied to agricultural fields to supplement required elements found naturally in the soil (Westermann, 1993). Fertilizers can be added from a variety of sources such as chemical fertilizers, organic matter and even by some plants. This maintains the soil fertility, so the farmer can continue to grow nutritious crops and healthy crops (Chang and Lee, 2016; Van Bruggen et al., 1996). So, application of fertilizers can promote plant growth and increase yield. Previous studies have referred to the role of nutrients in biological control of soilborne plant pathogens by competition for nutrients in addition to promotion of biocontrol activity (Benson and Baker, 1970; Huber, 1980).

Different nutrients have different effects on biocontrol agent-pathogen interactions. For example, in culture media, the use of potassium fertilizer can promote the activity of *Trichoderma* sp. against the pathogen growth of *Sclerotium cepivorum* (Ortega-Aguilar et al., 2011). The availability of three nitrogen sources such as urea, ammonium sulfate and potassium nitrate increased the activity of biocontrol *Trichoderma harzianum* against mycelial growth of *Sclerotium rolfsii* in the Petri-dishes (Khattabi et al., 2004). When nitrate was used as nitrogen source, the reduction of *S. rolfsii* was greater in soil by *T. harzianum* compared with ammonium and urea (Khattabi et al., 2004). Also, the availability of iron fertilizer increased the activity of *T. harzianum* and *T. hamatum* as biological control agents against the pathogen growth of *R. solani* (Tariq et al., 2010).

The combination between fertilizers and biological control agents can control plant diseases and promote plant growth (Benitez et al., 2004; Kavoo-Mwangi et al., 2013; Tariq et al., 2010). The addition of organic nutrition with biocontrol of *T. harzianum* to the soil significantly reduced the damping off disease on cucumber caused by *R. solani* under glasshouse conditions (Huang et al., 2011). Also, Abro et al. (2014) showed the effect of five concentrations of nitrate on biocontrol agent (*T. harzianum*) against *Botrytis cinerea* which causes tomato gray mold under glasshouse conditions. They found that high N fertilization increased the activity of biocontrol and protected the plants by 100%. It is noteworthy that fertilizers may inhibit the pathogen growth and affect the activity of biocontrol agents in

addition to plant growth promotion. However, there has been very little work published on testing this.

Therefore, this research project was to study whether fertilizers affected biocontrol and growth promotion of potato by *Trichoderma* spp. In addition, the effect of nutrients on interactions in culture was studied. The effects of selected nutrients were then tested in pot trials and a field trial for controlling *R. solani* AG-3PT and promoting potato plant growth.

5.2. Materials and Methods

5.2.1. Effect of nutrients in dual culture

Two isolates of *Trichoderma* (*T. harzianum* T5 and *T. hamatum* T8) were tested in a dual culture assay against *R. solani* AG-3PT on agar medium containing Hoagland's Nutrient Solution. The concentrations of seven elements were adjusted with five levels for each element independently, as elucidated below.

The composition of the standard Hoagland's nutrient solution was 5 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4.7H_2O$, 5 mM $CaNO_3$, 50 µM Fe EDTA, 45 µM H_3BO_3, 9 µM $MnCl_2.4H_2O$, 0.77 µM $ZnSO_4.7H_2O$, 0.32 µM $CuSO_4.5H_2O$ and 0.11 µM $H_2MoO_4.H_2O$.

The effects of nutrition on the interactions of the *R. solani* and *Trichoderma* spp. isolates were studied by increasing or reducing the concentration of one essential element (N, P, K, Ca, Mg, Fe and Mn) in Hoagland's nutrient solution for each trial. The solutions were adjusted to give 0.25, 0.5, 1, 1.5 and 2 times the standard concentration of each element (Table 5-1).

Table 5-1. Concentration of elements in seven series of Hoagland's solutions modified to give a range of rates of each of N, P, K, Ca, Mg, Fe and Mn.

Rate (x)	N (mM)	P (mM)	K (mM)	Ca (mM)	Mg (mM)	Fe (µM)	Mn (µM)
0.25	3.75	0.25	1.5	1.25	0.5	12.5	2.25
0.5	7.5	0.5	3	2.5	1	25	4.5
1	15	1	6	5	2	50	9
1.5	22.5	1.5	9	7.5	3	75	13.5
2	30	2	12	10	4	100	18

The concentration of N was adjusted by adding $NaNO_3$, or by replacing KNO_3 and $Ca(NO_3)_2$ with KCl and $CaCl_2$ as required to keep K and Ca concentrations constant. The

concentration of P was adjusted by adding NaH_2PO_4 or replacing KH_2PO_4 with KCl as required to keep K concentration constant. The concentration of K was adjusted by adding KCl or by replacing KNO_3 with $NaNO_3$ as required to keep N concentration constant. The concentration of Ca was adjusted by adding $CaCl_2$ or replacing $Ca(NO_3)_2$ with $NaNO_3$ as required to keep N concentration constant. The concentration of Mg was adjusted by adding $MgCl_2$ or replacing $MgSO_4$ with Na_2SO_4 as required to keep the concentration of S constant. The concentration of Fe was adjusted by varying the concentration of FeEDTA and adding NaEDTA as required to keep the EDTA concentration constant. The concentration of Mn was adjusted by varying the concentration of $MnCl_2$. It was assumed that the differences in concentration of Na and Cl in the solutions would not interact with the differences in essential nutrients.

Agar was added to each nutrient solution at 20 g/l before autoclaving and pouring into 9 cm Petri plates. Dual cultures (*T. harzianum* T5, and *T. hamatum*, T8 against *R. solani* AG-3PT) were inoculated on the same day and each combination was replicated three times. In controls the pathogen was plated alone on one side of the plate at the periphery. The plates were incubated at 20°C for 7 days. The growth of the pathogen was measured on the day before contact between colonies. The percentage inhibition of growth was calculated as in Chapter 3 (Terna et al., 2016; Whipps, 1987).

5.2.2. Effect of nutrients on antagonistic activity of culture filtrates

The effect of nutrition on the antagonistic activity of *Trichoderma* culture filtrates, as an indicator of antibiotic production, was examined by utilizing modifications of Hoagland's Nutrient Solution. The isolates of *Trichoderma* were grown in 250 mL plastic tubes, each of which contained 50 mL of sterilized liquid Hoagland solution with a range of concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, iron or manganese (Table 5-1), in a rotary shaker at 150 rpm and 22°C for 7 days. Because growth of fungi in the media may have altered nutrient content independently of antibiotic production, *R. solani* was also grown on the same media in order to provide an antagonist-free comparison. Culture filtrates were sterilized with a 0.45 μm filter. After that, 5 mL of culture filtrate was added to sterilized Petri-dishes (9 cm^3) and then 15 mL of molten PDA with low sugar (2 g of glucose) was added and mixed gently. The medium was allowed to cool, and inoculated with 5 diameter agar plugs from a 5 day old culture of *R. solani* AG-3PT. Cultures of the pathogen in PDB with low sugar (2 g of glucose) were also used as the control. There were three replicates for each treatment.

These plates were incubated at 20°C for 5 days. The radial growth of the pathogen was measured (Chen et al., 2012; Whipps, 1987).

5.2.3. Effect of nutrients on antagonism in planta

Based on dual culture and antibiotic production tests, the effects of different levels of potassium, nitrogen and manganese on biocontrol were selected for glasshouse trials. The inoculation treatments used were no inoculation (control); *R. solani* AG-3PT only; and *R. solani* AG-3PT in combination with *T. harzianum* T5 or *T. hamatum* T8. *Rhizoctonia solani* only with sterilized distilled water instead of nutrient solutions was used for checking both shoot dry weight and tuber fresh weight to compare with nutrient treatments.

Loamy sand soil was obtained from Smith Road, Newholme Farm, UNE. The soil at this site is a chromosol (loamy sand). In a depth of 1-10 cm, total C was 1.573%, N 0.161%, Silt 10.6%, Sand 74.3%, Clay 14.5%, Colwell P of 22.7 mg/Kg, pH of 5.6, EC of 60.6 µS/cm and exchangeable K of 0.39 $Cmol_c$/kg. Soil was sieved and placed into plastic pots (20 x 20 cm). Desiree seed potato tubers were sterilized by using 1% sodium hypochlorite for 2 minutes, and then washing with sterilized distilled water. The tubers were soaked in conidial suspension of *T. harzianum* T5 or *T. hamatum* T8 (10^6 conidia/mL water) for 1 hr, and dried for 3 hr at room temperature (Singleton et al., 1992). Pathogen inoculum on wheat seeds was prepared as in Chapter 3 (Balali et al., 1995; Williams, 1976). Pathogen inoculum (16 g) was mixed in soil around potato tubers in the pots (Abd-El-Khair et al., 2010).

The nutrient treatments were imposed by irrigating the potato plants with 300 mL of modified Hoagland's solution every week from seedlings until harvest. The standard treatment used normal Hoagland's solution as in section 5.2.1. High concentration of K had twice the K concentration obtained by adding KCl, while low concentration of K had half the K concentration and was adjusted by adding $NaNO_3$ instead of some of the KNO_3. Low concentration of N had 25% of the N concentration of the standard and was adjusted by replacing $Ca(NO_3)_2$ with $CaCl_2$ and some of the KNO_3 with KCl. Low concentration of Mn was obtained by omitting $MnCl_2$ from the solution.

There were 4 replicates for each treatment with 1 tuber/pot. This trial was carried out under controlled conditions in a glasshouse (20°C). Between fertilizer additions, plants were watered as required. Potato plants were harvested after 7 weeks of post emergence. The data

collected were stem canker disease severity which was measured at the time of harvest by measuring the length of lesion on stems based on a scale (0-4): 0 = no disease, 1 = less than 10% of stem area covered with lesions, 2 = 10-25% of stem area covered with lesions, 3 = 26-50% of stem area covered with lesions and 4 = stem girdled with lesions (Atkinson et al., 2010). The number of sclerotia on tubers was also observed. Measurements of growth for each plant were number of stolons, number of tubers, weight of tubers, and the dry weight of the shoots and roots.

5.2.4. Field experiment

5.2.4.1. Experimental site

A field experiment was conducted at the Trevenna Farm, University of New England, Armidale NSW, Australia. The soil at this site is a chromosol (loamy sand). In a depth of 1-10 cm, total C 1.573%, N 0.161%, Silt 10.6%, Sand 74.3%, Clay 14.5%, Colwell P of 22.7 mg/Kg, pH of 5.6, EC of 60.6 µS/cm and exchangeable K of 0.39 Cmolc/kg.

5.2.4.2. Artificial infestation of soil in the field experiment

R. solani AG-3PT was grown in mixed wheat and barley seeds incubated at 20°C in plastic oven bags for four weeks in the dark as in Chapter 3. The pathogen was added to soil at the rate of 100 g/m^2 one week before planting (Friberg et al., 2009). Based on previous results, the biocontrol agent *T. hamatum* T8 was selected for field experiments. T8 was grown on wheat bran. The bran was prepared in 30 × 45 cm polyester oven bags by adding 50 mL of distilled water to 100 g of bran in each bag and autoclaved at 121°C for 30 min. The bags were cooled and inoculated with T8. T8 inoculum was incubated at 28°C for 14 days. The inoculation of T8 into soil was at rate 50 g/m^2 of wheat bran one week before planting (Elad et al., 1980b).

5.2.4.3. Experimental design

A factorial field trial with commercial potato tubers (cv. Desiree) was planted on 3 October 2016 and harvested on 27 February 2017. A randomized complete block design with four replicates was used. Each plot was 100 x 90 cm in area and each block was 10 m long with buffers of 2 m between the blocks. Two potato tubers were sown in each plot. All plots were infested with *R. solani*. There were 4 fertilizer treatments: no fertilizer, low N, low K, and NPK; and 2 antagonist treatments: no *Trichoderma*, and T8. NPK fertilizer was as

recommended rate for potato growing on loamy sand soil (Wadas and Dziugieł, 2015), and low N or low K based on modifying of NPK standard level (Table 5-2). Inoculum of pathogen and antagonist were applied at 1 week before planting at a depth of 5-10 cm by removing the top layer of soil and spreading each inoculum evenly in depth before placing the soil again, and irrigated once to wet the soil. The fertilizer treatments were applied at 5 weeks after sowing the tubers by adding 500 mL of fertilizer solution by spraying (Table 5-2) to each plot.

Table 5-2. Formulation of fertilizer solutions used in field trial.

Component	Amount added (kg/ha)		
	NPK	Low N	Low K
Diammonium phosphate	262	262	262
Potassium sulphate	205	205	102.5
Ammonium sulphate	261		261

The site was irrigated as required to ensure maximum growth and avoid serious water stress. Stem canker disease severity and the number of sclerotia were measured as in section 5.2.3. The shoot dry weights, number of tubers and tuber fresh weight were recorded individually (Dahnke et al., 1989; Wu et al., 2013).

5.2.5. Effect of NPK fertilizers under glasshouse conditions

Based on field results, the response effect of nitrogen levels on plant growth was high. Therefore, this trial was repeated to check an observation on the effects of soil nitrogen levels on growth. The treatments were the same treatments as the field trial. Loamy sand was obtained from agricultural field of Trevenna Farm, and placed into 15 X 15 cm pots after sieving to remove the impurities. 2 g of biocontrol (*T. hamatum*, T8) inoculum on wheat bran and 4 g of pathogen (*R. solani*) inoculum on wheat seeds were incorporated into the soil 5 days prior to sowing seed potatoes of cv. Desiree. The fertilizer treatments were applied after emergence at 50 mL/pot of the solutions shown in Table 5-3.

Table 5-3. Formulation of fertilizer solutions used in pot trial.

Component	Amount added (g/l)		
	NPK	Low N	Low K
Diammonium phosphate	1.89	1.89	1.89
Potassium sulphate	1.48	1.48	0.74
Ammonium sulphate	1.88		1.88

There were three replicates for each treatment with 1 tuber/pot. Pots were grown at 20°C under natural light and were watered as required for three weeks. The results were expressed as the shoot dry weight.

5.2.6. Data analysis

All results were expressed as means and the impacts of *Trichoderma* spp. on *R. solani* AG-3PT and plant growth were checked by utilizing the two-way analysis of variance with statistical program SPSS version 22. Data were log-transformed if required to maintain homogeneity of variance. The least significant differences (LSD) at the 5% level was calculated to compare the means within and between the treatments. ANOVA tables are presented in Appendix 1.

5.3. Results

5.3.1. Effect of nutrients in dual culture

There was no significant effect of Ca concentration, or interaction between Ca and antagonist, on inhibition of *R. solani* in dual culture (Figure 5-1). There was a significant ($P < 0.03$) effect of antagonist on inhibition, which was significantly higher for T5 than for T8 (Figure 5-1). All isolates of *Trichoderma* overgrew the colonies of *R. solani* after 7 days in dual culture.

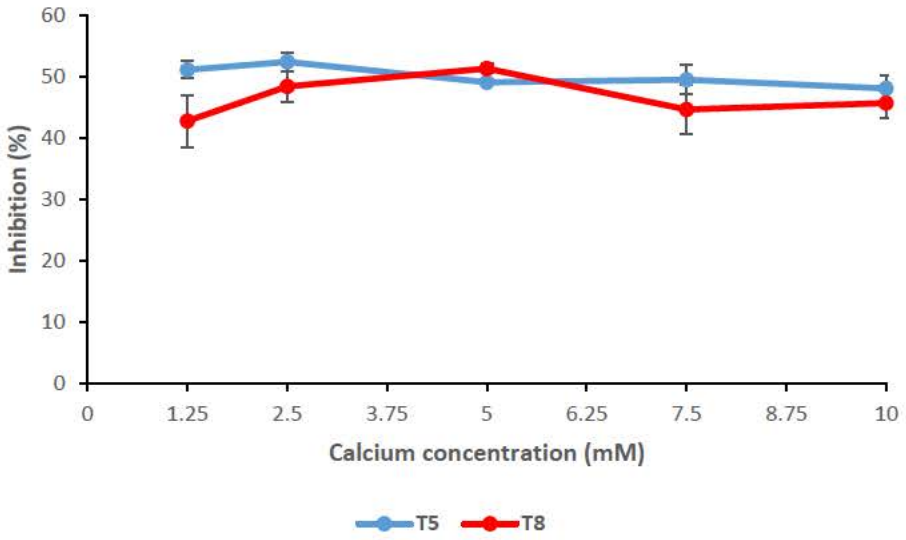

Figure 5-1. Effect of calcium (Ca) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There was no significant effect of Fe concentration, or interaction between Fe and antagonist, on inhibition of *R. solani* in dual culture (Figure 5-2). There was a significant ($P < 0.01$) effect of antagonist on inhibition, which was higher for T8 than for T5 (Figure 5-2).

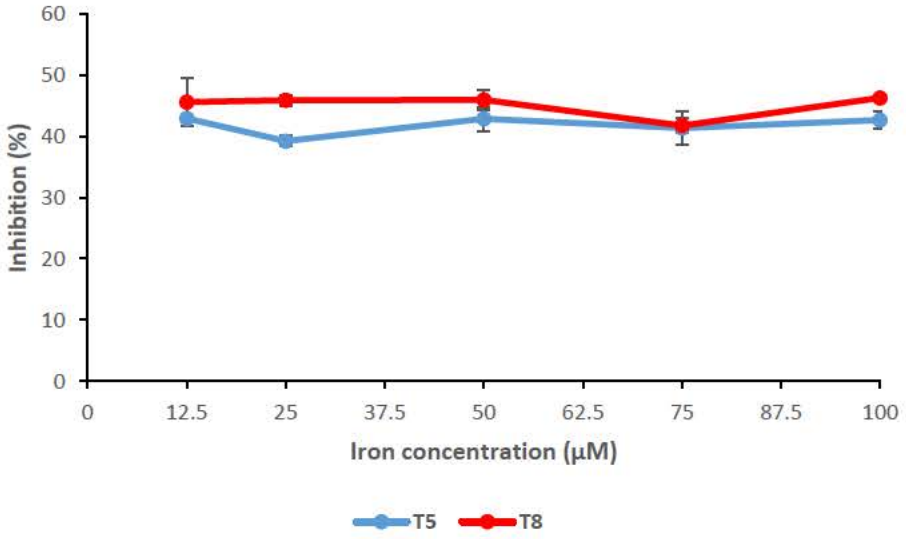

Figure 5-2. Effect of iron (Fe) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There were significant effects of concentration of K ($P < 0.001$), and interaction between antagonist and K concentration ($P = 0.002$), on inhibition of growth of *R. solani* in dual culture. There was no significant effect of antagonist on inhibition of pathogen growth. T5 showed significantly strongest inhibition at intermediate concentrations of K (Figure 5-3). T8 showed

lowest inhibition at both double and half of the standard concentration of K, and strongest inhibition at 1.5 times the standard concentration of K (Figure 5-3).

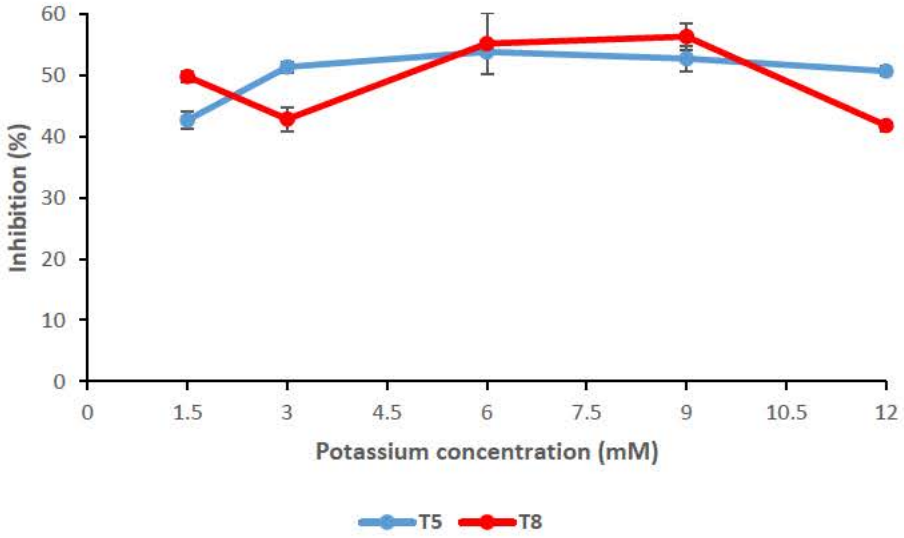

Figure 5-3. Effect of potassium (K) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There was a significant effect of Mg concentration ($P < 0.01$) on suppression of *R. solani* growth in dual culture. There were no significant effects of antagonist, or interaction between antagonist and concentration of Mg on inhibition of pathogen growth. Antagonism by both isolates was strongest and moderately low and standard concentration of Mg (Figure 5-4).

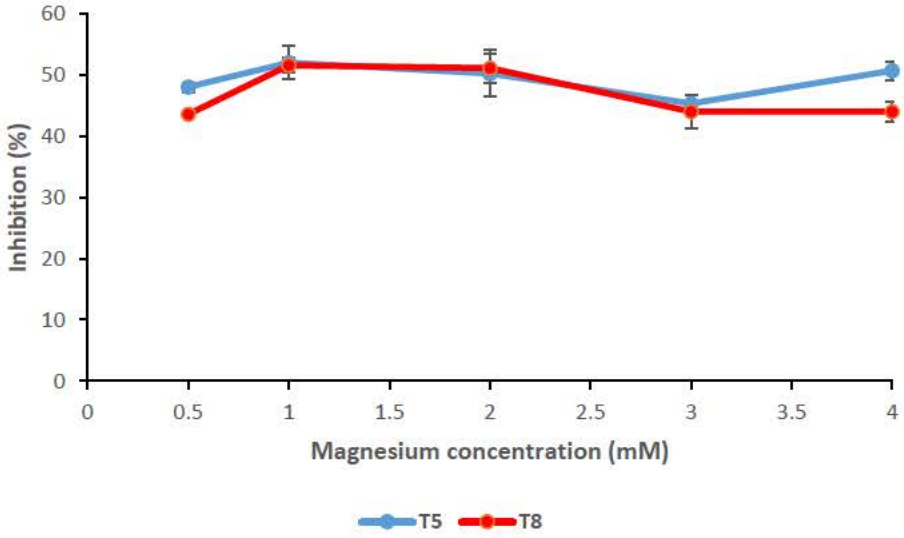

Figure 5-4. Effect of magnesium (Mg) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There were significant effects of antagonist ($P < 0.001$), concentration of Mn ($P < 0.001$), and interaction between antagonist and Mn concentration ($P = 0.001$), on inhibition of *R. solani*

growth in dual culture. T8 showed strongest inhibition at 1.5 times the standard concentration of Mn (Figure 5-5). The two isolates of *Trichoderma* showed lowest inhibition at half of the standard concentration of Mn. T5 represented strongest inhibition at double the standard concentration of Mn (Figure 5-5).

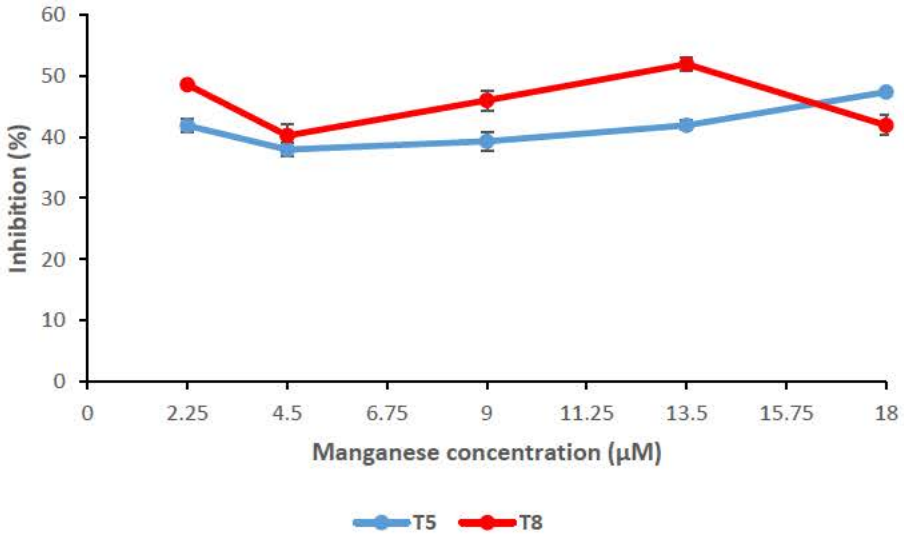

Figure 5-5. Effect of manganese (Mn) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There were significant effects of antagonist ($P < 0.001$), N concentration ($P < 0.02$), and interaction between antagonist and concentration of N ($P = 0.003$), on inhibition of *R. solani* growth in dual culture. T5 showed strongest inhibition at 1.5 times the standard concentration of N (Figure 5-6). T8 showed lowest inhibition at twice the standard concentration of N, and strongest suppression at 1.5 times the standard concentration of N (Figure 5-6).

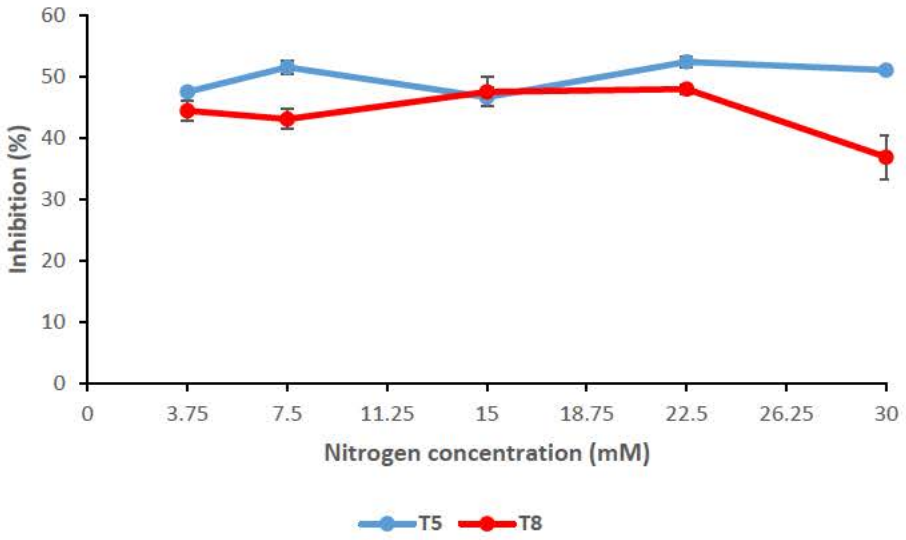

Figure 5-6. Effect of nitrogen (N) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

There were significant effects of antagonist ($P < 0.001$), and interaction between antagonist and P concentration ($P = 0.001$), on inhibition of growth of the pathogen. There was no significant effect of P concentration on inhibition of pathogen growth. T5 and T8 illustrated strongest suppression at a quarter and 1.5 times the standard concentration of P, respectively. T8 showed lowest suppression at a quarter of the standard concentration of P (Figure 5-7).

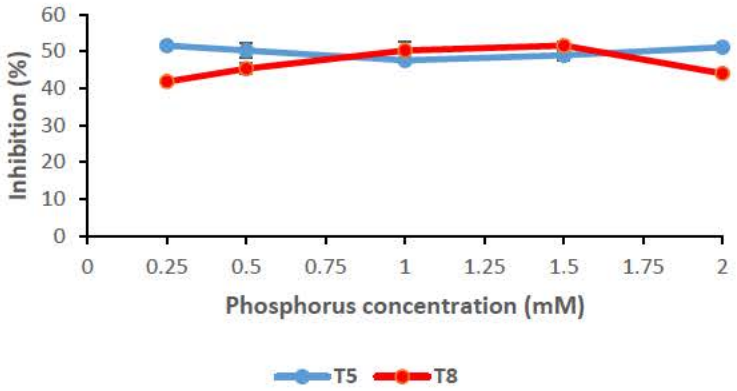

Figure 5-7. Effect of phosphorus (P) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture on adjusted Hoagland's nutrient agar after 7 days at 20°C. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3).

5.3.2. Effect of nutrients on antagonistic activity of *Trichoderma* culture filtrates

Rhizoctonia solani was grown on medium supplemented with culture filtrates of T5, T8 or *R. solani* (control) from liquid medium (Hoagland solution) with different concentrations of Ca, Fe, K, Mg, Mn, N or P. There were highly significant ($P < 0.01$) effects of isolate, nutrient

concentration, and interaction between isolate and nutrient concentration, for both isolates tested.

There were significant effects of isolate ($P < 0.001$), concentration of Ca ($P < 0.001$), and interaction between isolate and Ca concentration ($P = 0.001$), on reduction of pathogen growth in antibiotic test. Culture filtrates of both *Trichoderma* species significantly inhibited the radial growth of the pathogen at all concentrations of Ca, except for T5 at the lowest concentration (Figure 5-8). *T. harzianum* T5 was significantly the most effective inhibitor of the radial growth of the pathogen at the standard concentration of Ca.

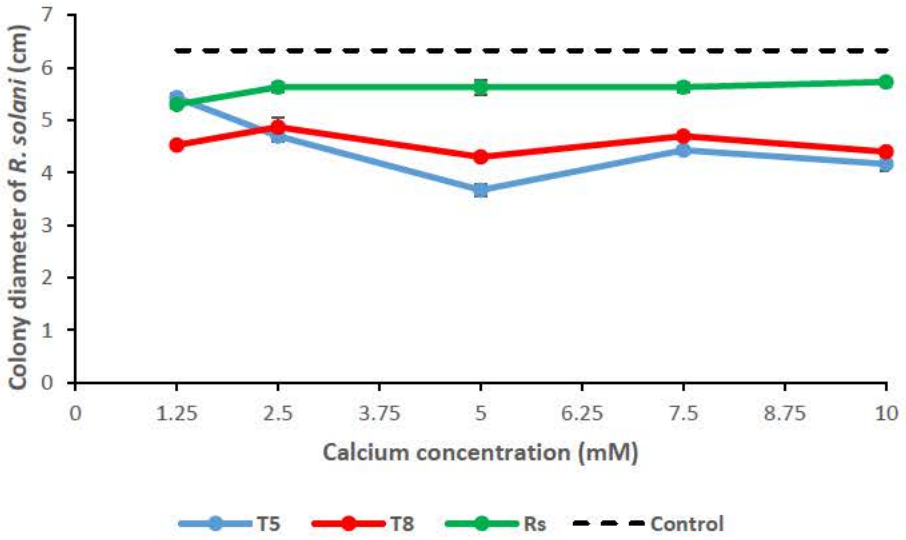

Figure 5-8. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of calcium (Ca). Calcium concentrations represent 0.25-2.0 times the standard concentration of 5 mM. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate ($P < 0.001$), concentration of Fe ($P < 0.001$), and interaction between isolate and Fe concentration ($P = 0.001$), on reduction of pathogen growth in antibiotic test. When filtrates from cultures on different concentrations of Fe were tested, *T. harzianum* T5 produced significantly high inhibition of the pathogen at a quarter of the standard concentration of Fe, whereas at double of the standard concentration of Fe T5 produced lower inhibition of pathogen, in comparison to the control treatment. Inhibition by *T. hamatum* T8 was greatest at both half and 1.5 times the standard concentration of Fe, and lowest at the lowest and highest concentrations of Fe, compared with control treatment (Figure 5-9).

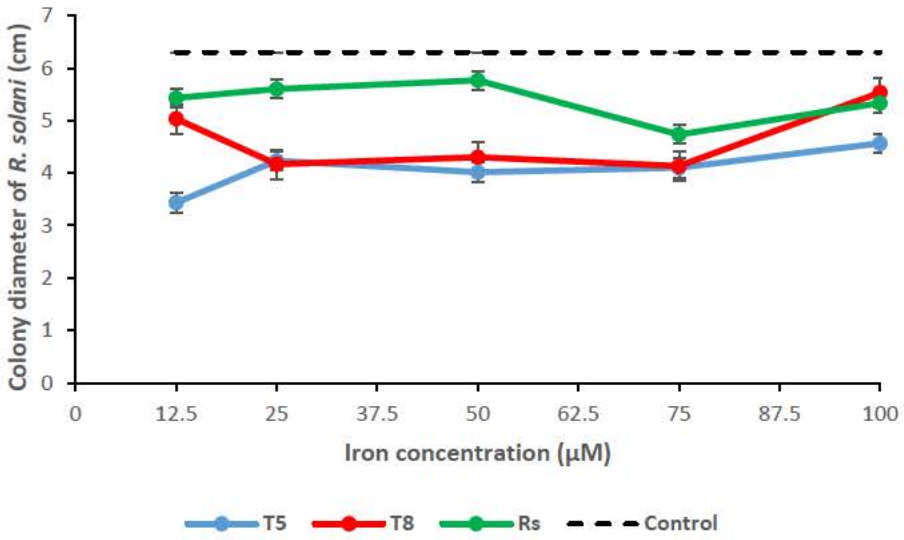

Figure 5-9. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of iron (Fe). Iron concentrations represent 0.25-2.0 times the standard concentration of 50 µm. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate ($P < 0.001$), concentration of K ($P < 0.001$), and interaction between isolate and K concentration ($P = 0.002$), on reduction of pathogen growth in antibiotic test. All *Trichoderma* fungi grown on different concentrations of potassium significantly inhibited the radial colony growth of *R. solani* (Figure 5-10). The highest radial mycelial growth inhibition was observed against *R. solani* for *T. harzianum* T5 at quarter of the standard concentration of K. The least radial mycelial growth inhibition was observed against the pathogen for T8 strain at half of the standard concentration of K followed by T5 strain at intermediate concentration of K; respectively, in comparison to the control treatment (*R. solani*) (Figure 5-10).

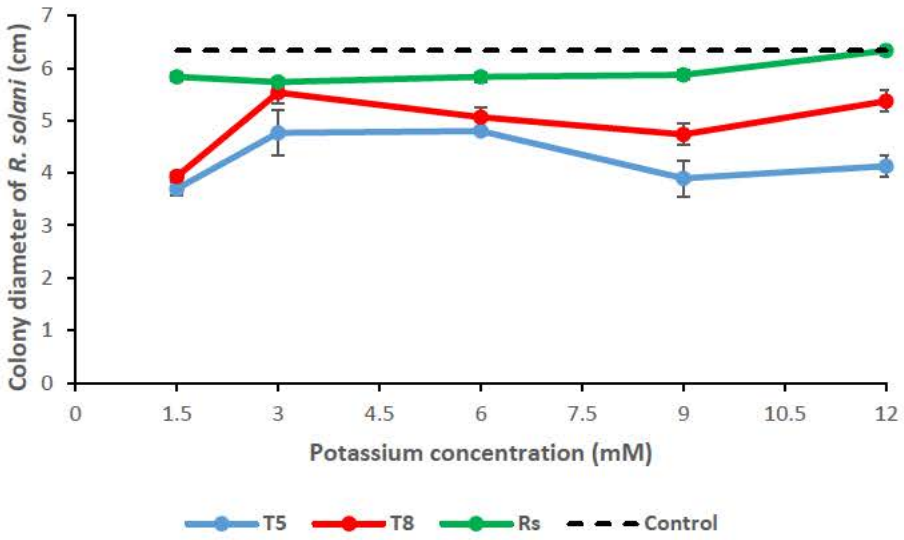

Figure 5-10. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of potassium (K). Potassium concentrations represent 0.25-2.0 times the standard concentration of 6 mM. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate (P <0.001), concentration of Mg (P <0.001), and interaction between isolate and Mg concentration (P =0.004), on reduction of pathogen growth in antibiotic test. Culture filtrate of *T. harzianum* T5 reduced the radial growth of pathogen at all concentrations of Mg, but the size of the effects was greatest at half of the standard concentration (Figure 5-11). *T. hamatum*, T8 was less effective at inhibition at 1.5 times the standard concentration of Mg, but also had their greatest inhibitory effect at the standard concentration, in comparison to the control treatments (Figure 5-11).

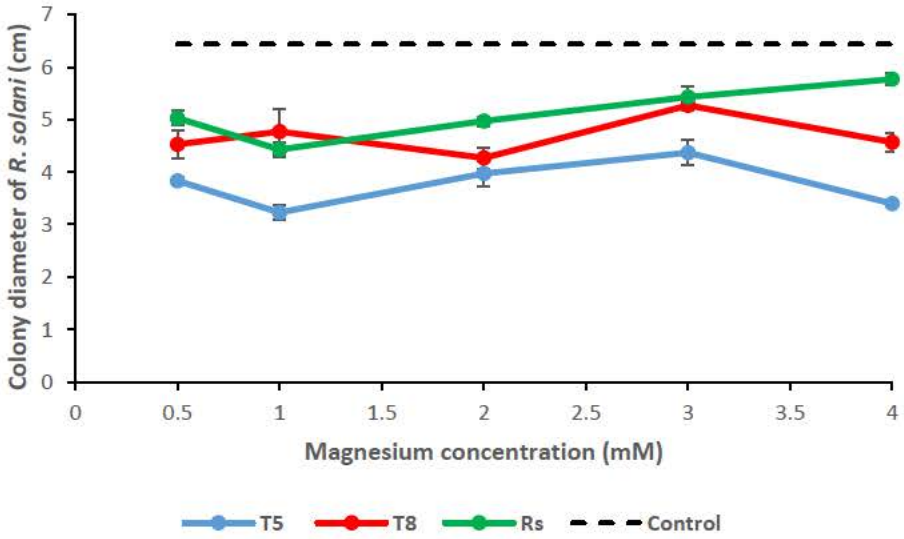

Figure 5-11. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of magnesium (Mg). Magnesium concentrations represent 0.25-2.0 times the standard concentration of 2 mM. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate (P <0.001), concentration of Mn (P <0.001), and interaction between isolate and Mn concentration (P =0.001), on reduction of pathogen growth in antibiotic test. Culture filtrate of *T. hamatum* T8 had significantly the highest radial mycelial growth inhibition of pathogen at a quarter of the standard concentration of Mn, whereas *T. harzianum* T5 had the least radial mycelial growth inhibition at double the standard concentration of Mn, compared with the control treatments of *R. solani* (Figure 5-12).

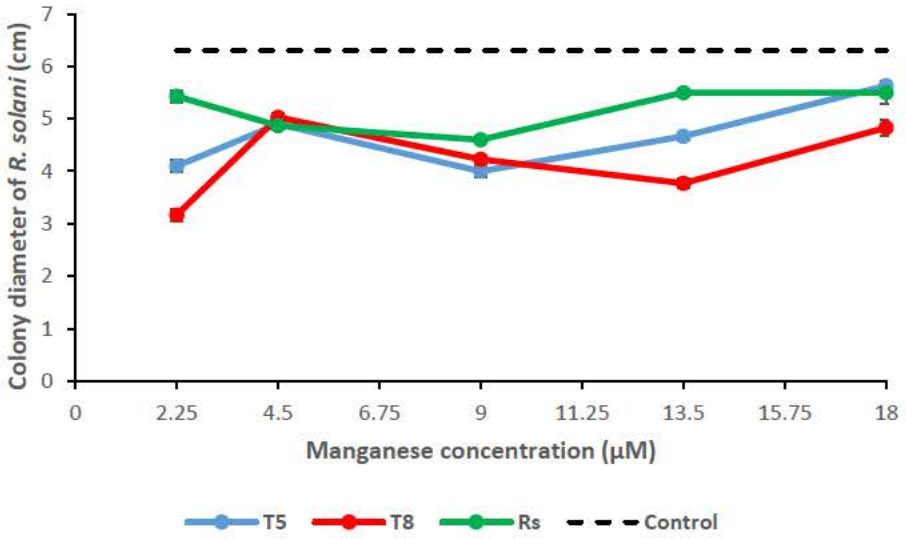

Figure 5-12. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of manganese (Mn). Manganese concentrations represent 0.25-2.0 times the standard concentration of 9 µm. T5: *T. harzianum*; T8: *T. hamatum*; Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate (P <0.001), concentration of N (P <0.001), and interaction between isolate and N concentration (P =0.001), on reduction of pathogen growth in antibiotic test. When different levels of nitrogen were tested, the highest radial mycelial growth inhibition of the pathogen was significantly for *T. harzianum* T5 at a quarter of the standard concentration of N, whereas *T. hamatum* T8 had the lowest inhibition of the pathogen at twice the standard concentration of N, to compared with the control treatment (*R. solani*) (Figure 5-13).

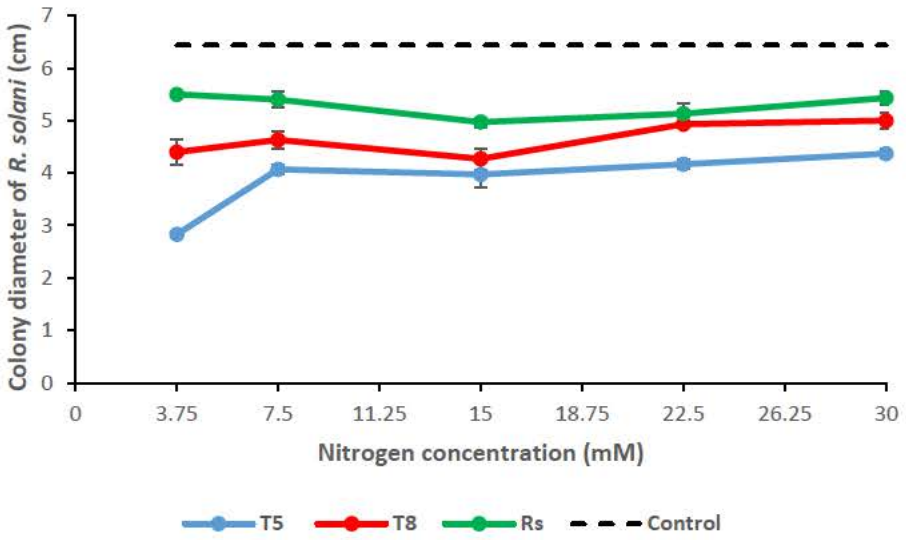

Figure 5-13. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of nitrogen (N). Nitrogen concentrations represent 0.25-2.0 times the standard concentration of 15 mM. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

There were significant effects of isolate ($P < 0.001$), concentration of P ($P < 0.001$), and interaction between isolate and P concentration ($P = 0.02$), on reduction of pathogen growth in antibiotic test. When P concentrations were tested, at a quarter of the standard concentration of P, *T. harzianum* T5 strain had significantly the highest inhibition of the pathogen, while the lowest inhibition was for isolates of *T. harzianum* T5 and *T. hamatum* T8 at twice the standard concentration of P, in comparison to the control treatment (*R. solani*) (Figure 5-14).

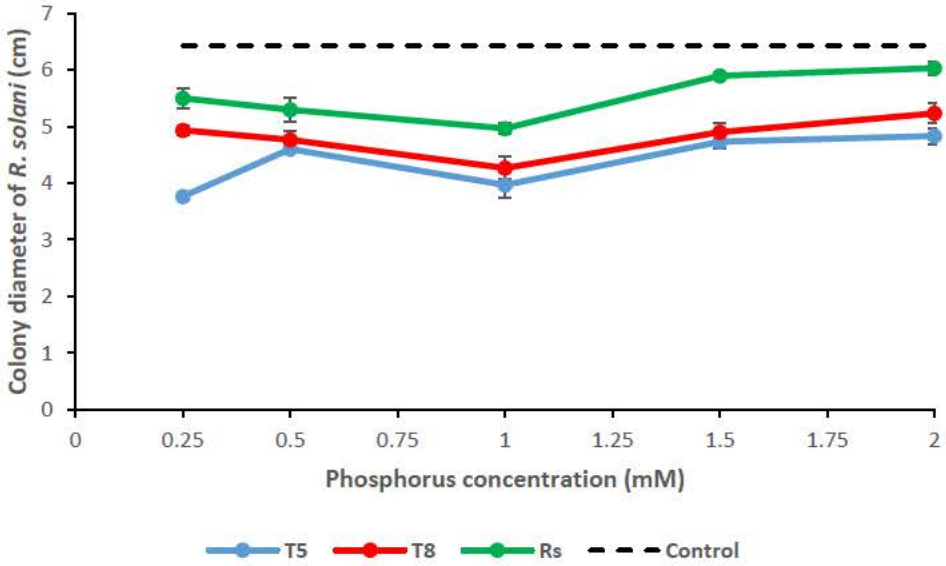

Figure 5-14. Colony growth after 5 days growth at 20°C of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of phosphorus (P). Phosphorus concentrations represent 0.25-2.0 times the standard concentration of 1 mM. T5: *T. harzianum*; T8: *T. hamatum;* Rs: *R. solani*; Control: *R. solani* on potato dextrose agar. Error bars show standard errors (n=3).

5.3.3. Effect of nutrients on antagonism in planta

In control treatments without T5 or T8, *R. solani* significantly (P = 0.012) reduced the shoot dry weight (Figure 5-15). Hoagland's solution significantly (P < 0.01) increased shoot dry weight (Figure 5-15). There was no significant interaction between inoculation with *R. solani* and application of Hoagland's solution in their effects on shoot dry weight. There was a significant interaction between inoculation with *R. solani* and fertilizer in their effects on tuber fresh weight (Figure 5-16). Inoculation of unfertilized plants with *R. solani* significantly reduced the tuber fresh weight by 50% compared with other treatments (Figure 5-16). In general, disease symptoms were only seen in the treatment with *R. solani* AG-3PT and sterilized distilled water only, with lesion severity at 0-10% on stem and with just a few sclerotia on potato tubers, 8.67 (standard error 1.453) sclerotia per plant.

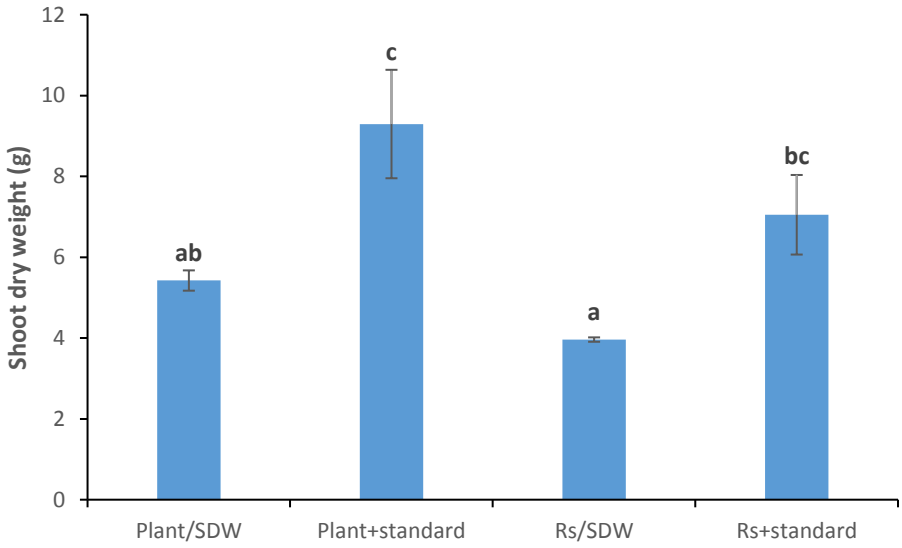

Figure 5-15 Effect of standard concentration of Hoagland solution on shoot dry weight of potato plants (cv. Desiree) with/without *R. solani* (Rs) at 20°C. SDW: sterilized distilled water. Plant: uninfested soil (plant only). Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

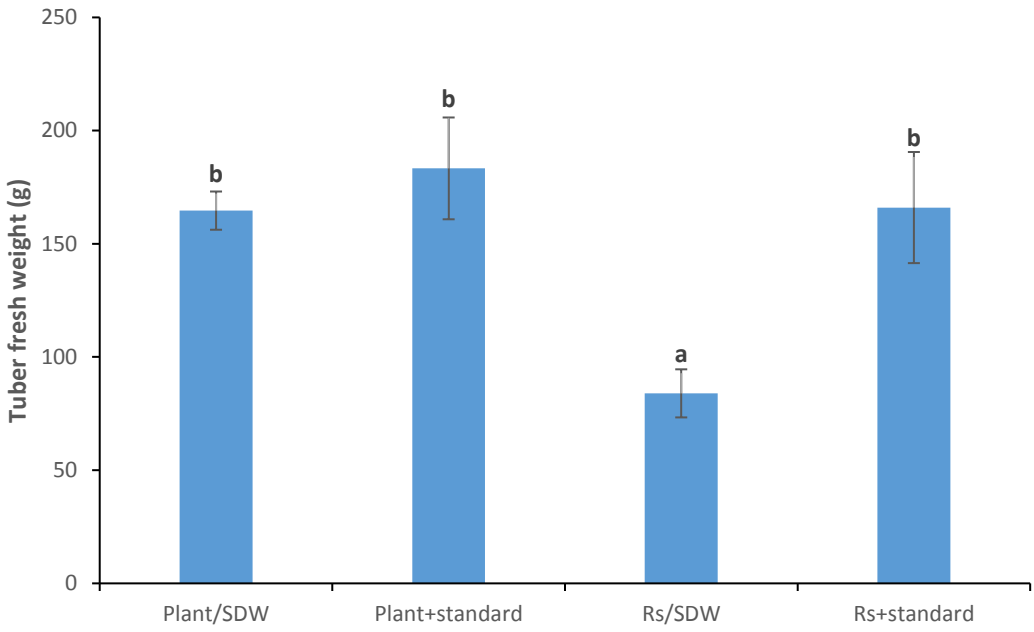

Figure 5-16 Effect of standard concentration of Hoagland solution on tuber fresh weight of potato plants (cv. Desiree) with/without *R. solani* (Rs) at 20°C. SDW: sterilized distilled water. Plant: uninfested soil (plant only). Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Inoculation treatment had a significant effect on all plant growth parameters in the potassium experiment (Table 5-4). Treatment with *Trichoderma* isolates promoted plant growth in comparison with the *R. solani* only treatment (Table 5-4). There were no significant interactions between potassium concentrations and inoculation of *Trichoderma* strains on the shoot dry weight, number of stolons and tubers and tuber weight (Table 5-4). There were significant interactions between K concentrations and *Trichoderma* in their effects on the root dry weight and plant height (Table 5-4). The highest root weight was found in the low K treatment with *T. hamatum* T8 plus *R. solani* and in the standard treatment with *T. harzianum* T5 plus *R. solani*. The root dry weight in the *R. solani* treatments was significantly higher at high and low K compared with standard K. Also for T5 plus *R. solani* the root dry weight in the standard K was higher than at the low K. Plant height increased as K concentration increased in the *R. solani* only treatment and in T5 plus *R. solani*, but there was no significant effect of K on plant height in the uninoculated control or in the T8 plus *R. solani* treatment (Table 5-4).

Table 5-4 Effect of different concentrations of K by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production (cv. Desiree) when introduced into conductive soil infested with pathogen *R. solani* at 20°C. Nil: uninfested soil; T5: *T. harzianum*; T8: *T. hamatum*.

Inoculation	Potassium (mM)	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)	Plant height (cm)
Nil	3	8.44	3.56	11.00	7.75	214.87	46.88
	6	9.29	4.26	14.00	11.50	183.32	53.43
	12	8.29	3.65	12.00	10.50	208.79	47.25
R. solani	3	6.80	3.32	8.50	7.25	193.98	33.50
	6	7.05	2.27	9.50	6.50	166.01	41.38
	12	10.30	3.89	11.50	7.75	172.54	50.50
R. solani + T5	3	8.91	3.81	12.00	8.25	228.78	53.75
	6	11.13	5.02	13.00	6.75	213.68	66.25
	12	11.79	4.78	14.75	9.25	248.63	70.08
R. solani + T8	3	11.23	6.95	15.50	11.50	250.26	58.63
	6	10.79	4.77	14.25	8.00	194.62	65.50
	12	11.76	4.80	14.00	8.67	251.09	57.67
P (Inoculation)		<0.001	<0.001	0.003	0.019	0.037	<0.001
P (potassium)		0.021	ns[a]	ns	ns	ns	0.001
P (interaction)		ns	<0.001	ns	ns	ns	0.041
L.S.D.		2.28	0.95	4.17	3.17	71.54	9.41

[a] ns = not significant; [b] least significant difference at P = 0.05 for comparing any two values within each column. LSD has not been calculated where there are no significant treatment effects.

Inoculation treatment had a significant effect on shoot dry weight, root dry weight, tuber fresh weight and plant height in the nitrogen experiment (Table 5-5). These were all higher in the T5 and T8 treatments than in the *R. solani* only treatment. There were no significant interactions between concentration of N and inoculation in their effects on any of the growth parameters measured (Table 5-5). Root dry weight and the number of tubers were significantly increased at the low level of N, while plants at standard N were marginally significantly taller than those at low N (Table 5-5).

Table 5-5 Effect of different concentrations of N by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production (cv. Desiree) when introduced into conductive soil infested with pathogen *R. solani* at 20°C. Nil: uninfested soil; T5: *T. harzianum*; T8: *T. hamatum*.

Inoculation	Nitrogen (mM)	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)	Plant height (cm)
Nil	3.75	10.48	5.60	14.00	10.75	190.34	50.63
	15	9.29	4.26	14.00	11.50	183.32	53.43
R. solani	3.75	8.37	4.63	12.00	10.00	111.18	42.38
	15	7.05	2.27	9.50	6.50	166.01	41.38
R. solani + T5	3.75	11.81	5.61	15.50	11.25	215.31	55.68
	15	11.13	5.02	13.00	6.75	213.68	66.25
R. solani + T8	3.75	11.76	5.54	15.00	10.25	200.11	58.63
	15	10.79	4.77	14.25	8.00	194.62	65.50
P (Inoculation)		0.002	<0.001	ns[a]	ns	0.009	<0.001
P (nitrogen)		ns	<0.001	ns	0.008	ns	0.047
P (interaction)		ns	ns	ns	ns	ns	ns
L.S.D.		2.71	1.13	-	3.38	60.68	5.93

[a] ns = not significant; [b] least significant difference at P = 0.05 for comparing any two values within each column. LSD has not been calculated where there are no significant treatment effects.

Inoculation treatment had a significant effect on shoot dry weight, root dry weight, and plant height in the manganese experiment (Table 5-6). These were all higher in the T5 and T8 treatments than in the *R. solani* only treatment. There were significant interactions between inoculation and concentration of Mn in their effects on root dry weight and plant height (Table 5-6). Root and shoot dry weight were significantly greater at low Mn than standard Mn in the *R. solani* only treatment, but did not differ between concentrations of Mn in the other inoculation treatments. Plant height was significantly lower at low Mn than standard Mn in the T5 plus *R. solani* treatment, but did not differ between levels of Mn in the other inoculation treatments (Table 5-6). Tuber fresh weight was higher at low Mn than standard Mn (Table 5-6).

Table 5-6 Effect of different concentrations of Mn by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production (cv. Desiree) when introduced into conductive soil infested with pathogen *R. solani* at 20°C. Nil: uninfested soil; T5: *T. harzianum*; T8: *T. hamatum*.

Inoculation	Manganese (mM)	Shoot dry weight (g)	Root dry weight (g)	No. of stolons	No. of tubers	Tuber fresh weight (g)	Plant height (cm)
Nil	0	12.05	4.96	12.25	9.50	196.26	61.88
	9	9.29	4.26	14.00	11.50	183.32	53.43
R. solani	0	9.49	4.65	16.25	8.25	209.05	38.78
	9	7.05	2.27	9.50	6.50	166.01	41.38
R. solani + T5	0	10.71	5.06	13.75	10.75	234.59	53.75
	9	11.13	5.02	13.00	6.75	213.68	66.25
R. solani + T8	0	12.72	5.38	17.25	12.25	272.51	74.20
	9	10.79	4.77	14.25	8.00	194.62	65.50
P (Inoculation)		<0.001	0.001	ns[a]	ns	ns	<0.001
P(manganese)		0.003	0.003	ns	ns	0.018	ns
P (interaction)		ns	0.042	ns	ns	ns	0.006
L.S.D.		2.08	1.16	-	-	62.9	9.18

[a] ns = not significant; [b] least significant difference at P = 0.05 for comparing any two values within each column. LSD has not been calculated where there are no significant treatment effects.

5.3.4. Field experiment

Stem canker and black scurf disease were found in the *R. solani* only treatment at 0-10% of stem area covered with lesions, and with just a small number of sclerotia on tubers, with 11.5 (standard error 1.041) sclerotia per plant. Stem canker also appeared in treatment with NPK fertilizer inoculated with *R. solani* only at 0-10%. In this treatment, there were 3.5 (standard error 0.5) sclerotia per plant on potatoes. There was no stem canker or sclerotia on potatoes in treatment with low K or low N fertilizer. There were no symptoms on *Trichoderma* treated plants. Application of *T. hamatum* T8 treatments or fertilizer treatments had significant effects on all plant growth parameters, while the interaction between the effects of *Trichoderma* and fertilizers was significant for shoot dry weight and tuber fresh weight (Table 5-7).

Table 5-7 Probabilities from ANOVA of effects on growth parameters of potato cv. Desiree in a field trial investigating the interaction between treatment with *Trichoderma hamatum* T8 and fertilizers. Shoot dry weight and tuber fresh weight were log-transformed before analysis.

Factor	Shoot dry weight	Tubers	Tuber fresh weight
Trichoderma	< 0.001	0.018	< 0.001
Fertilizer	< 0.001	0.008	< 0.001
Interaction	0.004	ns	0.002

In plots not treated with *T. hamatum* T8, fertilizers (low K, low N and NPK standard) significantly increased the shoot biomass production of potato by 7.4, 8.9 and 3.5 times, respectively, compared to plants without fertilizers (Figure 5-17). In unfertilized plots, treatment with *T. hamatum* T8 significantly increased the shoot dry weight by 5.9 times. The relative effect of fertilizers on shoot dry weight was less for plants treated with T8 compared to plants with pathogen only (Figure 5-17).

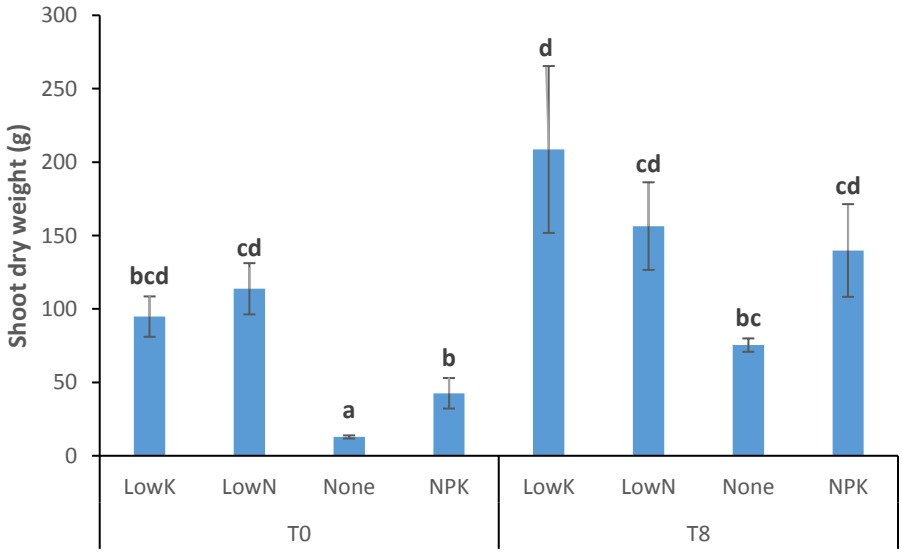

Figure 5-17 Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on shoot dry weight of potato plants (cv. Desiree) grown in field plots inoculated with *Rhizoctonia solani* after 20 weeks growth. None: uninoculated control; T0: *R. solani* only; T8: *T. hamatum* + *R. solani*. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

There were significant effects of *T. hamatum* T8 (P <0.01) and fertilizer levels (P <0.008), but no interaction between them, on the number of tubers (Figure 5-18). Treatment with T8 increased the number of tubers (Figure 5-18). The number of tubers was increased by application of fertilizer, and this effect was greater for low N and low K treatments than for NPK (Figure 5-18). Analysis with Tukey test showed that the number of tubers was increased by Low K and Low N compared with untreated control in the absence of T8, while there were no significant differences between fertilizer treatments when T8 was applied (Figure 5-18).

However, the ANOVA had shown that the interaction between fertilizer and *Trichoderma* treatments was not significant.

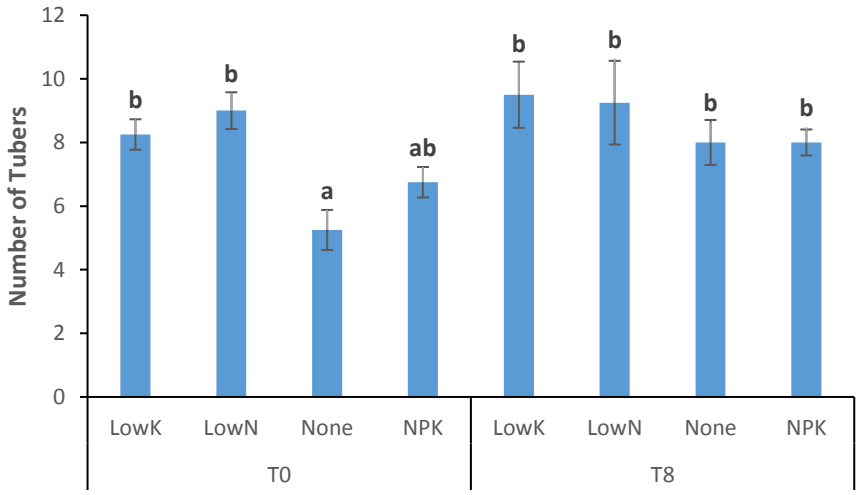

Figure 5-18 Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on number of tubers of potato plants (cv. Desiree) grown in field plots inoculated with *Rhizoctonia solani* after 20 weeks growth. None: uninoculated control; T0: *R. solani* only; T8: *T. hamatum* + *R. solani*. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The fresh weight of tubers was significantly affected by treatment with *T. hamatum* T8, fertilizers, and their interaction (Figure 5-19). The application of *Trichoderma* significantly increased the tuber fresh weight in none, low K and NPK treatments (Figure 5-19). Application of fertilizers (NPK, Low N and Low K) increased significantly the tuber fresh weight, but the effect of fertilizer was lower in the plants treated with T8 (Figure 5-19).

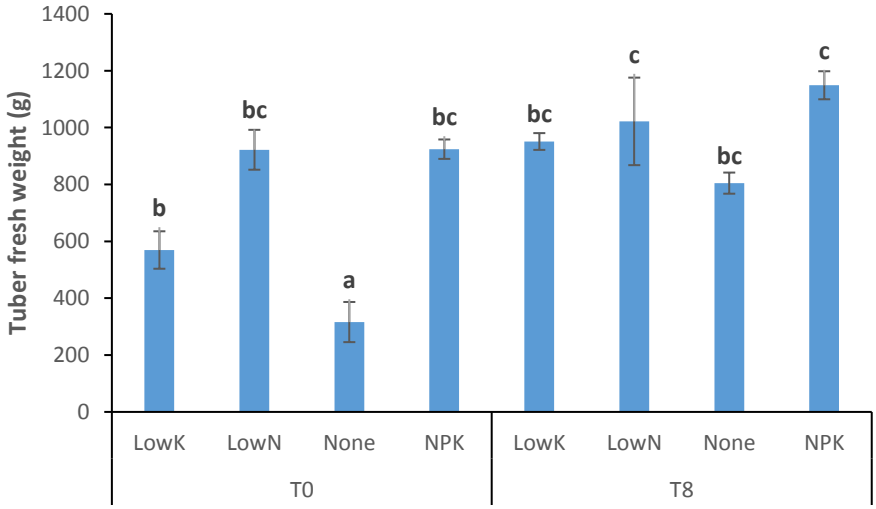

Figure 5-19 Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on tuber fresh weight of potato plants (cv. Desiree) grown in field plots inoculated with *Rhizoctonia solani* after 20 weeks growth. None: uninoculated control; T0: *R. solani* only; T8: *T. hamatum* + *R. solani*. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

5.3.5. Effect of NPK fertilizers under glasshouse conditions

There were significant effects of treatment with fertilizer and with *T. hamatum* T8, and a significant interaction between them, on shoot dry weight of potatoes in pots (Figure 5-20). Shoot dry weight was significantly increased by treatment with *T. hamatum* T8. Shoot dry weight was increased by application of low K, Low N and NPK fertilizers (Figure 5-20). The effect of T8 on growth promotion was greatest in the no fertilizer treatment.

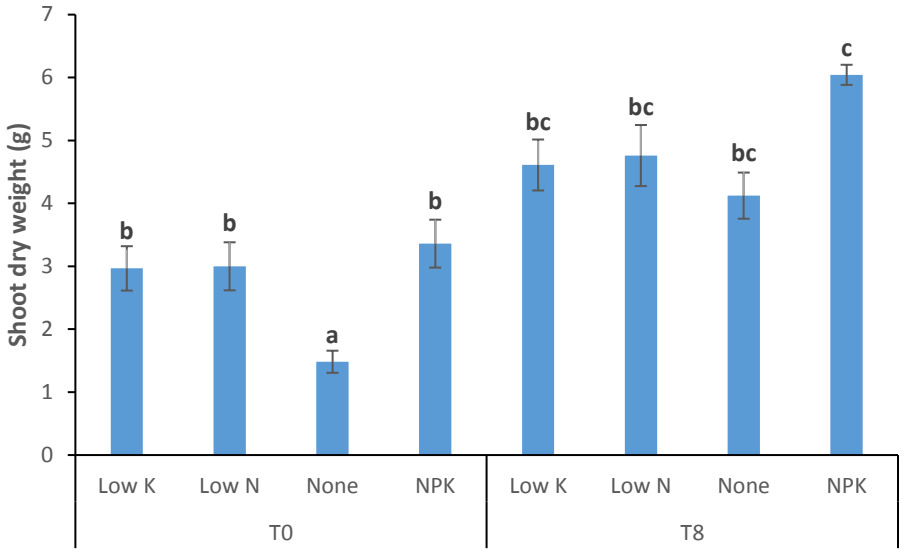

Figure 5-20 Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on shoot dry weight of potato plants (cv. Desiree) grown in glasshouse conditions inoculated with *Rhizoctonia solani* in a pot trial. None: uninoculated control; T0: *R. solani* only; T8: *T. hamatum* + *R. solani*. The error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

5.4. Discussion

This research detected that all nutrients except Ca and Fe had significant effects in dual culture, and all had significant effects on antibiosis. In pot trials, there was no interaction between K, N or Mn fertilizer and the effects of *Trichoderma* isolates on growth and yield of plants. In the field trial in the presence of *R. solani*, fertilizer had much less effect on growth and yield of plants inoculated with *T. hamatum* T8 than in controls. This was confirmed for shoot dry weight of potato in a pot trial.

In vitro antagonism and colony diameter tests have become a standard procedure of most methods for checking strains of *Trichoderma* for inhibitory activity. The effect of Ca concentration on inhibition was not significant in the dual culture method, whereas in the

antibiotic test, the effect was significant. *T. harzianum* T5 with standard level of Ca gave the highest suppression of pathogen growth, while at lower level of Ca gave the lowest reduction of growth compared to the other treatments. It is suggested that calcium concentration in hydroponics can interact with biocontrol *T. harzianum* T5 isolate as culture filtrates (Lei and Ya-qing, 2015).

The effect of iron concentrations (Fe EDTA) on inhibition was not significant in the dual culture technique. However, there was a high significance level for the effect of Fe and *Trichoderma* on mycelial growth in colony diameter in the antibiotic test. *T. harzianum* T5 isolate grown on lower concentration of Fe had a negative effect on hyphal growth rate of the pathogen, while *T. hamatum* T8 at twice the standard level of Fe had little impact on radial growth of the pathogen compared to the control treatments. It is possible that *T. harzianum* grew considerably faster with lower Fe concentration and stimulated fungal biomass in the liquid medium, and as a result it produced high concentration of antibiotics and fungitoxic metabolites that can inhibit the fungus *R. solani*. Some strains of *Trichoderma* produce numerous siderophores under iron limiting conditions (iron deficiency) in culture filtrates to deprive soilborne pathogens of this important element, and therefore affect the growth of pathogens (Anke et al., 1991; Qi and Zhao, 2013; Tariq et al., 2010). Also, soil pH has an effect on availability of Fe in the soil, however, it could be quite different from that of Fe in the culture media (Jin et al., 2013).

The antagonistic effect of *Trichoderma* isolates on *R. solani* AG-3PT was maintained at all KCl concentrations, but there was more growth suppression at high level of K with *T. hamatum* T8, while at low level of K *T. harzianum* T5 and *T. hamatum* T8 gave less growth inhibition in comparison to the other different concentrations. The probability is that high concentrations of potassium had more effect on the growth of soilborne pathogens than the *Trichoderma* isolates in the PDA plates (Ordóñez-Valencia et al., 2009).

In the antibiosis study, *T. harzianum* T5 and *T. hamatum* T8 at lower K level significantly inhibited growth of the pathogen more than other K compared with control treatments. The results suggest that *T. harzianum* T5 was sensitive to increasing KCl concentrations decreasing the release of secondary metabolites, which are responsible for inhibiting the growth of *R. solani* (Erper et al., 2011).

The effect of magnesium concentrations ($MgCl_2$) on the efficacy of antagonists against pathogen was not significant in the dual culture technique. While in the colony diameter test, *T. harzianum* isolate, T5 exerted significant suppression of the growth of mycelium of *R. solani* at low concentration of $MgCl_2$, although this fungus behaved variably when exposed to different concentrations of magnesium. However, *T. hamatum* isolate, T8 at high Mg concentration gave the lowest radial growth repression of pathogen, compared with the control treatment. There is little information about the amendments of Mg concentrations in hydroponic culture, but it is possible that low concentrations of Mg increase both growth and antibiotic production by *T. harzianum* T5, and improve reliability and level of biocontrol activity (Duffy and Défago, 1997). In contrast, the high concentrations of Mg seem to affect the antibiotic production or growth of the BCAs against *R. solani*, or it is more likely that it is growth that is reduced that affects antibiotic production due to less fungal biomass present. These results concur with Slininger and Jackson (1992) who reported that antibiotic productivity and growth by strain *Pseudomonas fluorescens* 2-79 as a biocontrol agent increased with addition of H_3BO_4, $MgSO_4$ and $FeSO_4$ in the liquid culture.

The isolate of *T. hamatum* T8 gave significantly the highest inhibition of the pathogen at a high concentration of Mn in dual culture, whereas *T. harzianum* T5 at low concentration of Mn gave the lowest inhibition. It could be that high levels of manganese are toxic to pathogens (Graham and Webb, 1991; Mortvedt et al., 1963).

In the antibiotic test, the effect of a low concentration of Mn on the efficacy of T8 was significantly inhibited the mycelial growth of *R. solani*, while T5 with double the standard level of Mn increased the colony diameter of the pathogen compared with the control treatment. This could be because the Mn may reduce fungal growth of T8 at high concentration of Mn. So, it is possible that antibiotic production by *Trichoderma* isolates was affected by Mn concentration.

When the antagonistic activity with different levels of nitrogen against *R. solani* was evaluated through the dual culture technique, maximum inhibition of pathogen growth was seen with *T. harzianum* isolate, T5 with high levels of nitrogen, and least inhibition of pathogen growth was observed in *T. hamatum* isolate, T8 with double the standard level of nitrogen. It is possible that the role of nitrogen is in inducing the activity of *T. harzianum* to release cell wall degrading enzymes such as chitinase, protease and glucanases which are key factors in the cell wall lysis of the pathogen and mycoparasitism on it (Alamri et al., 2016; Gajera et al.,

2011). This result is consistent with those of others Kredics et al. (2005); Šimkovič et al. (2008); Szekeres et al. (2004) who found that the addition of nitrogen to the media is crucial for biocontrol through releasing enzymes, particularly proteases that improve the mycoparasitism by various *T. harzianum* strains.

T. harzianum T5 with lower level of nitrogen induced significant suppression of colony diameter of *R. solani* in vitro, while T8 with double the standard level of nitrogen gave least suppression of the pathogen, compared with control treatment, suggesting that nitrogen limitation combined with *Trichoderma* as biocontrol agents induced the hydrolytic enzymes that are responsible for inhibiting the growth of *R. solani* (Olmedo-Monfil et al., 2002). In contrast, *Trichoderma virens* strains had a negative role under limitation of nitrogen for mycoparasitism in solid media or antibiotics in liquid media (Mendoza-Mendoza et al., 2003).

The *Trichoderma* strains of T5 and T8 interacted differently with lower and high phosphorus levels in their antagonistic effects against *R. solani* growth. T8 showed least inhibition at the highest and lowest P concentration, while T5 had the highest inhibition at these concentrations. It is probably that biocontrol agents of T5 and T8 was affected by P concentration to inhibit the pathogen growth. Castagno et al. (2011) showed that different isolates of *Rhizobium* as biocontrol agents had different effects of P availability.

The filtrate of T5 at lower level of P reduced the pathogen growth highly. This could be because the production of diffusible and/or volatile metabolites may be stimulated in the lower level of P by *T. harzianum* T5. *T. hamatum* T8 does not seem to be inhibitory at lower phosphate levels for inhibiting the pathogen growth, compared with the control treatment. It was suggested that *Trichoderma* strains in Hoagland solution differ in the uptake of P at the media (Rashid et al., 2004); as a result of this, the inhibition metabolites responsible for diminishing *R. solani* growth will be produced at different amounts in broth media. Illmer and Schinner (1995) found that there were highly significant correlations between microbial biomass production and P content in the culture media.

In glasshouse experiments, potato plants were harvested after 7 weeks post emergence, and before full maturity stage. Treatment with *R. solani* AG-3PT by itself caused canker lesions on stems, and sclerotia (scurf) on tubers. The effect of nutrients on antagonism and plant growth parameters was tested. The measurement characteristics were shoot and root dry weight, number of stolons and tubers and tuber weight. High K concentrations along with *Trichoderma*

isolates proved efficient in promoting plant growth. The above treatments significantly increased root weight and plant height, but with other parameters were not significant. It is possible that the high root-uptake capacity of potato buffered the *Trichoderma* biostimulation in fertilized pots. The *Trichoderma* biostimulation increased fertilizer K use efficiency of potato in the soil to improve the plant height, and also improved K uptake by roots under K availability conditions (Fiorentino et al., 2018; Shunka et al., 2017). It is noticeable that the K availability had a slight effect on interactions between the *Trichoderma* isolates and the host on disease in the soil.

The interaction of N and biocontrol agents were not significant for all plant growth parameters, except tuber size which was significantly increased with increasing nitrogen supply at recommended rates (15 mM) in a Hoagland solution plus biocontrol agents. The growth of tubers is influenced by the nitrogen supply to the potato plant in the presence of biocontrol inoculum into soil (Mäck and Schjoerring, 2002), and may be because high nitrogen supply increased the total chlorophyll content. Nitrogen is a major component of the photosynthesis system which is involved in the yield (Bassi et al., 2018; Oliveira, 2000). In results similar to these, Sonnewald et al. (1997) found that high concentrations of nitrate resulted in few tubers, but at large size, while low concentrations of nitrate resulted in many tubers, but at small size. Crozier et al. (2000) detected that low N level might lead to preferable tuber quality and diminished Rhizoctonia stem canker disease incidence caused by *R. solani*, whereas application of high N levels increased disease incidence and decreased yield. They recommended to use N at standard rates with potato plants.

The growth parameters did not show a marked response when adding either Mn levels plus *Trichoderma* isolates to the pots, except dry matter from roots and height of plant which were significantly increased by *Trichoderma*. There was some evidence that plant growth was reduced by the high level of Mn in the absence of *Trichoderma*. High amounts of Mn in the soil are considered toxic for the plants, particularly in acid soils which increase its toxicity to organisms, so that the *Trichoderma* as a fungal biocontrol agent acts better at low Mn concentrations than high Mn concentrations, because high Mn level could affect the amount of metabolites (Millaleo et al., 2010).

In the field study, treatment with *R. solani* AG-3PT by itself caused canker lesions on stems, and sclerotia (scurf) on tubers, but the appearance of lesions on surviving stems was inconsistent. Using symptoms the effect of disease would be rated as mild, but the pathogen

had more effect on growth. Therefore, the assessment of inhibition of disease relied on the comparison of growth between treatments and control *R. solani* only. *T. hamatum* T8 was chosen based on the pot trial and results in the previous chapters. The fertilizer treatments reduced or eliminated the symptoms of disease caused by *R. solani* AG-3PT. This could be because fertilizers may increase plant resistance to *R. solani* (Chaudhary et al., 2015; Ros et al., 2008). The application of fertilizer NPK was in the middle of the season in a solution at the recommended rate and half rate of N and K per plant. Thus, as expected, the control plants which were NPK fertilized significantly registered better growth parameters than those unfertilized. T8 treatment in comparison to the control treatment increased the growth parameters in both NPK fertilized and unfertilized plants. The interaction between fertilizer levels and T8 significantly affected the shoot dry weight and tuber fresh weight, whereas there was no significant interaction in number of tubers. Numerous studies have reported that plants respond to the presence of *Trichoderma*-plant root colonization which directly enhance the plant growth characteristics through different mechanisms (Domínguez et al., 2016; Martínez-Medina et al., 2014; Morán-Diez et al., 2012; Nieto-Jacobo et al., 2017; Rubio et al., 2012). While NPK fertilizer was very crucial for plant growth and yield, particularly the balanced supply to the plants, shortage of one of these fertilizers like N or K might induce a specified nutrient deficiency and delay total growth of plants. The response of plants to the NPK fertilizer with different rates were diverse possibly due to the differences in soil fertility (Yousaf et al., 2017). Similarly, Fakhry (2016) found that the interaction between NPK levels and *Bacillus* spp. and *Pseudomonas fluoresens* as biocontrol agents significantly increased the sesame plant growth promotion and reduced wilt disease caused by *F. oxysporum* in two seasons under field conditions. Rubio et al. (2017) also found that the growth promotion (shoot and root dry weight) of tomato plants by a biocontrol *Trichoderma harzianum* isolate was greater in plants with NPK fertilizer compared to the control plants. Hridya et al. (2013) also reported that there was no significant interaction between NPK fertilizer and *Trichoderma* strains on the suppression of root rot infection of cassava caused by *Phytophthora palmivora*, while *Trichoderma* application alone significantly controlled the root rot disease, and NPK fertilizer alone did not influence the risk of infection.

A glasshouse test repeated the field trial with the same treatments to check the results. The results indicated that the interaction between *T. hamatum* T8 and NPK fertilizer significantly affected the shoot weight of potato plants, with *Trichoderma* having a greater growth promotion effect when fertilizer was not added.

The outcomes show partial support for the hypothesis which suggested that the interaction with nutrients can alter the effect of biocontrol *Trichoderma* spp. and promote potato plant growth in addition of elimination the stem canker and black scurf disease. Data indicate the impacts of some nutrients did affect dual culture and antibiosis, but effects were small. Pot trials with individual nutrients generally did not show an interaction between fertilizers and *Trichoderma* biocontrol agents. The field trial showed that fertilizers had less effect on growth of plants inoculated with *Trichoderma*, and that conversely *Trichoderma* had greater effects at lower fertilizer levels. Fertilizer treatments do not affect the effect of biocontrol on disease. Therefore, growers could reduce levels of fertilizer when *Trichoderma* is applied, but this needs more study in the future. Overall, although nutrients could affect biocontrol agent-pathogen interactions, the effects were small and there was little evidence of an interaction between nutrient levels and biological control agent action in the pot trials. In the field, fertilizers had less effect when *T. hamatum* T8 was present and T8 had more effect at low fertilizer levels.

Chapter 6. Effect of *Brassica* plant tissues on antagonism and growth promotion

Part I. Preliminary experiments

6.1. Introduction

There are several soilborne potato diseases which cause a decrease in plant growth and vigour, lower quality of potato tubers, and decrease the marketable crop (Larkin and Griffin, 2007; Muzhinji et al., 2018; Tsror, 2010). The most important soilborne diseases in potato growing areas are stem canker and black scurf caused by *Rhizoctonia solani* AG-3PT, which is widespread in the world (Larkin and Griffin, 2007; Yang et al., 2017). Disease is identified by the presence of black sclerotia on the potato tuber surface, called black scurf disease and at the same time the damage caused by the pathogen to the base of the stem at soil level is called stem canker disease (Kumar et al., 2017a).

In some cases, seed treatment chemicals have been shown to successfully control potato diseases. However, they are not beneficial due to environmental contamination through improper handling of chemicals (Farooq et al., 2012), therefore sustainable potato disease control choices are required. For example, a well-known method to control soilborne plant pathogens and diseases is the utilization of *Brassica* spp. as rotation, cover, intercrop and green manure crops with potential biofumigation effects for lowering populations of pathogens (Bernard et al., 2014). *Brassica* crops, including cabbage, radish, canola, broccoli, cauliflower, kale, turnip, rapeseed, and mustards, produce sulphur-containing compounds called glucosinolates (GSL) which are broken down to liberate isothiocyanates (ITC) which are considered toxic to numerous soilborne fungal plant pathogens (Hanschen et al., 2015; Olivier et al., 1999; Sarwar et al., 1998; Smolinska and Horbowicz, 1999). They can be used to make enriched soil, and increase agricultural sustainability and crop production (McGuire, 2003).

Smolinska (2000) and Larkin and Honeycutt (2006) noted that rotation with *Brassica napus* resulted in suppression of stem canker of potato caused by *R. solani*, and tomato wilt caused by *F. oxysporum* f. sp. *lycopersici*, through the alteration of microbial communities in soil, which was unrelated to glucosinolate concentrations. Also, isothiocyanate and volatile compounds released from *Brassica* spp. inhibit potato plant pathogens, including *R. solani*,

Pythium ultimum, Verticillium dahliae and *Fusarium sambucinum* (Charron and Sams, 1999; Mayton et al., 1996). Galletti et al. (2008) incorporated *Brassica* crops or seed meal into soil to produce biofumigation in combination with a biological control agent (*Trichoderma* spp.) which was more effective than chemical fumigation to control soilborne disease in sugar beet. *Trichoderma* isolates were shown to be tolerant to *Brassica* species for combined utilization in biofumigation in order to eliminate soilborne pathogens, particularly *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum* (Deng and Cao, 2017; Galletti et al., 2015). Likewise the application of strain 17S of *Bacillus amyloliquefaciens, Brassica carinata* seed meal and essential oil from different aromatic plants inhibited the soilborne fungal plant diseases of *Fusarium oxysporum* f.sp. *lycopersici* on tomato, *Sclerotinia minor* on lettuce and *Rhizoctonia solani* on bean in vitro and glasshouse conditions (Pane et al., 2017).

The specific objectives of this study were to evaluate selected *Brassica* species and biocontrol agent *Trichoderma* spp. for their ability to inhibit the development of soilborne potato pathogen (*R. solani* AG-3PT) in the laboratory. These were preliminary tests done before selecting the best combinations of *Brassica* and *Trichoderma* to use in pot and field trials.

6.2. Materials and Methods

6.2.1. Effect of *R. solani* AG-3PT on *Brassica* plants

Rhizoctonia solani AG-3PT was evaluated for its effect against two *Brassica* varieties which were cabbage (Sugarloaf) and broccoli (Green Sprouting). Loamy sand soil (Kirby Farm, UNE; pH 5.50, EC 21.0 µS/cm, S (KCl-40) 1.3 mg/kg, Colwell P 2.7 mg/kg) was used in this trial. Seeds were sown in small trays (5 x 5 cm) into sand-potting mix and placed in a plastic house for four weeks and then the healthy seedlings were transplanted into plastic pots (20 x 20 cm) to use for glasshouse trial. Inoculum of *R. solani* on wheat seeds (as in Chapter 3) was mixed into the soil at the rate of 16 g around seedlings of broccoli and cabbage individually. A set of pots were used with the same rate of un-colonized wheat seeds as the control treatment. There were three replicates for each treatment in a completely randomized design. The experiment was done twice. The first experiment was harvested 2 weeks after transplanting and the second experiment was harvested 4 weeks after transplanting. At the end of experiment, the root and stem of *Brassica* plants were examined for visual symptoms to

check if there were any effects or symptoms on plants (El-Mohamedy, 2012; Singleton et al., 1992). Experimental measurements were shoot and root dry weights.

6.2.2. Effects of *Trichoderma* on growth of *Brassica* plants and potato plantlets from tissue culture

T. harzianum T5 and *T. hamatum* T8 were grown on PDA in Petri-dishes and incubated for 7 days at 25°C. Conidial suspensions (100 mL) of each isolate in sterilized distilled water were adjusted to 10^6 spore/mL by haemocytometer (Jegathambigai et al., 2009). Cabbage (Sugarloaf) and broccoli (Green Sprouting) seeds were grown in germination trays containing sand-potting mix for 4 weeks under greenhouse conditions (Rabeendran et al., 2000). Potato plantlets (cv. Desiree) were obtained from tissue culture (as previously described in Chapter 3) which were grown on fresh MS_3 medium. The cabbage and broccoli seedlings were inoculated at 4 weeks old, when they had 2-5 leaves, and potato plantlets also with 2-5 leaves were obtained from tissue culture. The seedlings were sown into two types of media (sand:potting mix (2:1) and Kirby soil only) in 15 cm diameter plastic pots, and then irrigated once with spore suspension of *Trichoderma* at 100 mL/kg soil (Rabeendran et al., 2000). The untreated control was irrigated with SDW (100 mL/ kg soil). There were three replicates for each treatment. The glasshouse trial ran for 4 weeks at 20°C. The shoots and roots were dried in an oven at 60^{o}C until constant weight, then weighed individually (Rabeendran et al., 2000).

6.2.3. Effects of volatiles and non-volatiles from *Brassica* plants on the growth of *R. solani* AG-3PT and inhibition in dual culture

Broccoli (Green Dragon), cauliflower (Large White) and cabbage (Green Coronette) seedlings were bought from a local market (Bunnings, Armidale) and then transferred into big plastic pots (20 x 20 cm) in a glasshouse at 18-20°C for 4 weeks before sampling. Tissues from six plants of each species were combined. Leaf, root and stem pieces 20 mm long were cut with a scalpel from fully expanded leaves, roots and stems of broccoli, cauliflower and cabbage. They were put in a freezer at -18°C for one week, and then transferred to a freeze drier machine for 3 days. A coffee grinder was used to mill the freeze-dried material into powder. Five-mm-

diameter agar plugs from the margins of 5-day-old actively growing cultures on PDA containing hyphae of *R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8 were inoculated separately in the centre of Petri-dishes which were filled with a 20 mL of 1/4-strength PDA. Then 0.1 g powder of each part of a *Brassica* plant and 1 mL of sterilized distilled water were placed in a small weighing boat in the upturned lid of the Petri-dish (Kirkegaard et al., 1996). The inverted Petri-dish was sealed with two layers of Parafilm. In controls the *R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8 were separately plated on the centre of the plate on 1/4-strength PDA only. In this way, only volatile hydrolysis products produced by the *Brassica* tissues came into contact with the cultures (Rahmanpour et al., 2009; Rahmanpour et al., 2010). The diameters of the colonies were measured after 24 hr incubation. There were three replicates for each treatment.

A dual-culture experiment was set up in the same way on 1/4-strength PDA. A 5-mm-diameter disc of antagonists T5 and T8 was positioned diametrically opposite a 5 mm diameter disc of pathogen (*R. solani*) to measure the inhibition zone. In controls the pathogen was plated alone on one side of the plate at the periphery. A completely randomized design with three replications was used for the experiment. The diameters of the exposed fungal colonies were measured after 24 and 48 hrs of incubation (Kirkegaard et al., 1996; Rahmanpour et al., 2009).

The effects of non-volatiles derived from *Brassica* plants on the growth of the pathogen and *Trichoderma* isolates was determined by adding 0.1 g of each experimental plant sample to the Petri-dishes. Cooled 1/4-strength PDA was poured into the Petri plate with gentle mixing, and then left for 45 min without covering inside a laminar flow cabinet to allow volatiles to escape. The plates were then inoculated with the pathogen or *Trichoderma* sp. isolate alone to measure the radial growth. In controls the *R. solani* AG-3PT, *T. harzianum* T5 and *T. hamatum* T8 were separately plated on the centre of the plate on 1/4-strength PDA only.

The interaction between *Trichoderma* and *R. solani* in dual culture was tested by adding 0.1 g of powder in tubes with 1 mL of sterilized distilled water for each replicate and leaving for 3 hrs to remove any volatile compounds. The tubes were centrifuged at 13000 rcf for 10 min, and then the supernatant was placed in Petri-dishes before pouring 1/4-strength PDA. In controls the pathogen was alone plated on one side of the plate at the periphery. The Petri-dishes were inoculated with T5, T8 and *R. solani* as described previously and incubated without being sealed (Yadav et al., 2011).

6.2.4. Effects of powder of *Brassica* plant tissue on the growth of *Trichoderma* sp. and *R. solani* AG-3PT in liquid media

Each part of the plants (leaf, stem and root) for cauliflower, cabbage and broccoli (same varieties as in section 6.2.3) were screened to determine their effects upon growth of *R. solani* and biocontrol agents (*T. harzianum* T5 and *T. hamatum* T8) in liquid culture. 0.1 g of powder of *Brassica* plants was added to autoclavable tubes (size 150 mL) which contained 20 mL of potato dextrose broth (PDB). 0.22 µm sterile Millipore filters were used to sterilize the medium with the powder to transfer to clean tubes. After sterilization, these tubes were inoculated with mycelium of *R. solani* and biocontrol agents (T5 and T8) separately (0.5 cm in diameter discs) from 7 days old cultures. In controls the *R. solani*, T5 and T8 were inoculated on ¼ PDB only for each fungus. Each treatment had three replicates. The cultures were shaken at 120 rpm, at 22°C for 10 days. Cultures were filtered and the mycelial dry weights were determined after drying to a constant weight at 60°C (Khallil, 2001).

6.2.5. Effect of root exudates on antagonism

Seedlings of broccoli, cabbage and potato from tissue culture were grown in sterilized glass jars (450 mL) in a sterile Hoagland solution in a sterile growth chamber with 12 hr light and 12 hr dark photoperiod, at a temperature of 18-20°C. The seedlings were sterilized with 1% NaOCl with two drops of Triton X-100 detergent as a mix for 2 minutes for surface disinfestation for each plant separately. The seedlings were washed three times with sterilized distilled water. Root exudates were collected from plants after 16 days of growth. Broccoli, cabbage and potato seedlings were removed manually from the Hoagland solution in a laminar flow cabinet to avoid contamination (Dechassa and Schenk, 2004; Hayder, 2017). 1 mL was taken from each root exudate and cultured on PDA medium and incubated at 25ºC for 5 days to check the microbial contamination. The solution was stored frozen until used in trials.

225 mL of root exudates of each plant was mixed with sterilized water agar (4.5 g/50 mL water) that had been held in a water bath at 60°C for 2 hrs to cool before mixing to examine the microbial interactions between *Trichoderma* sp. and *R. solani*. The medium was poured into sterilized Petri-dishes and inoculated with 5 mm diameter disc of antagonist diametrically opposite a 5 mm diameter disc of pathogen. In controls the pathogen was alone plated on one

side of the plate at the periphery for each root exudate. There were three replicates for each treatment. The plates were incubated at 20°C for 7 days. The growth of the pathogen was measured on the day before contact. The percentage inhibition of growth was calculated relative to growth on Hoagland solution alone by using the same equation as in Chapter 3 (Sivakumar et al., 2000).

6.2.6. Testing the effect of carrier materials on growth promotion

Wheat bran was used in this experiment to evaluate whether the nutrients in the bran carrier were responsible for the high growth promotion or related to *T. hamatum* T8 for all previous experiments. Cabbage seedlings (Sugarloaf) were used to check the growth parameters relative to treatments. The bran carrier material was prepared in 30 × 45 cm polyester oven bags as in Chapter 5. There were four treatments. The first treatment was bran inoculated with T8 and incubated at 28°C for 14 days; the second treatment was bran inoculated with T8 and autoclaved at 121°C for 30 min after incubation; the third treatment was uninoculated bran incubated for 14 days; the fourth treatment was untreated soil as a control. This experiment was carried out in pots (15 X 15 cm) with Trevenna soil (loamy sand). 2 g of T8 inoculum or bran was mixed into the soil in each pot around the cabbage seedlings. There were four replicates for each treatment. This experiment was prepared by the modified method of El-Fattah et al. (2013). This test was carried out under controlled conditions in a glasshouse at 20°C for 20 days. The shoots and roots were dried in an oven at 60°C until constant weight, then weighed individually.

6.2.7. Statistical analysis

Pathogenicity tests, shoot and root dry weight were analysed using one-way T-Tests. Data were analysed by one way ANOVA and two way (analysis of variance) with statistical program SPSS version 22. The criterion for significance was $P < 0.05$. Log_{10} transformation was used when necessary to correct for non-homogeneity of variance. Tukey's test was used for mean separation at the 5% level.

6.3. Results

6.3.1. Effect of *R. solani* AG-3PT on *Brassica* plants

In the first pot trial on 2-week old plants, *R. solani* significantly reduced the shoot dry weight of broccoli plants, compared with the uninoculated control (Figure 6-1). There were no symptoms in all treatments either *R. solani* only or uninoculated control. There was no significant effect of *R. solani* on cabbage or broccoli parameters in a repeat experiment on 4-week old plants (Figure 6-2).

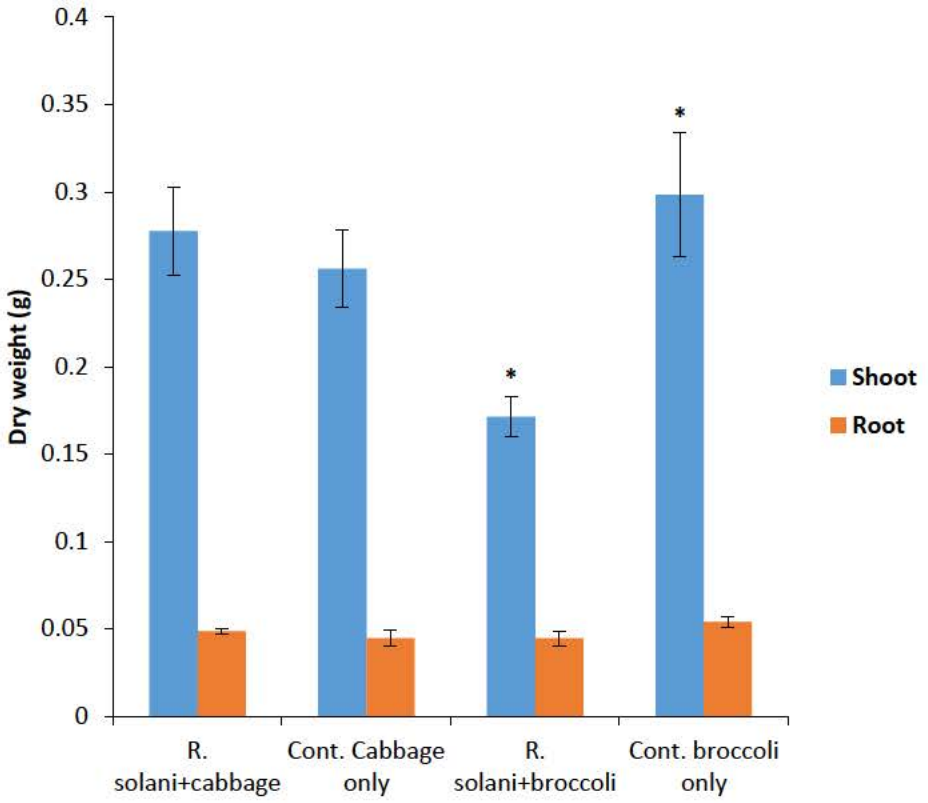

Figure 6-1. Dry weight of shoots and roots inoculated with *R. solani* AG-3PT on two-week-old broccoli and cabbage seedlings under glasshouse conditions at 20°C. * indicates that effect of *R. solani* is significant (T test, P = 0.05) for this tissue on this plant. Error bars show standard errors (n=3).

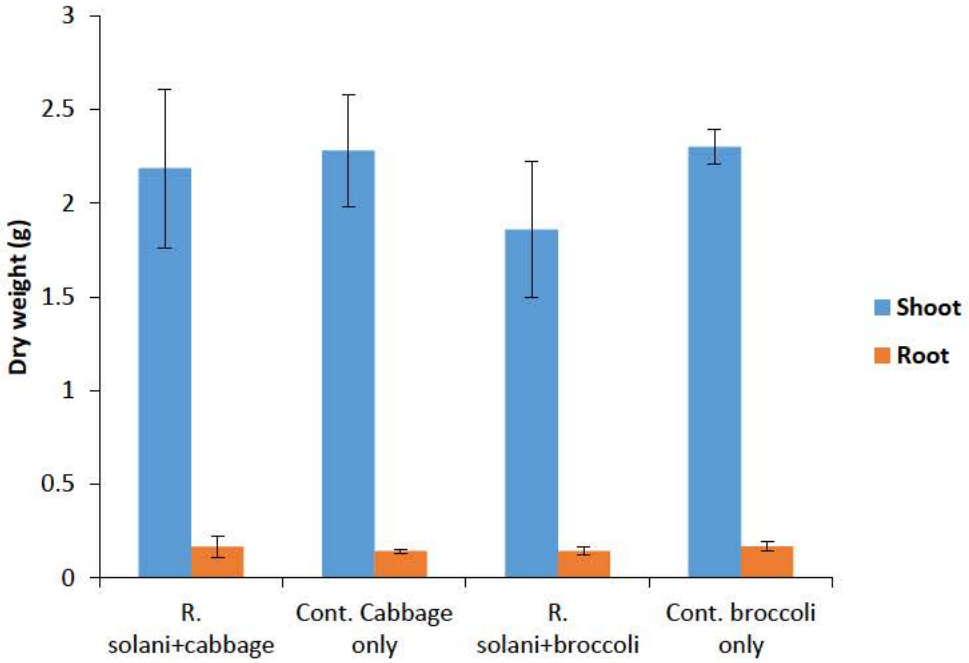

Figure 6-2. Dry weight of shoots and roots inoculated with *R. solani* AG-3PT on four-week-old broccoli and cabbage seedlings under glasshouse conditions at 20°C. Error bars show standard errors (n=3).

6.3.2. Effects of *Trichoderma* on growth of *Brassica* plants and potato plantlets from tissue culture

Trichoderma hamatum T8 and *Trichoderma harzianum* T5 significantly increased the shoot and root dry weights of broccoli, cabbage and potato in potting mix (Figure 6-3) and Kirby soil (Figure 6-4). In most cases, the effect of T8 was significantly greater than that of T5. Growth promotion has been presented as a ratio by comparing dry weight of plants inoculated with *Trichoderma* isolates to untreated control (sterilized distilled water) (Figure 6-3 and Figure 6-4).

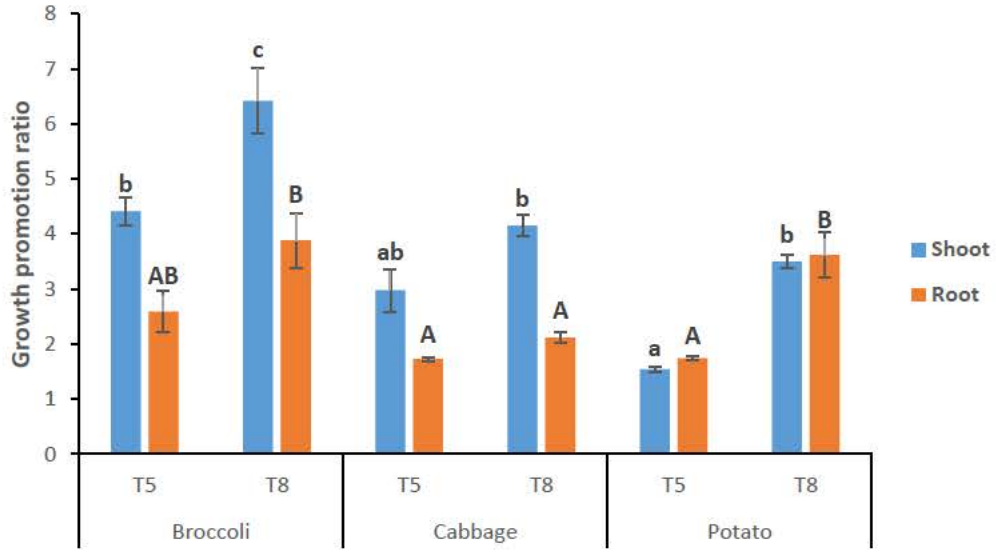

Figure 6-3. Ratio of dry weights of broccoli, cabbage and potato seedlings inoculated with *Trichoderma* strains to dry weights of control plants in potting mix. Ratios above 1 indicate promotion of growth. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

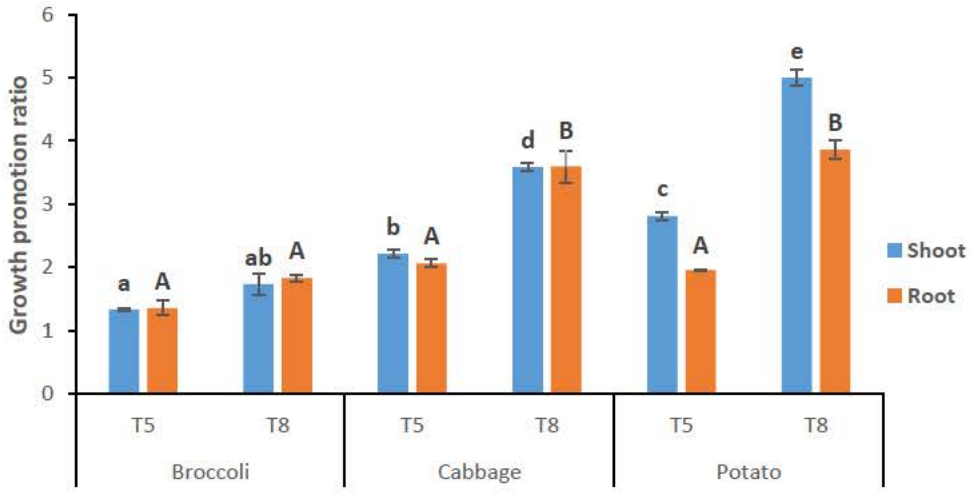

Figure 6-4. Ratio of dry weights of broccoli, cabbage and potato seedlings inoculated with *Trichoderma* strains to dry weights of control plants in Kirby soil. Ratios above 1 indicate promotion of growth. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

6.3.3. Effects of volatiles and non-volatiles from *Brassica* plants on the growth of *R. solani* AG-3PT

There were significant ($P < 0.05$) main effects of plant, tissue type and fungus on growth of fungi exposed to volatiles. Most interactions were also significant. Generally, growth was less with volatiles from stems than leaves, and least with roots. Root volatiles of cabbage and cauliflower were more inhibitory to T5 and T8 than broccoli.

In general, volatiles from all tissues of brassica plants reduced the radial growth of *R. solani* compared with control treatment. Growth of *R. solani* was completely inhibited by root volatiles of all plants and stem volatiles of cauliflower. Volatiles from cabbage stem were highly inhibitory, while those from broccoli stem and leaves of all plants showed least inhibition of growth (Figure 6-5).

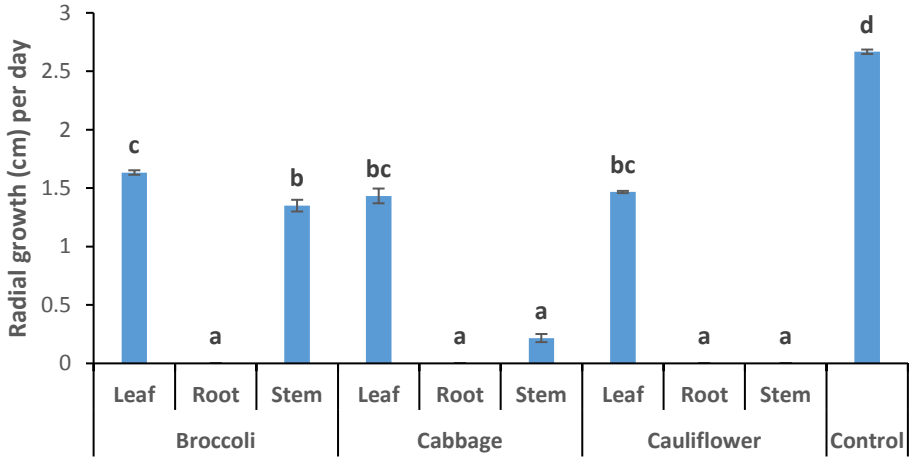

Figure 6-5. Mean radial growth of *R. solani* (Rs) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Volatiles from root tissues of all plants reduced the growth of *T. hamatum* T8 in comparison to the control treatment. Root volatiles of cabbage and cauliflower were more inhibitory to T8 than broccoli. Volatiles from cabbage stem caused a slight reduction in growth of T8 (Figure 6-6).

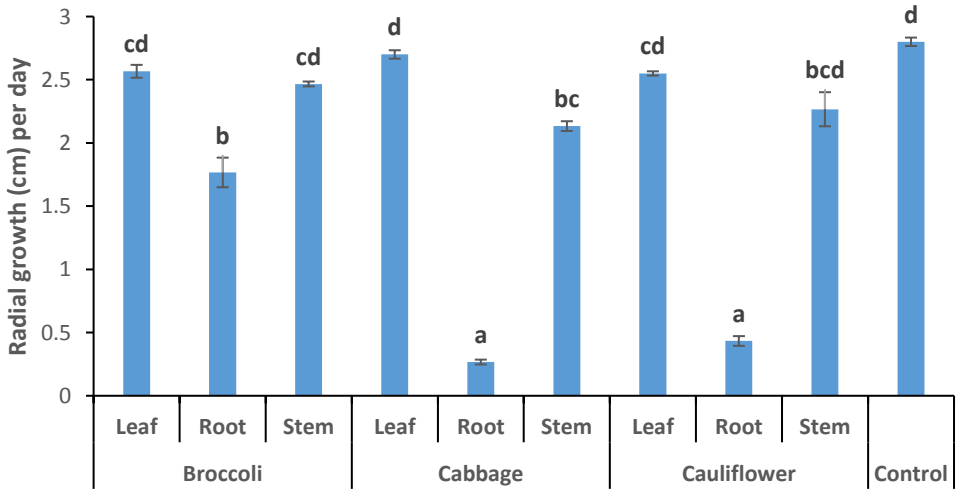

Figure 6-6 Mean radial growth of *T. hamatum* (T8) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Volatiles from leaf tissues of all plants and stem of broccoli had no effect on the growth of *T. harzianum* T5 compared with control treatment. Root tissues of brassica plants were more inhibitory to T5 than leaf and stem tissues of plants. Also, root tissue of cabbage had a higher inhibitory effect than broccoli root (Figure 6-7).

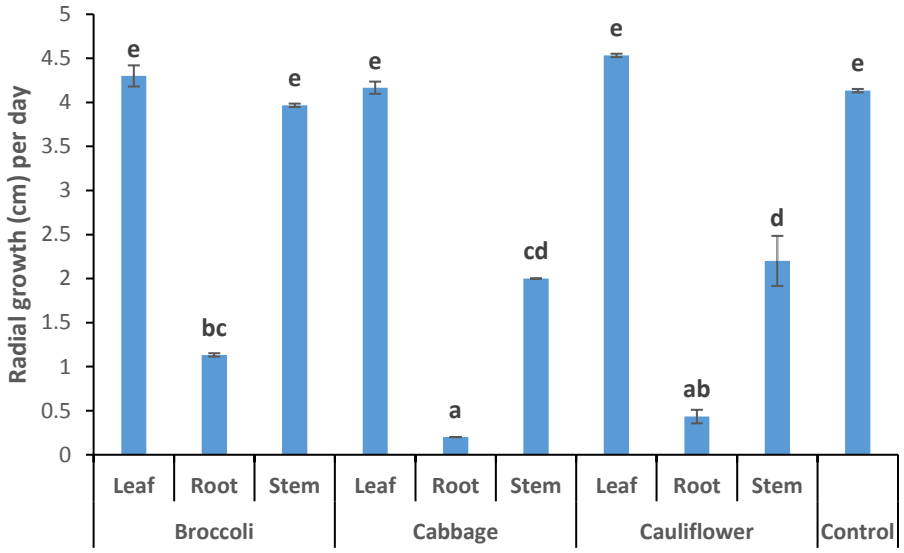

Figure 6-7 Mean radial growth of *T. harzianum* (T5) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

When the tissue powders were added to the medium all tissues of brassica plants reduced the growth of *R. solani* in comparison to the control treatment. Growth of *R. solani* was more inhibited by root tissues than leaf and stem tissues of brassica plants. *R. solani* was completely inhibited by cabbage roots but grew slowly with broccoli and cauliflower roots. Also, stem tissues of plants had higher inhibition to *R. solani* than leaf tissues of plants (Figure 6-8).

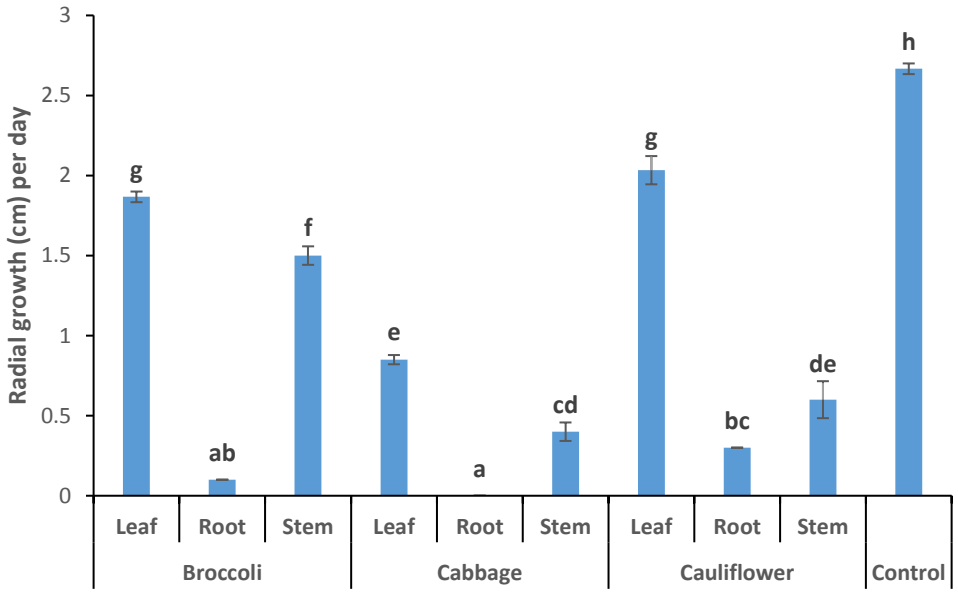

Figure 6-8 Mean radial growth of *R. solani* (Rs) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

T. hamatum T8 was relatively unaffected by non-volatiles from all plants and tissue types. All tissues of broccoli and leaf tissue of cauliflower increased the growth of *T. hamatum* T8 compared with control treatment. Root tissues of broccoli and cabbage were less inhibitory to T8 than cauliflower. Cabbage leaf had higher inhibition to T8 than broccoli and cauliflower (Figure 6-9).

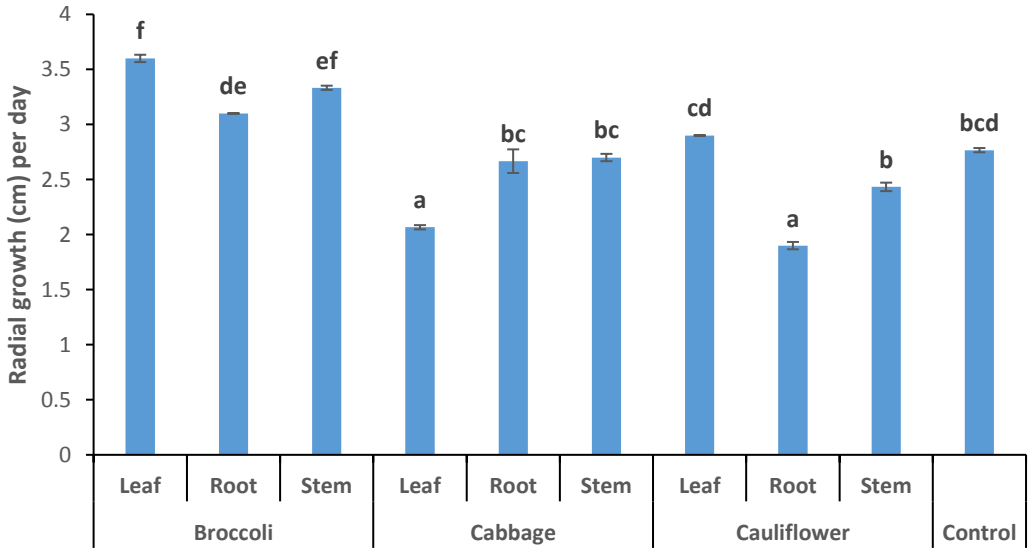

Figure 6-9 Mean radial growth of *T. hamatum* (T8) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Non-volatiles from all tissues of brassica plants reduced the growth of *T. harzianum* T5 compared with control treatment. Root tissues of all plants was more inhibitory to T5 than leaf and stem tissues of plants. However, root tissue of cabbage had higher inhibition to T5 than root tissues of other plants (Figure 6-10).

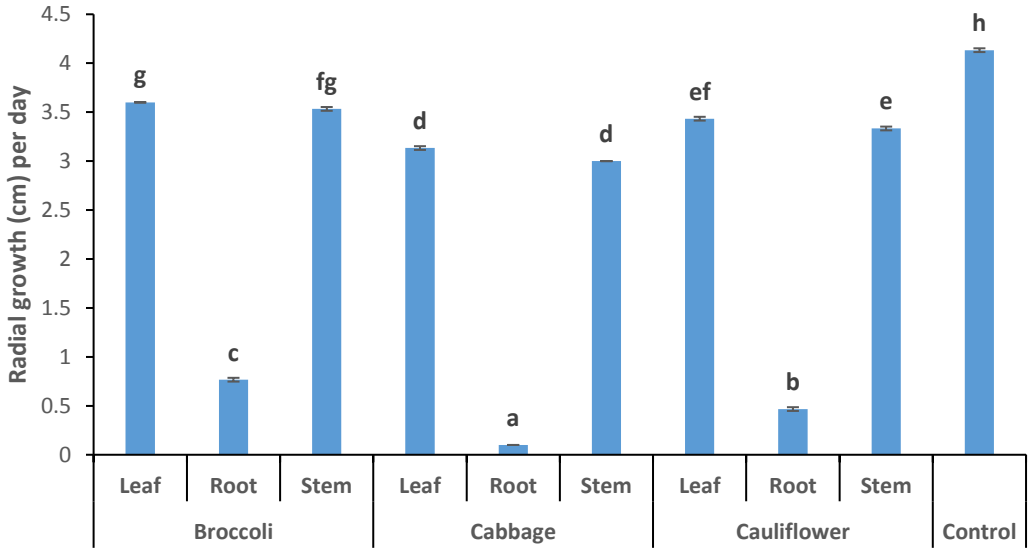

Figure 6-10 Mean radial growth of *T. harzianum* (T5) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants. Control: 1/4-strength PDA only. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

6.3.4. Effects of volatiles and non-volatiles from *Brassica* plants in dual culture against *R. solani* AG-3PT

There was a highly significant ($P < 0.001$) interaction between plant, tissue type and *Trichoderma* isolate in their effects on growth of *R. solani* in dual culture when exposed to volatiles from *Brassicas* (Figure 6-11). *T. harzianum* T5 and *T. hamatum* T8 inhibited growth in the presence of volatiles from all tissues except cabbage roots, which had very high inhibition by volatiles in the controls (Figure 6-11).

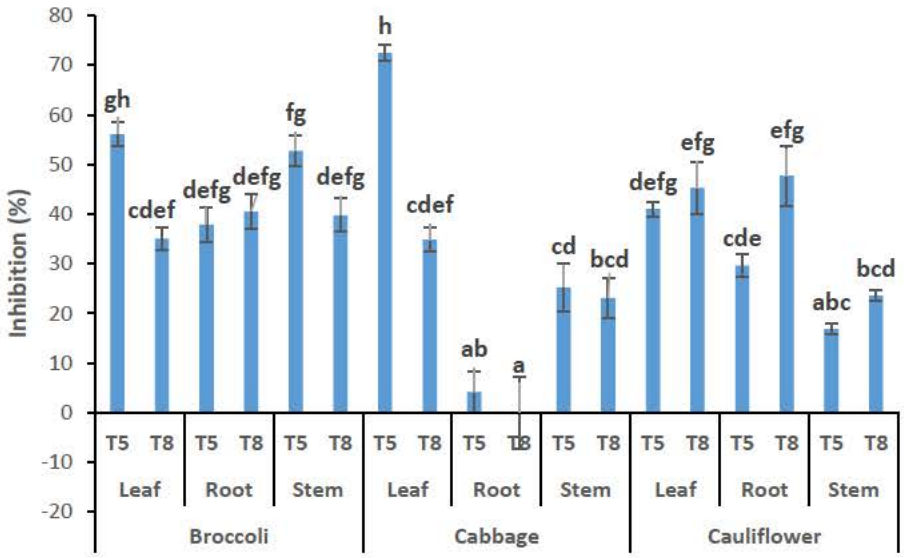

Figure 6-11 Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to volatiles from freeze-dried Brassica tissues. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

T. harzianum T5 and *T. hamatum* T8 inhibited growth of the pathogen in the presence of non-volatiles from all tissues. There was a highly significant ($P < 0.001$) interaction between plant, tissue type and *Trichoderma* isolate on growth of *R. solani* in dual culture when exposed to non-volatiles from *Brassicas*, but the interaction between plant and *Trichoderma* isolate were not significant (Figure 6-12). *T. hamatum* T8 was more inhibitory in the presence of non-volatiles from cauliflower stems than T8 with stem tissue of broccoli or T5 with root tissue of cabbage and cauliflower or stem tissue of broccoli, but other comparisons were not significant (Figure 6-12).

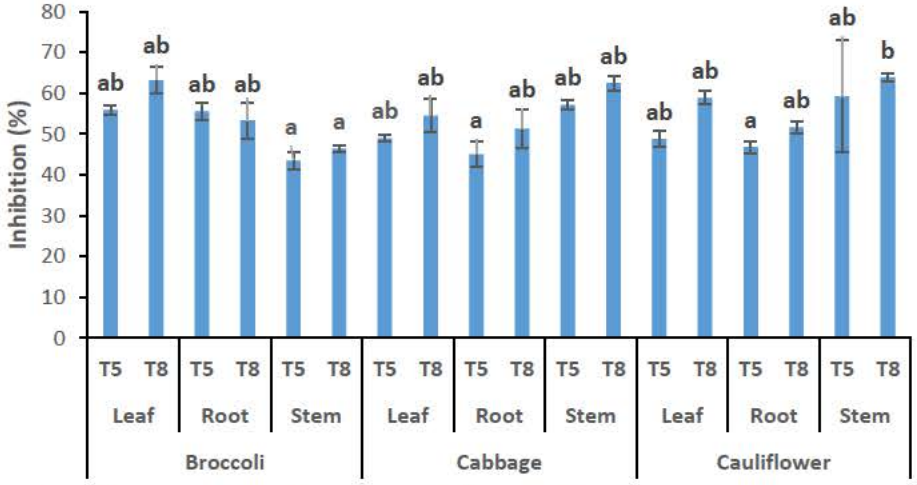

Figure 6-12 Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to non-volatiles from freeze-dried Brassica tissues. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The dual culture method with non-volatile substances from root tissues of broccoli and cabbage was re-tested to confirm the previous results (Figure 6-13). There was a highly significant (P =0.001) interaction between plant and *Trichoderma* treatment. The combinations of T5 with cabbage roots had significantly less inhibition of the pathogen that the controls or non-volatiles of broccoli roots (Figure 6-13).

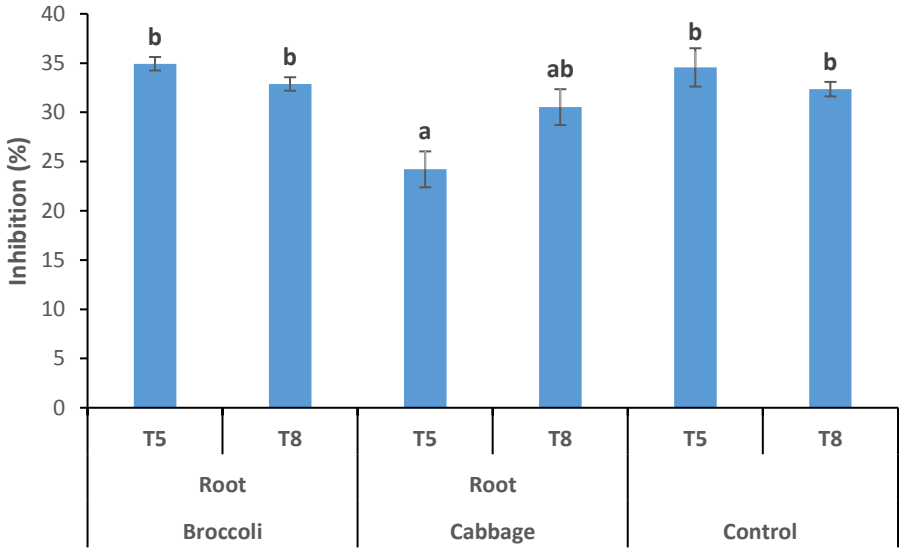

Figure 6-13 Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to non-volatiles from cabbage and broccoli roots. T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

6.3.5. Effects of powder of *Brassica* plant tissue on the growth of *Trichoderma* sp. and *R. solani* AG-3PT in liquid media

The mycelial dry weight of *T. hamatum* T8 was significantly increased in media supplemented with powder of all tissues except for root tissues of broccoli and cauliflower (Figure 6-14). The mycelial dry weight of *T. harzianum* T5 was significantly increased by powder of all tissues from all species, except for broccoli leaf and root, cabbage leaf, and cauliflower root, which did not differ significantly from the control (Figure 6-15). Stem tissue of cabbage and leaf tissue of cauliflower gave the largest amount of fungal biomass dry weight (Figure 6-15). The mycelial dry weight of *R. solani* was significantly reduced by the addition of all tissues from all species to PDB, except for cauliflower leaf (Figure 6-16). Root tissue of cabbage reduced the pathogen biomass significantly in comparison to all other treatments (Figure 6-16).

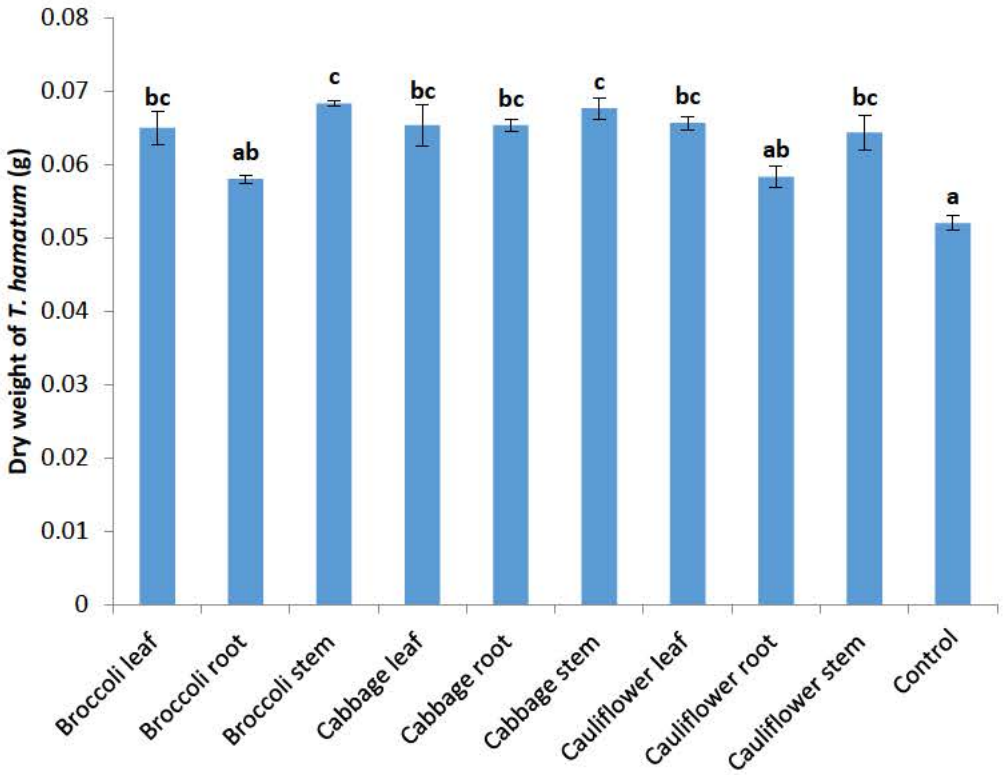

Figure 6-14 Dry mycelial biomass from *T. hamatum* T8 after 10 days growth in a liquid medium supplemented with various parts of plant tissue (*Brassica* species), at 22°C. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

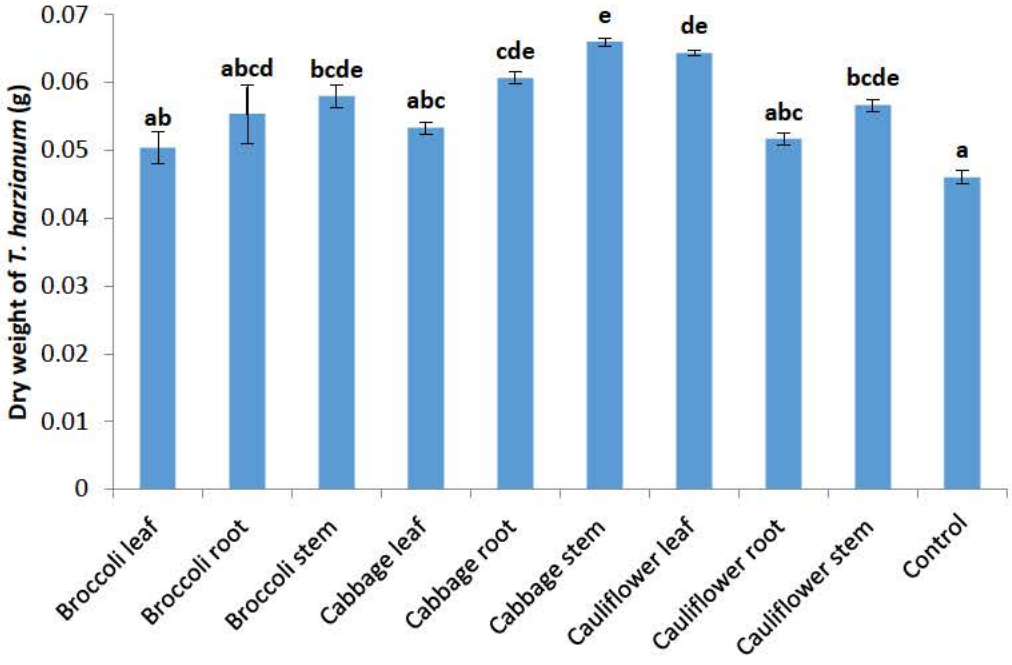

Figure 6-15 Dry mycelial biomass from *T. harzianum* T5 after 10 days growth in a liquid medium supplemented with various parts of plant tissue (*Brassica* species), at 22°C. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

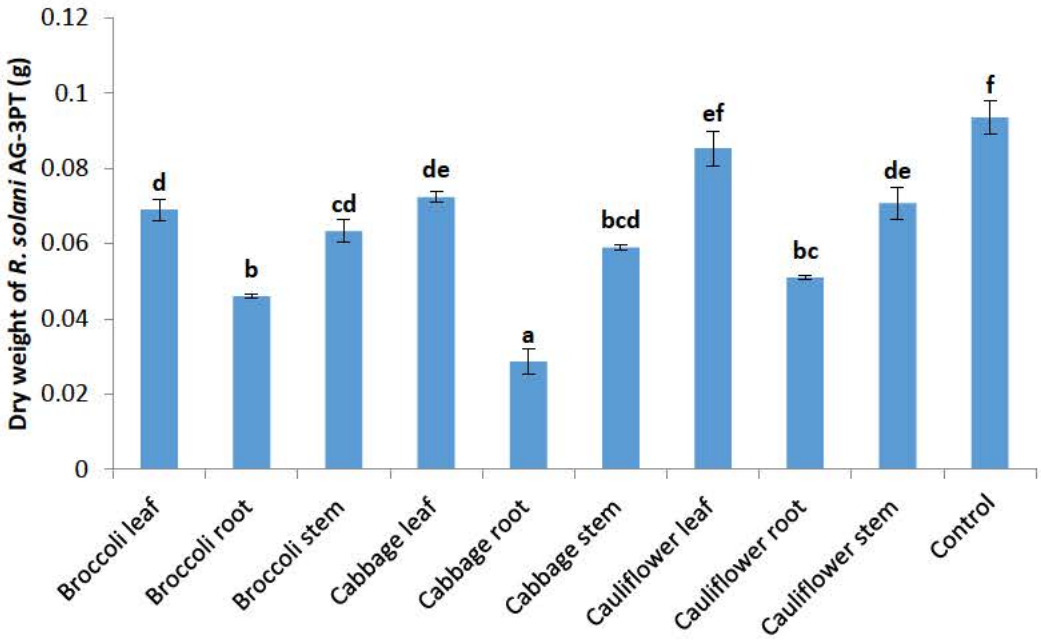

Figure 6-16 Dry mycelial biomass from *R. solani* AG-3PT after 10 days growth in a liquid medium supplemented with various parts of plant tissue (*Brassica* species), at 22°C. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

6.3.6. Effect of root exudates on antagonism

There was a significant interaction between root exudates and *Trichoderma* isolates on inhibition of growth in dual culture (Figure 6-17). In controls without *Trichoderma*, broccoli and cabbage root exudates inhibited growth, but potato exudates promoted growth. Growth inhibition by T5 and T8 was significantly decreased by potato exudates. The combination of broccoli or cabbage root exudates with either T5 or T8 resulted in greater inhibition than broccoli or cabbage residues alone (Figure 6-17).

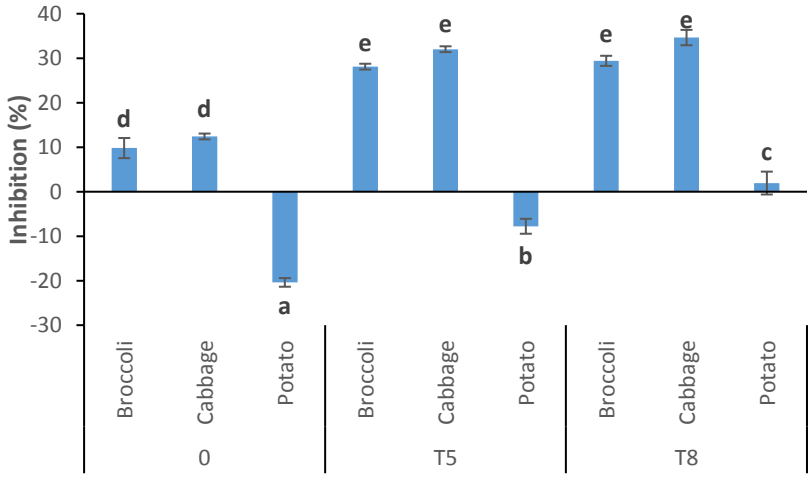

Figure 6-17 Inhibition of *R. solani* in dual culture by root-exudates derived from broccoli, cabbage and potato plants, and isolates of *Trichoderma*. 0: *R. solani* only; T5: *T. harzianum*; T8: *T. hamatum*. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

6.3.7. Testing the effect of carrier materials on growth promotion

Growth promotion activity of the bran formulation of T8 was assessed under glasshouse conditions after 20 days of growth of cabbage seedlings. Sterilized bran without *Trichoderma hamatum* T8 gave higher shoot and root dry weights than the control treatment, but the difference was not significant (Figure 6-18 and Figure 6-19). Live inoculum of T8 on wheat bran gave significantly higher root and shoot dry weights than sterilized wheat bran only and plant only (control treatment). Autoclaved inoculum significantly increased both shoot and root dry weight compared with the control (Figure 6-18, Figure 6-19 and Figure 6-20).

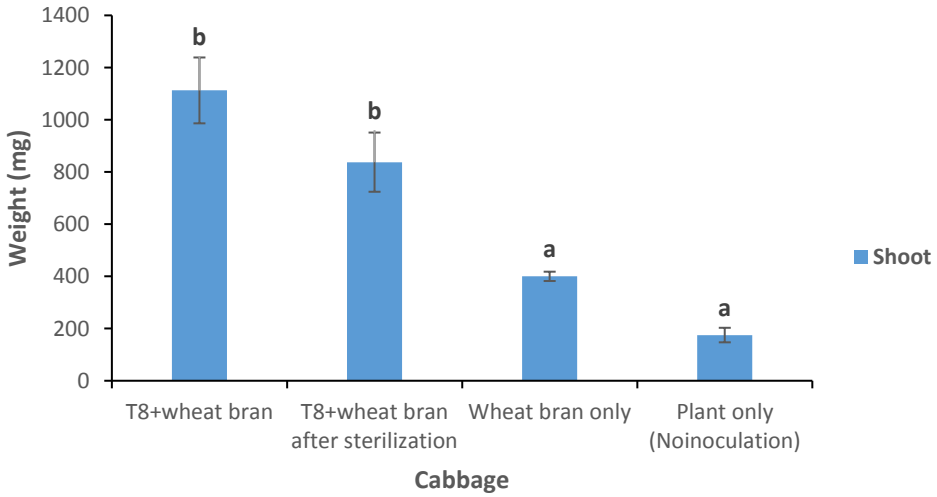

Figure 6-18 The effect of carrier materials on biocontrol agent of *T. hamatum* (T8) on shoot dry weight of cabbage seedlings. Each column shows the mean of four seedlings. The error bars show standard errors. Columns labelled with the same letter are not significantly different P=0.05 (Tukey test).

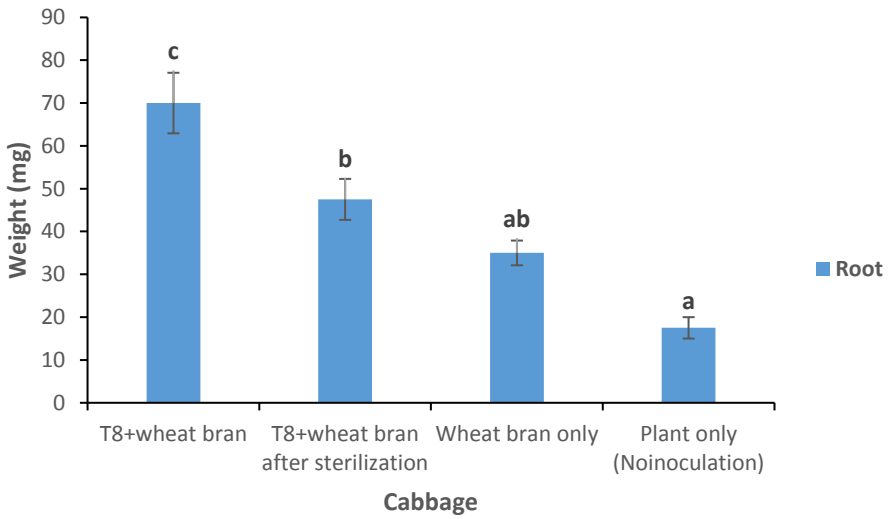

Figure 6-19 The effect of carrier materials on biocontrol agent of *T. hamatum* (T8) on root dry weight of cabbage seedlings. Each column shows the mean of four seedlings. The error bars show standard errors. Columns labelled with the same letter are not significantly different P=0.05 (Tukey test).

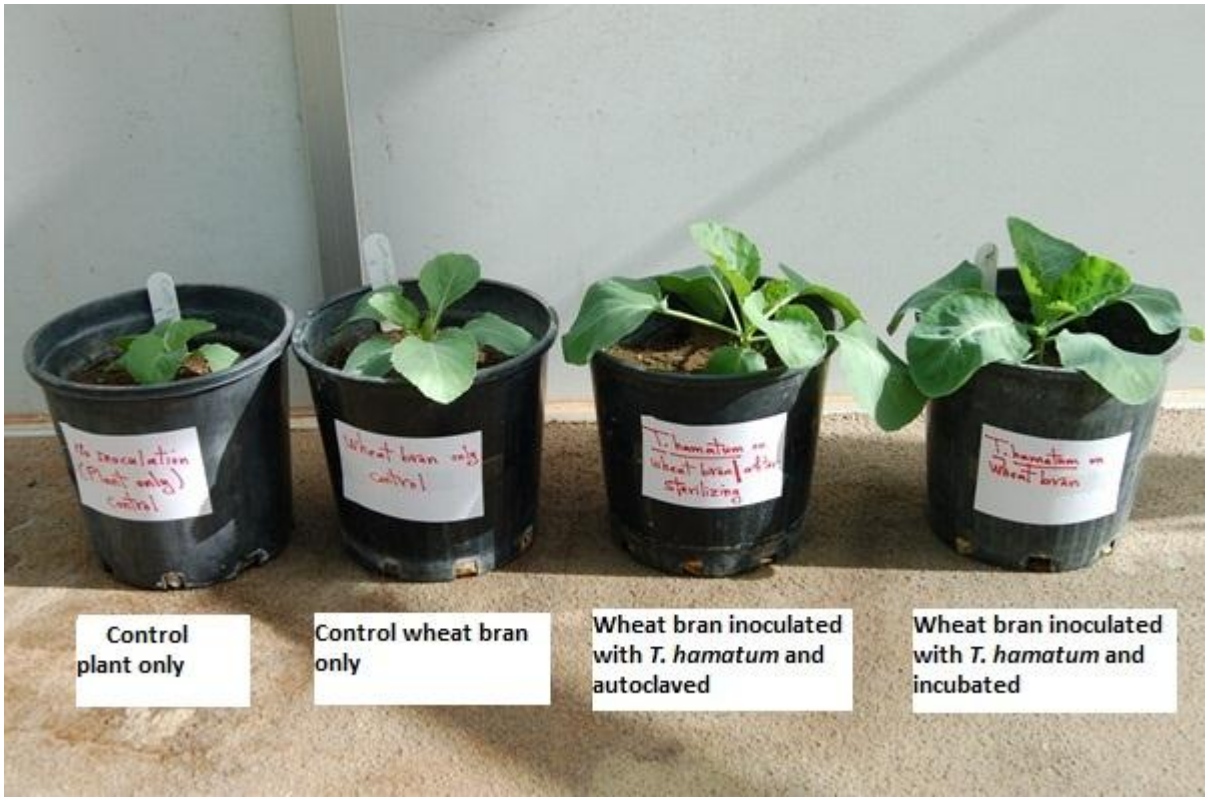

Figure 6-20 . The effect of carrier materials on *T. hamatum* (T8) on growth promotion of cabbage seedlings.

6.4. Discussion

This present study tested the suppressive capacity of some *Brassica* crops and interaction with biocontrol agents on reduction of pathogen growth. The isolates tested in this chapter were chosen according to their ability to inhibit the pathogen and promote growth of potato plants. In previous chapters, *T. hamatum* T8 promoted the growth of potato plants significantly higher than other isolates. Although *T. harzianum* T5 and *T. hamatum* T8 were chosen so far because they belonged to different species, they were used in this chapter to see which application of T5 or T8 was more compatible or tolerant to the volatile and non-volatile compounds derived from brassica crops. The pathogen isolate originated from a potato plant and was tested on *Brassica* crops (cabbage and broccoli) in this study. There were no significant symptoms in the *Brassica* crops. There was slight growth inhibition of young broccoli seedlings, but older seedlings grew out of this. This could be related to the glucosinolates that are released from plants in the soil. Similarly, Budge et al. (2009); Pannecoucque et al. (2008) found that no *R. solani* AG-3PT isolates were retrieved from any *Brassica* crops which were collected from various areas.

Growth promotion in this study by the two *Trichoderma* sp. isolates was tested under glasshouse conditions. Broccoli, cabbage and potato plantlets treated with two *Trichoderma* isolates (*T. hamatum* T8 and *T. harzianum* T5) had greater shoot and root weights compared with the untreated plantlets; however, isolate T8 gave a higher growth enhancement of all plantlets than isolate T5. Clouston et al. (2010) tested the effects of 62 *Trichoderma* isolates for their ability to enhance growth in *Impatiens walleriana* plants, and the authors detected that isolate LU556 (IT160) augmented root dry weight and root length compared with untreated control, but isolate IT167 was superior at increasing shoot dry weight. Plant growth responses to biocontrol *Trichoderma* isolates might be due to both the inhibition or control of minor plant pathogens and production of growth regulating substances such as phytohormones (either by the plants or microbes), vitamin production, enhanced development of plant roots, increased solubilisation and soil nutrient uptake and rises in the carbohydrate metabolism rate, photosynthesis and plant defence mechanism (Baker, 1988; Harman, 2006; Harman et al., 2004; Inbar et al., 1994; Kleifeld and Chet, 1992). A report by da Silva et al. (2012) who recorded that *Trichoderma* strain T-52 increased the biomass production in rice plants (*Oryza sativa*) by approximately 38% in comparison to the untreated control. Rabeendran et al. (2000) reported that *Trichoderma* spp. isolates increased the rate of biomass production of cabbage

and lettuce plants. Chang et al. (1986) reported the induction of growth response by *Trichoderma* spp. in different horticultural and floricultural crops. This experiment was repeated with the same treatments, but it was planted into Kirby soil. Similar results were found by *Trichoderma* applications which increased the plant growth enhancement in the Kirby soil under glasshouse conditions. However, the plant growth promotion in potting mix was more than in Kirby soil in all test plants (broccoli, cabbage and potato plantlets), which could be related to the higher nutrients in the potting mix.

Volatile substance tests on colony diameter of *R. solani* and *Trichoderma* spp. isolates by freeze-dried *Brassica* tissues (leaf, root and stem) from three species (broccoli, cabbage and cauliflower) demonstrated that toxic volatiles were produced by root tissue of all plants as well as stem tissue of cauliflower. It has been shown in previous studies that extracts from root tissues of different *Brassica* plants produce significant amounts of antifungal compounds that inhibit pathogens (Schreiner and Koide, 1993; Vierheilig and Ocampo, 1990). Interestingly, volatiles produced by root tissues significantly reduced or prevented the growth of *R. solani*, while *Trichoderma* isolates were much less sensitive to suppressive effects from the root tissues. Smith and Kirkegaard (2002) mentioned that strains of *Trichoderma* were less susceptible to a specific isothiocyanate released from *Brassica* tissues than many genera of fungal plant pathogens such as *Pythium* spp. and different anastomosis groups of *R. solani*. Also, Galletti et al. (2008) showed that *Trichoderma* isolates as biocontrol agents were more tolerant to *Brassica* tissues than soilborne plant pathogens (*Pythium ultimum*, *R. solani* and *Fusarium oxysporum*) in the soil.

Rhizoctonia solani had no growth when exposed to volatile compounds released from stem tissue of cauliflower and also grew very slowly with volatiles from stem tissue of cabbage. Variation in efficiency of *Brassica* species for suppressing mycelium growth of *R. solani* in this assay is presumably related to glucosinolate content. The different *Brassica* species or varieties have different concentrations and types of GSLs (Daxenbichler et al., 1991; Kirkegaard and Sarwar, 1999; Kirkegaard et al., 1996). It was noticeable that the radial growth of *R. solani* and *Trichoderma* colonies were higher with the leaf tissue of all *Brassica* plants compared with other tissues. This suggest that GSL content is varied among the tissues, plant and growth stages (Bhandari et al., 2015), and in the current study seedling/young plants used and the effect may be more pronounced if more mature plants had been used. Also, the fungal growth depends fundamentally on the fungi sensitivity to isothiocyanates (ITCs) (Sarwar et al.,

1998). This is in agreement with Morra and Kirkegaard (2002) and Villalta et al. (2016) who reported that isothiocyanates can reduced the growth of soilborne fungal plant pathogens.

This study also showed that the tissues of cabbage gave better repressive effectiveness than the other *Brassica* plants to the fungi, suggesting that parts of this plant contain different types of biofumigation compounds (Fan et al., 2008), and also probably because total GSL content in cabbage and cauliflower were higher than in broccoli (Bhandari et al., 2015; Kushad et al., 2004; Rangkadilok et al., 2002). The major GSLs in cabbage roots are glucoerucin (ERU) and gluconasturtiin (NAS), while cauliflower has almost no ERU; broccoli has less NAS than either cabbage or cauliflower so that these contents are toxic to the soilborne fungal growth (Bhandari et al., 2015). It is also reported that ERU produces erucin, or 4-(methylthio) butyl ITC, which is an aliphatic ITC and will be volatile, but it will tend to have low toxicity when incorporated into agar, whereas NAS produces nasturtiin, or 2-phenyl ethyl ITC which is aromatic and will have low volatility and high toxicity in agar or soil (Sarwar et al., 1998). This could explain why cabbage root, with high NAS, was more inhibitory than broccoli root.

In tests on the effect of non-volatile substances on colony diameter, it would appear that *R. solani* was most sensitive to the root tissue of cabbage which allowed no growth. *T. harzianum* T5 was slightly less sensitive to the inhibition of growth by root tissue of cabbage. It was clear that resistance of *T. hamatum* T8 to inhibition was greater for all tissues from the *Brassica* species compared to *T. harzianum* T5. This makes T8 a better candidate for combining with *Brassica* intercropping than T5.

In volatile substances test by dual culture technique, *Trichoderma* isolates inhibited the mycelial growth of *R. solani* significantly over the control. Generally, strains of *Trichoderma* and root tissues of all *Brassica* plants showed the highest percentage of growth inhibition of *R. solani*. However, cabbage root was significantly superior in suppressing the pathogen with and without biocontrol agents, which may be associated with the cabbage root which highly inhibited the pathogen growth, but not with the biocontrol agents. While, the suppression mechanism for biocontrol agent may be competition for space and food (Kumar et al., 2017c), and furthermore, each parts of *Brassica* plants has different amounts of glucosinolates (Fan et al., 2008). The maximum growth suppression of *R. solani* was caused by *T. harzianum* T5 followed by *T. hamatum* T8 with leaf and stem tissues of broccoli and cabbage plants, whereas leaf and stem tissues of cauliflower with *T. hamatum* had more effect than *T. harzianum* to inhibit the pathogen growth. All *Trichoderma* isolates with stem tissue of broccoli inhibited

mycelial growth of the test pathogen more than the other stem tissues of *Brassica* plants. Maximum overgrowth is observed when a biocontrol isolate shows higher rate of growth and higher ability of antibiotic production (Mathivanan et al., 2000), and higher tolerance against toxic volatiles produced by glucosinolates which is released from each part of plant (Rahmanpour et al., 2009). Volatile compounds produced by *Trichoderma* spp. and other biocontrol agents and volatile compounds produced by green manure have been confirmed to be repressive against *S. rolfsii* and *R. solani* (Kamil et al., 2009; Srinivasa et al., 2014).

In non-volatile substances test by dual culture technique, both *Trichoderma* isolates tested with non-volatile compounds derived from *Brassica* species, were found to inhibit the growth of *R. solani* over control. All of the *Trichoderma* treatments reduced *R. solani* growth, with activity being related to non-volatile compounds of *Brassica* plants compared with control treatments, but root powder of cabbage with and without *Trichoderma* spp. had maximum growth zone inhibition of pathogen. The highest efficiency of non-volatile substances produced by cabbage root tissues for inhibition in growth zone of plant pathogen may be due to the presence of high antifungal compounds that affect pathogen growth. In a repeated trial, growth inhibition of *R. solani* showed the same pattern with root tissues of broccoli and cabbage, but not exactly the same size of effects. This could be because the activity of powder was less than in the beginning when it was fresh. It is not necessary to obtain the same numbers when repeating the trial, but the pattern of growth inhibition was the same.

When mycelial biomass on different liquid media that contained various *Brassica* powders was measured, *T. hamatum* and *T. harzianum* appeared less sensitive to toxicity from freeze-dried *Brassica* tissues than *R. solani* inoculum in the in vitro system. Surprisingly, all *Brassica* tissues augmented the mycelial dry weight of both isolates of *Trichoderma*, whereas *R. solani* was shown to be negatively affected by *Brassica* tissues compared with the control treatments. Importantly, the cabbage roots whilst increasing mycelial growth of both *Trichoderma* isolates, reduced strongly the dry weight of *R. solani*.

Biocontrol efficacy of *T. hamatum* in different inoculants on wheat bran as a carrier was tested after 20 days in a glasshouse experiment on cabbage growth. *T. hamatum* on bran significantly increased the biomass productivity of cabbage plants in the pot trials followed by *T. hamatum* on bran after sterilization in comparison to the sterilized wheat bran only and plant only (no inoculation). The results suggest that wheat bran can provide nutrients, so that the plant growth promotion is partially due to the presence of nutrients such as nitrogen,

phosphorus, potassium or carbon. However, previous chapters also showed that *Trichoderma* spores alone can promote growth so the effect of the inoculation is not just due to the nutrients or organic matter added with the bran.

Overall biocontrol agents increased the plant growth of potato and *Brassica* plants in two soil types. The interaction of *T. hamatum* T8 with *Brassica* crops significantly inhibited the growth of *R. solani* AG-3PT. Preliminary tests showed that *Trichoderma* and brassica roots were compatible so it should be possible to combine them as management techniques for the disease. *Trichoderma* spp. were tolerant to the *Brassica* plants, but the *R. solani* was sensitive to the tissues of *Brassica* species, especially the root tissues. Pot and field trials combining the effects of *Trichoderma* and *Brassica* species are described in the next chapter.

Chapter 7. Interaction between intercropping with *Brassica* crops and biological control

Part II. Glasshouse and Field trials

7.1. Introduction

The previous chapter showed that the effects of biocontrol agents *T. harzianum* T5 and *T. hamatum* T8 were compatible with the volatile and non-volatile effects of *Brassica* species, although the combination of these isolates and biofumigation was not yet tested against stem canker in planta. Potato growers are more and more interested in growing and using cover crops such as brassicas as green manures with soil biofumigation activity which may replace soil fumigants before sowing (Collins et al., 2006). Larkin and Griffin (2007) showed that several brassica plants used as green manures and incorporated prior to planting reduced scurf caused by *R. solani* AG-3PT in pot and field trials. *Brassica napus* (rapeseed) has been used as a rotation crop with potato crops and also used as fresh biomass incorporated into the soil with or without compost and biological control organisms (*Trichoderma virens, Bacillus subtilis* and *Rhizoctonia solani* Rhs 1A1) against soilborne diseases on potato plants (stem canker, black scurf, common scab and silver scurf), and these treatments either alone or in combination can inhibit disease and increase yield (Bernard et al., 2014). *Trichoderma harzianum* has proved to be tolerant to *Brassica carinata* (Galletti et al., 2008), and application of *Brassica* materials with melon seed treatment with *T. harzianum* led to increased root length by 20% under glasshouse conditions (Galletti et al., 2015). Ahmad and Shah (2012) investigated the combination of *Trichoderma* species and intercropping for control of *Fusarium oxysporum* in gladiolus. No similar work has been done on potato.

Most work on biofumigation has used either rotation or green manuring. An alternative that has rarely been examined is intercropping, where the biofumigation crop is grown at the same time as the crop it is designed to protect. *Brassica* as green manure or cover crops are grown to be directly incorporated for the purpose of enriching soil, especially increased nitrogen content, and releasing biofumigants in the soil to inhibit the soilborne plant pathogens (Collins et al., 2006; Gimsing and Kirkegaard, 2006; Larkin and Griffin, 2007). Also, green manure and biofumigation crops are easy to include in potato cropping systems, while

intercrops are more difficult to apply between two different plants, with different requirements for management and for harvesting. This problem may be overcome by hand-harvesting the brassica crops either cabbage or cauliflower or broccoli before the potato crop is later machine harvested. The intercropping crops also offer opportunity for better use of fertilizers, water use efficiency and solar radiation in comparison to monoculture, and increase yield stability, vegetable production, nutrient availability, financial benefits over sole crops (Jeromela et al., 2017; Singh et al., 2010). Furthermore, intercropping systems give the opportunity to use the differences between crops when the intercropped plants have different resource demands in space or time (Mucheru-Muna et al., 2010). The concept of an intercropping system is assisting to avoid the hazards from insects and diseases in addition to overcoming the impact of adverse environmental conditions. Intercropping is an alternative way of mixing brassicas and potatoes and the expectation that intercropping will perform better than a monoculture with *Brassica* as either green manure or a cover crop. However, no one has examined interactions between the use of brassica plants and *Trichoderma* species with potato plants at the same time against stem canker and black scurf until harvest as mature plants for each crop in the field.

The aim of this chapter was to test the use of an intercropping system between potato plants and *Brassica* plants with the interaction of biocontrol agent for managing the effect of stem canker and black scurf disease and increasing yield production in glasshouse and field trials.

7.2. Materials and Methods

7.2.1. Production of inoculum

Rhizoctonia solani AG-3PT was grown on mixed wheat and barley seeds incubated at 20^{o}C in plastic oven bags for four weeks in the dark as in Chapter 3. The pathogen inoculum was added to soil at rates of 25 g per crate (Nally IH-026 50 litre vented base solid crates with external dimensions of 58 cm length X 38 cm width X 32 cm depth) for the glasshouse trial, and 100 g/m^2 for the field trial one week before sowing (Friberg et al., 2009). Based on results from the previous chapter, the biocontrol agent *T. hamatum* T8 was selected for glasshouse and field experiments. *T. hamatum* T8 was grown on wheat bran as in Chapter 5, and incubated for two weeks. The inoculum was added to soil two days before sowing at rates of 12.5 g per crate for glasshouse experiment, and 50 g/m^2 for field experiment (Abd-El-Khair et al., 2010; Elad

et al., 1980a). Application in a carrier like wheat bran is more practical for large-scale field use than spore suspensions, so is closer to potential commercial practice.

7.2.2. Glasshouse experiment

The Trevenna soil was used in this experiment which is a Chromosol (loamy sand soil). In a depth of 1-10 cm, total C 1.573%, N 0.161%, Silt 10.6%, Sand 74.3%, Clay 14.5%, Colwell P of 22.7 mg/Kg, pH of 5.6, EC of 60.6 µS/cm and exchangeable K of 0.39 $Cmol_c$/kg. This soil was collected from Trevenna Farm, University of New England. The soil was placed in crates at 45 kg of soil per crate.

The potato plants (cv. Desiree) were intercropped with secondary crops of cabbage (Sugarloaf) (*B. oleracea* subsp. *capitata*) and broccoli (Green Sprouting) (*B. oleracea* subsp. *italica*). Cabbage and broccoli seeds were grown in germination trays containing sand-potting mix for 4 weeks under greenhouse conditions (Rabeendran et al., 2000), and planted in crates. There were six plants in each crate. In the intercropping treatments two potato tubers were grown between two pairs of the same *Brassica* plant whether cabbage or broccoli with six tubers of potato in a crate as a control treatment. There were three inoculation treatments: uninoculated soil; soil inoculated with *R. solani* AG-3PT only; and soil treated with *T. hamatum* T8 and *R. solani* AG-3PT. Grain inoculum of *R. solani* was added at 50 g/crate 2 weeks before planting, and bran inoculum of *T. hamatum* T8 at 12.5 g/crate 2 days before planting. Each inoculum was added separately into soil at a depth of 3-5cm by removing the top layer of soil and spreading each inoculum evenly in depth before replacing the soil again, and irrigated once to wet the soil. There were three replicates for each treatment, and the experiment was set up as a randomized complete block design. This study was conducted under glasshouse conditions at temperature 20°C for 13 weeks after emergence. Stem canker severity was measured as the length of lesion on stems based on a scale (0-4): 0 = no disease, 1 = less than 10% of stem area covered with lesions, 2 = 10-25% of stem area covered with lesions, 3 = 26-50% of stem area covered with lesions and 4 = stem girdled with lesions (Atkinson et al., 2010). Black scurf was measured as the number of sclerotia on tubers. Shoot and root dry weights of potato plants, number of tubers, fresh weight of tubers, and shoot and root dry weights of cabbage and broccoli were determined (Yildirim and Guvenc, 2005).

This trial was repeated to confirm the results. The same crates and soil were replanted with a total of three plants per crate (one potato tuber with two cabbage or broccoli plants) to get more space and reduce the competition between plants to check the effect of *Brassica* roots and biocontrol agent on pathogen growth. Crates were re-inoculated with *R. solani* AG-3PT to increase disease severity, but no further inoculum of *T. hamatum* T8 was added.

7.2.3. Field experiment

The field experiment was conducted at the Trevenna Farm, University of New England, Armidale, NSW, Australia. The soil at this site is the Chromosol (loamy sand soil) used in the crate experiments.

Cabbage seeds (Sugarloaf) were sown in small pots (5 x 5 cm) then placed in the green house for four weeks and then the seedlings were transplanted to the field. Tubers of potato cv. Desiree were planted directly into the field.

The trial had a factorial design with three factors: intercropping, inoculation with *R. solani* AG-3PT, and inoculation with *T. hamatum* T8. Desiree potato plants were grown as a monoculture or intercropped with cabbage. Plot size was 100 X 100 cm. Each plot contained 3 rows of plants, with three plants in each row. Plants were spaced 30 cm apart within rows, and 30 cm between rows. In the intercrop treatment, the potatoes were grown in the centre row and cabbages in the outside rows. There was a 1 m buffer around each plot. A randomised complete block design with 4 blocks was used.

There were four soil treatments; 1) uninoculated soil; 2); soil inoculated with *R. solani* AG-3PT; 3) soil inoculated with *T. hamatum* T8; 4) soil inoculated with T8 and *R. solani*. Inoculum of *R. solani* AG-3PT (100 g/m^2) and *T. hamatum* T8 (50 g/m^2) were applied at 1 week before planting at a depth of 5-10 cm by removing the top layer of soil and spreading each inoculum evenly in depth before placing the soil again, and irrigated once to wet the soil. The field trial was set up in early October. Cabbage seedlings were transplanted to the field site on 16 October 2016 with potato tubers sown at the same time. The site was irrigated as required to ensure maximum growth and avoid serious water stress. The trial was harvested on 15 February 2017. Experimental measurements were disease symptoms (stem canker and black scurf) as in section 7.2.2. Measurements of growth for each plant were shoot dry weight of potato and cabbage plants, and fresh weight of potato tubers.

7.2.4. Statistical analysis

Data were analysed by factorial analysis of variance with statistical program SPSS version 22. The criterion for significance was $P < 0.05$. Log_{10} transformation was used when necessary to correct for non-homogeneity of variance. ANOVA tables are presented in Appendix 1.

7.3. Results

7.3.1. Glasshouse experiment

The symptoms of stem canker and black scurf appeared on potato plants in monoculture that were inoculated with *R. solani*. Stem canker symptoms were only seen in the treatment with *R. solani* alone at 0-10% of stem area infected, and were not found in other treatments. There were 13.9 (standard error 2.12) sclerotia per plant. The disease was significantly decreased by the sowing of *Brassica* crops as an intercrop. There were 1.0 (standard error 0.29) sclerotia per plant on potatoes intercropped with broccoli and inoculated with *R. solani* only, and no sclerotia on potatoes intercropped with cabbage, or treated with *T. hamatum* T8, or in uninoculated controls.

The inoculation treatment had significant effects on shoot dry weight (Figure 7-1), tuber fresh weight of potatoes (Figure 7-2) and root dry weight (Table 7-1). All of these parameters were increased in the *R. solani* plus T8 treatment compared with *R. solani* alone or the controls. There were no significant differences between the *R. solani* alone treatment and the controls. The intercrop treatment had significant effects on shoot dry weight and tuber fresh weight, which were lowest in the potato monoculture and highest in the potato-cabbage intercrop (Figure 7-1 and Figure 7-2). There were no significant interactions between inoculation and intercrop treatments for any parameter (Table 7-1).

Table 7-1 Effects of intercropping with cabbage or broccoli and inoculation with *Rhizoctonia solani* AG-3PT and *Trichoderma hamatum* T8 on root dry weight and number of tubers of potato plants in two repeats of a glasshouse experiment.

Inoculation	Intercrop[A]	Experiment 1 Root dry weight (g)	No. of tubers	Experiment 2 Root dry weight (g)	No. of tubers
Control	PP	0.45	2.56	1.08	8.56
Control	PC	0.59	4.33	1.62	11.33
Control	PB	0.65	3.83	1.62	11.00
R. solani	PP	0.58	4.11	0.88	5.89
R. solani	PC	0.62	4.33	1.29	12.33
R. solani	PB	0.65	4.00	1.58	19.33
R. solani+T8	PP	0.79	5.39	1.47	10.22
R. solani+T8	PC	1.26	3.5	1.50	12.67
R. solani+T8	PB	1.10	3.67	1.83	11.67
P (inoculation)		0.012	ns[a]	0.007	ns
P (intercrop)		ns	ns	0.001	0.004
P (interaction)		ns	ns	0.046	0.032
L.S.D.		0.57	-	0.38	5.50

[A]P potato; C cabbage; B broccoli. [a] ns = not significant; [b] least significant difference at P = 0.05 for comparing any two values within each column. LSD has not been calculated where there are no significant treatment effects.

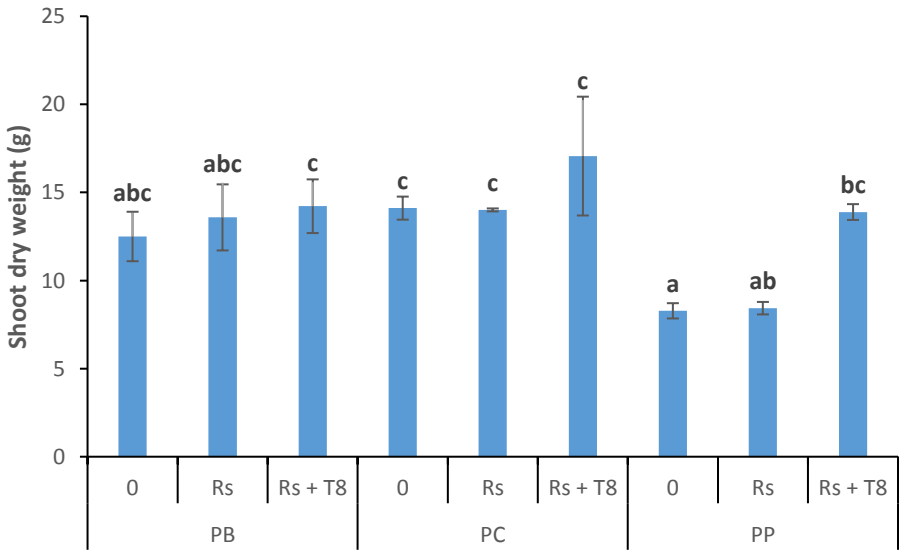

Figure 7-1. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato intercropped with broccoli and cabbage. 0: uninfested soil (plant only); P: potato plants; B: broccoli plants; C: cabbage plants. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

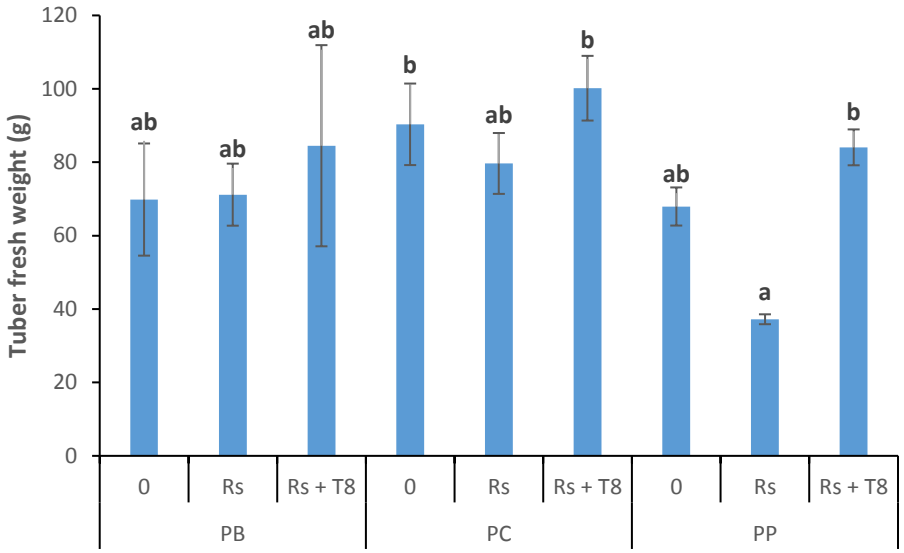

Figure 7-2 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato intercropped with broccoli and cabbage. 0: uninfested soil (plant only); P: potato plants; B: broccoli plants; C: cabbage plants. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The inoculation treatment had a significant effect on shoot and root dry weights of cabbage (Figure 7-3 and Figure 7-4). Cabbage plants in the *R. solani* plus T8 treatment were significantly larger than in the controls or *R. solani* only treatment, which did not differ. Broccoli plants with inoculation treatment had no significant effect on shoot and root dry weights (Figure 7-3 and Figure 7-4).

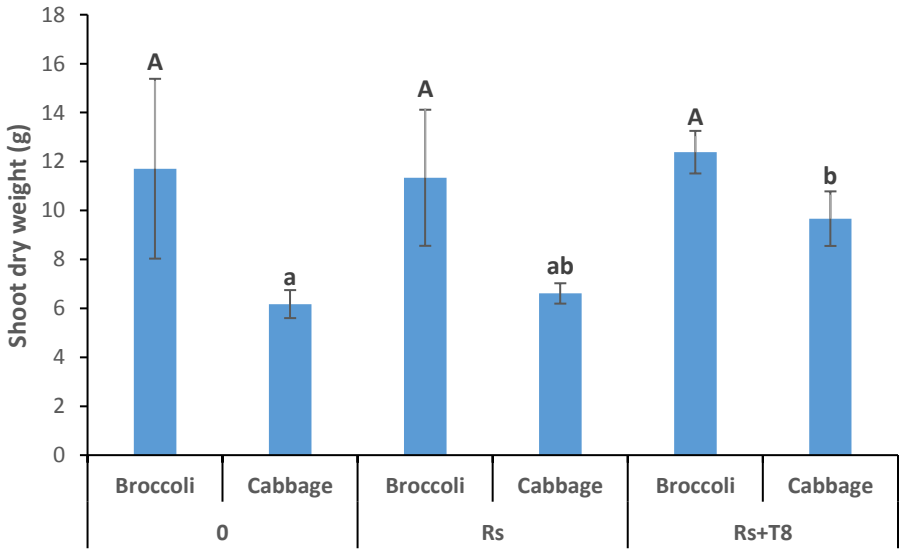

Figure 7-3 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage and broccoli intercropped with potato. 0: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

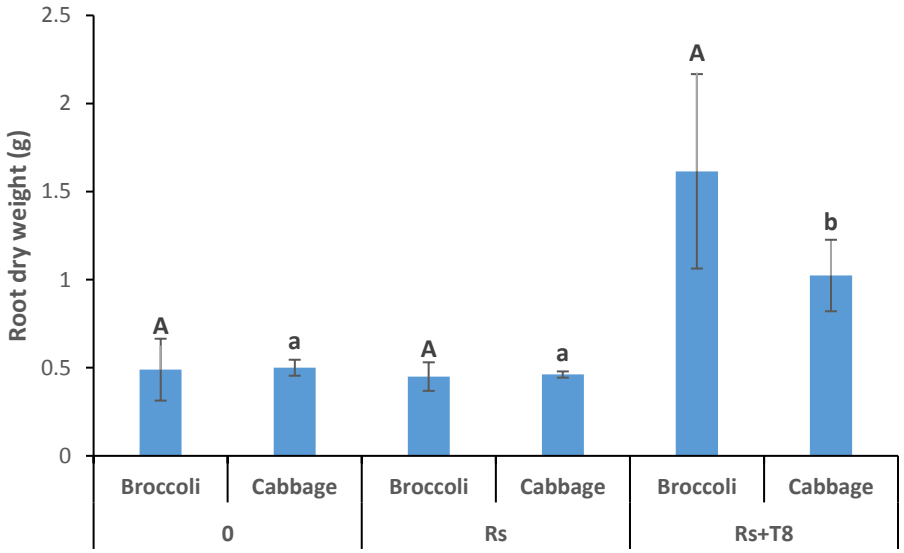

Figure 7-4 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on root dry weight of cabbage and broccoli intercropped with potato. 0: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Because there was little effect of *R. solani* in the experiment, and evidence of competition between potato plants, the crates were re-sown with the same plants at lower density and with additional inoculum of *R. solani*. In the repeat experiment, sclerotia were found only on tubers from the potato monoculture in the *R. solani* only treatment with 13.7 (standard error 1.88) sclerotia per plant. There was no stem canker in any treatment, but the effect of pathogen could be seen through the comparison of growth between treatments and control (pathogen only). There were significant main effects of inoculation and intercrop, and a significant interaction between them, for shoot dry weight (Figure 7-5) and root dry weight (Table 7-1). Potato plants were significantly smaller in the monoculture treatment than in either of the intercrop treatments. Inoculation with *R. solani* only reduced the shoot dry weight in the monoculture, but not in either the broccoli or cabbage intercrop treatments. Inoculation with T8 as well as *R. solani* increased the shoot and root dry weights to significantly above the controls in the monoculture treatment, but had only a small or no effect in the intercrops with broccoli and cabbage. There was a significant main effect of intercrop, and a significant interaction between intercrop and inoculation, on the number of tubers (Table 7-1). There were significantly fewer tubers on potato plants from monoculture than from the intercrops with broccoli or cabbage in the *R. solani* only treatment.

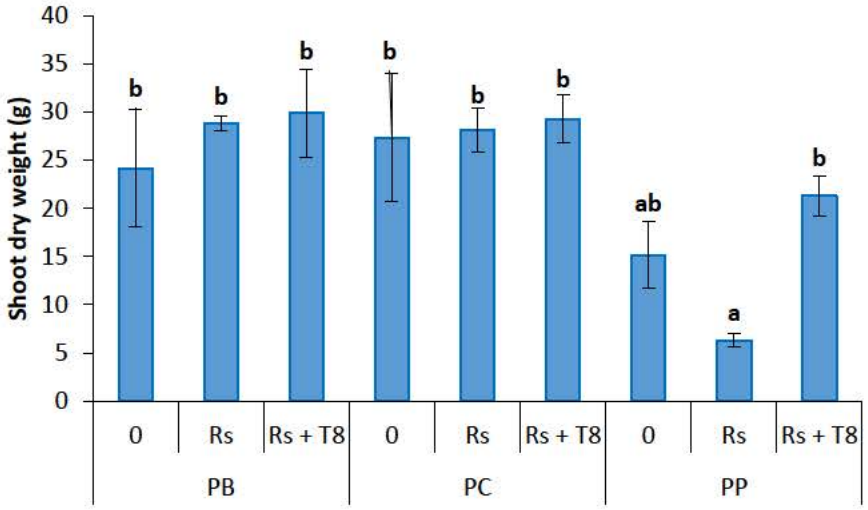

Figure 7-5 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato intercropped with broccoli and cabbage in a repeat assay. 0: uninfested soil (plant only); P: potato plants; B: broccoli plants; C: cabbage plants. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

There was a significant main effect of inoculation, and a significant interaction between inoculation and intercrop, on the tuber fresh weight (Figure 7-6). Tuber fresh weight was halved in the potato monoculture inoculated with *R. solani* only, but there was no significant effect of inoculation with *R. solani* in either broccoli or cabbage intercrop treatments. *T. hamatum* T8 increased the tuber fresh weight of monoculture potato plants inoculated with *R. solani* so that it was not significantly different from the controls, but had no effect in the intercrops with broccoli or cabbage.

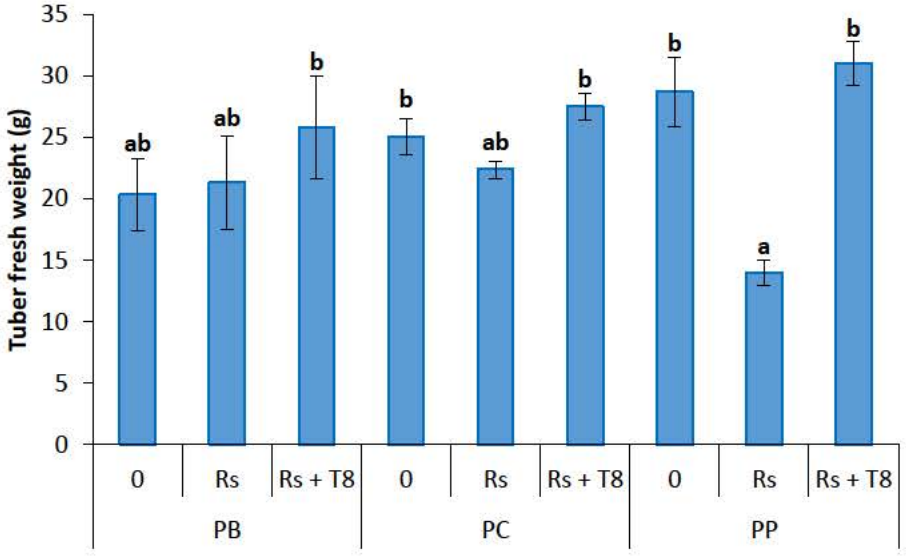

Figure 7-6 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato intercropped with broccoli and cabbage in a repeat assay. 0: uninfested soil (plant only); P: potato plants; B: broccoli plants; C: cabbage plants. Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

There was a significant main effect of inoculation on the shoot and root dry weight of both cabbage and broccoli intercropped with potato (Figure 7-7 and Figure 7-8). Both shoot and root dry weight were increased by inoculation with *R. solani*, and increased again in the *R. solani* plus T8 treatment. Shoot dry weight of cabbage was greater than for broccoli, but there was no significant interaction between inoculation and plant species.

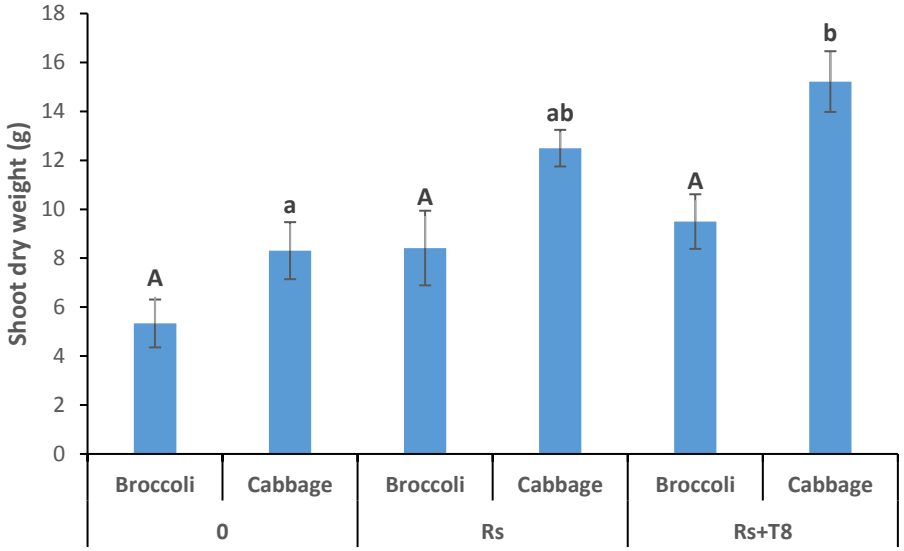

Figure 7-7 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage and broccoli intercropped with potato in a repeat assay. 0: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

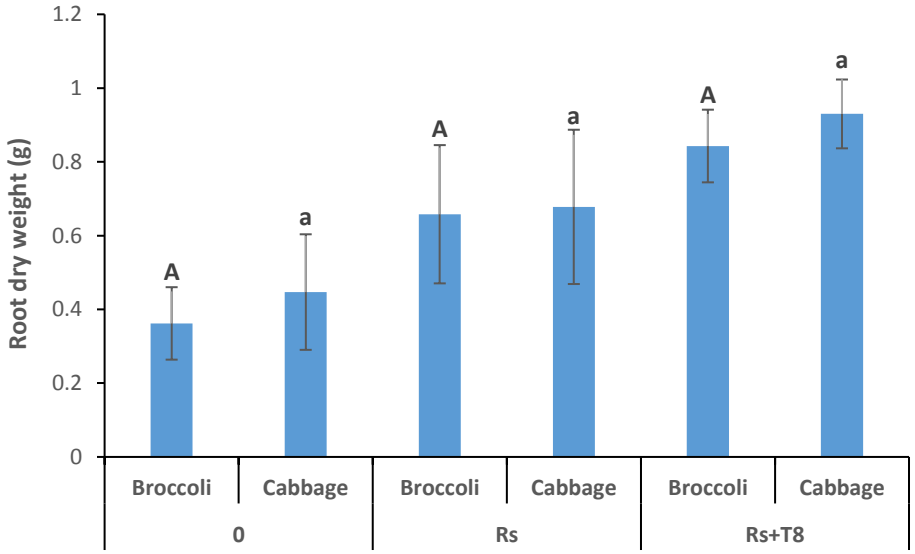

Figure 7-8 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on root dry weight of cabbage and broccoli intercropped with potato in a repeat assay. 0: uninfested soil (plant only). Error bars show standard errors (n=3). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

7.3.2. Field experiment

The typical symptoms of stem canker and black scurf of potato caused by the pathogen were recorded only in the monoculture potato in the *R. solani* only treatment. No stem lesions or sclerotia were seen in uninoculated controls, or in intercrops with cabbage or in plots treated with T8. Because symptoms were only seen in one treatment, which was potato monoculture inoculated with *R. solani* but not with *T. hamatum*, disease data could not be analysed or used to compare the effects of treatments. Stem canker severity occurred in loamy sand soil at 0-10%. There were 12.09 (standard error 1.686) sclerotia per plant, and no stem canker or sclerotia on potatoes intercropped with cabbage, or treated with *T. hamatum* T8 or in uninoculated control.

Generally, the potato plants grew better and yielded more in monoculture than intercrop, and grew better when inoculated with T8. Growth and yield were generally reduced by inoculation with *R. solani*. However, there were many interactions between these factors, including significant pathogen by *Trichoderma* by intercrop interactions for each of shoot dry weight, number of tubers, and tuber fresh weight.

Inoculation with *R. solani* alone decreased the shoot dry weight of potato plants in monoculture by 43%, but did not have a significant effect on dry weight in the potato-cabbage intercrop (Figure 7-9). In the absence of *R. solani*, inoculation with T8 significantly increased the shoot dry weight of potatoes in the intercrop treatment, but not in the monoculture treatment. In the presence of *R. solani*, inoculation with T8 increased shoot dry weight 2-fold in the intercrop treatment and 3-fold in the monoculture treatment (Figure 7-9).

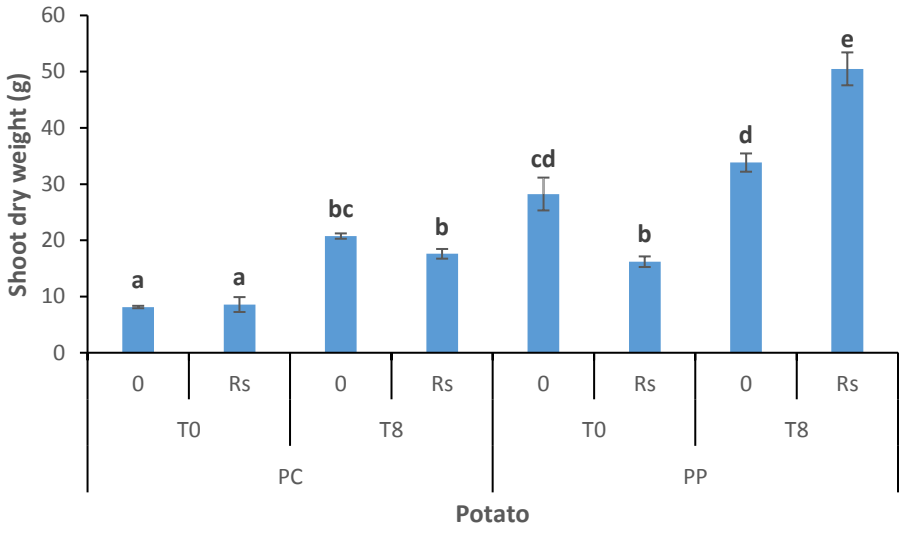

Figure 7-9 Effects of intercropping with cabbage and inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato plants in a field trial. P: potato plants; C: cabbage plants; T0: untreated soil. Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Inoculation with *R. solani* alone significantly reduced the number of tubers in the monoculture treatment, but not in the potato-cabbage intercrop (Figure 7-10). In plots inoculated with T8, *R. solani* did not have a significant effect on the number of tubers in either the monoculture or intercrop treatments. Inoculation with T8 and *R. solani* did significantly increase tuber numbers relative to *R. solani* only without T8 in monoculture, but not intercrop.

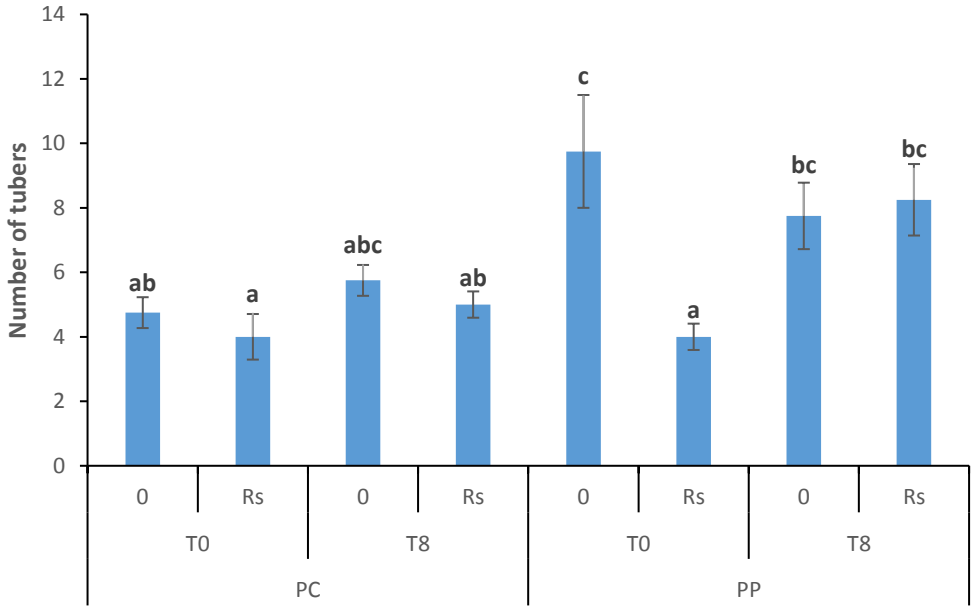

Figure 7-10 Effects of intercropping with cabbage and inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on number of tubers of potato plants in a field trial. P: potato plants; C: cabbage plants; T0: untreated soil. Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

Inoculation with *R. solani* alone reduced the tuber fresh weight by 76% in the potato monoculture, but had no significant effect in the potato-cabbage intercrop (Figure 7-11). In plots inoculated with *R. solani*, T8 increased tuber fresh weight by 5.3 times in the potato monoculture, but did not have a significant effect in the potato-cabbage intercrop.

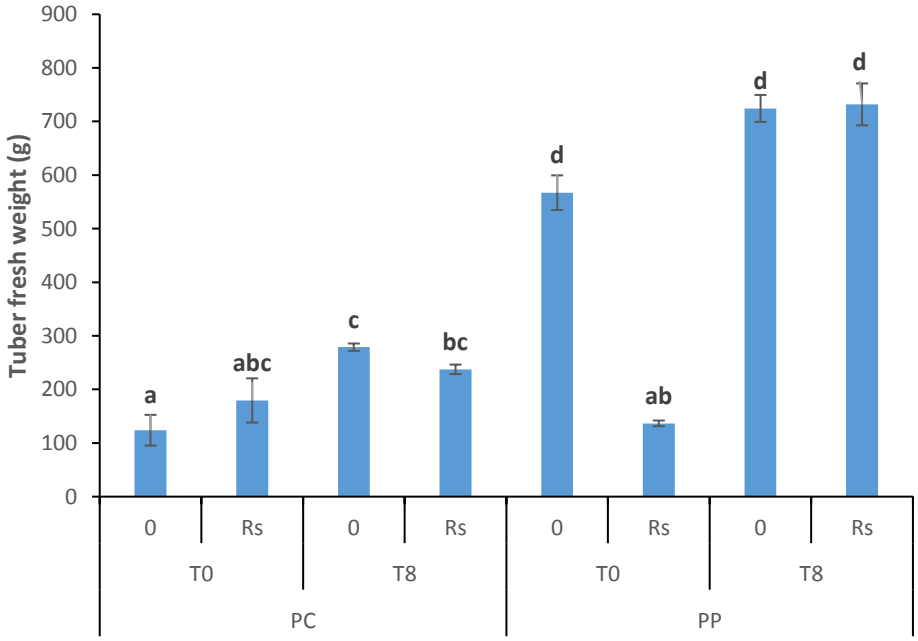

Figure 7-11 Effects of intercropping with cabbage and inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato plants in a field trial. P: potato plants; C: cabbage plants; T0: untreated soil. Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

The shoot dry weight of cabbage plants was significantly increased approximately 6-times by inoculation with T8 (Figure 7-12). There was no significant effect of *R. solani* on shoot dry weight of cabbage, and no significant interaction between T8 and *R. solani*.

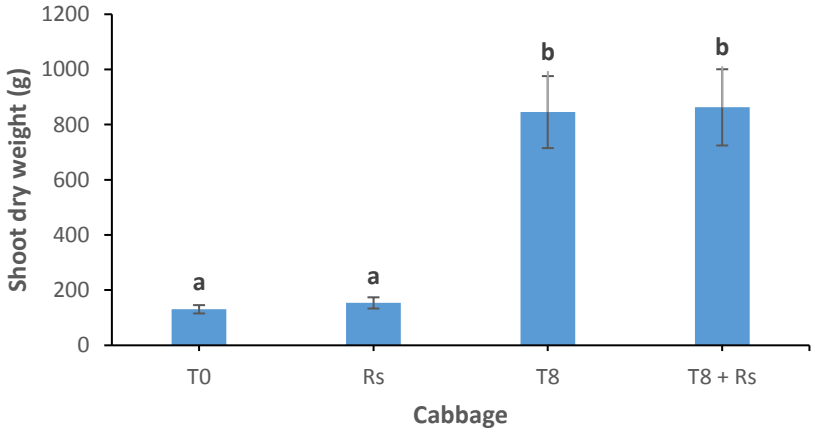

Figure 7-12 Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage intercropped with potato in a field trial. T0: untreated soil. Error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different at P= 0.05 (Tukey test).

7.4. Discussion

This chapter was based on the intercropping between *Brassica* plants and potato plants plus inoculum of *Trichoderma* and *R. solani* AG-3PT in glasshouse and field tests on the management of Rhizoctonia disease of potato. In the previous chapter, the biocontrol agent was more tolerant to the volatile and non-volatile products that were released from *Brassica* plants, which otherwise were toxic to the pathogen. Therefore, it should be tested in the glasshouse and field trials.

In the crate trials, intercropping with broccoli and cabbage greatly reduced or eliminated the symptoms of disease caused by *R. solani* AG-3PT as assessed by the number of sclerotia on tubers and stem canker lesions. This shows that the intercrops suppressed the pathogen. As a result, plant growth and tuber yield were not reduced by *R. solani* in the intercrop treatments, compared with large reductions in growth and yield due to inoculation with the pathogen in the monoculture treatment. The probability is that fungitoxic ITCs might be delivered into the soil from cabbage or broccoli via root exudates that affect the *R. solani* growth; and therefore, protected the potato plants from the effect of *R. solani* AG-3PT (Gimsing et al., 2007; Matthiessen and Kirkegaard, 2006; Motisi et al., 2010). Snapp et al. (2007) found that using roots and shoots of mustard as a cover crop treatment with potato tubers in a field trial reduced mycelial growth of *R. solani* and *P. ultimum* and enhanced healthy root and tubers of potato. Previous work has studied rotations or incorporation of green manure crops. The crate trials have shown for the first time that intercropping with brassicas can also protect potatoes against Rhizoctonia diseases.

In the crate trials, T8 significantly increased the yield in potato monoculture inoculated with *R. solani*. This is similar to results from previous chapters. The mechanism of *T. hamatum* produces many biologically active substances including secondary metabolites and cell wall degrading enzymes (Kumar et al., 2017c; Verma et al., 2007; Vinale et al., 2008) which may inhibit pathogen activity. There was no significant effect of T8 on potato growth and yield in cabbage or broccoli intercrop treatments that had been inoculated with *R. solani*. This is mostly because of the absence of a disease effect in these treatments because of the suppression by the cabbage or broccoli. There was no significant additional growth promotion effect on potato, unlike what had been found in previous chapters. This may have been because of the soil and environmental conditions in the trial. It may be that plants were grown in suboptimal conditions

due to the existence of minor pathogens and even though nutrients and water were not limiting (Rabeendran et al., 2000).

This experiment was repeated with the same treatments to confirm the results, but it was limited to half of the number of plants in each crate. The growth and yield of potato plants in the control monoculture was similar to that in the intercrops, unlike the reduction in growth seen in the first assay. It is probable that there was less competition between the plant roots for nutrients and space compared with the first experiment. The effects of intercropping and *Trichoderma* gave similar results to that seen with first assay.

Cabbage plants were selected as an intercrop with potato in the field experiment based on preliminary tests and glasshouse tests in order to test the hypothesis that biofumigant crops can alter the balance between pathogens and their antagonists. *R. solani* inoculum in the potato monoculture significantly suppressed the biomass and yield of potato, whereas the number of tubers was equal to the potato-cabbage intercrop. As was seen in the present study that the pathogen inoculum in the potato monoculture reduced the number of tubers. This is supported by Van den Brink and Wustman (2014); Wharton et al. (2007) who found that *R. solani* inoculum decreased number of tuber and size in comparison to the untreated control. In the field, as in the crate trial, yield was reduced by *R. solani* in potato monoculture but not in the potato cabbage intercrop, showing that cabbages protected the potatoes from disease in the field. This confirms what was found in the glasshouse experiment. While previous studies indicated that the use of *Brassica* crops as green manures or rotation crops reduced the disease level and promoted plant growth (Bernard et al., 2014; Collins et al., 2006; Larkin and Griffin, 2007).

In the field, potato growth and yield per plant was lower in the intercrop than the monoculture, presumably because of competition between the cabbage and potato. This was not seen in the crate trials, and may have been because the cabbages established faster than the potatoes or space between rows in these plants was narrow in the field or the glucosinolates and ITCs given off from the Brassica roots may have directly inhibited the potato plants to some degree. In a series of experiments in England, Opoku-Ameyaw and Harris (2001) found that in most cases there was evidence of competition between cabbages and potatoes in intercrops of these two species. These workers and Santos et al. (2002) suggested that competition between potato and cabbage or broccoli was mostly due to overlap of canopy, with the species that established its canopy fastest coming to dominate. Competition between brassica and potato needs to be

managed to make intercropping work. Further experiments would be required on time of sowing and variety to coordinate canopy development in the two species. Also, the effect of Brassica roots on potato plants need to be tested.

There was a small growth promotion effect of *T. hamatum* T8 on potatoes in the field, but a larger growth promotion effect on the cabbages. Growth promotion of cabbage could not be tested with the experimental design used in the crate trials, but had been shown in Chapter 6. This result may be because the existence of *T. hamatum* on plant roots can improve the growth of cabbage leaves, as has been shown for *T. viride* on cabbage (Topolovec-Pintaric et al., 2013). It is also possible that T8 inhibited cabbage pathogens that may have been present in the field, as previously reported by Alkooranee et al. (2015); Montfort et al. (2011) who noted that the use of biocontrol *Trichoderma* spp. on *Brassica* crops induced resistance to soilborne pathogens.

The conclusion from these experiments is that either intercropping with brassicas, or biocontrol with *T. hamatum* T8, control *R. solani* disease in the field. There was no additional disease control from using both, because either were highly effective by themselves. However, applying T8 to intercrops had a growth promotion effect, particularly on the cabbage, which is additional to the effect on the disease.

The concept of combining intercropping with *Trichoderma* needs future tests in glasshouse and field trials with these and other *Brassica* crops to determine the best ways to apply it, and to determine if it is economical to utilize. This would include finding ways to reduce the competition effect between cabbages and potatoes. In this experiment, cabbage plants were not harvested until the potatoes were harvested. However, in a commercial situation the cabbages would be harvested first, and the timing of harvest is one thing that needs further investigation. Because T8 had a large promotion effect on cabbage growth, it could allow the cabbages to be harvested earlier which would reduce competition with the potatoes during tuber growth. These experiments also only tested the protective effect of *Brassica* species in the current season, and future work could examine whether their effects on disease last more than one season.

Chapter 8. General discussion

The principal aim of this thesis was to improve biological control agents and natural materials for control of the fungal potato pathogen *R. solani* AG-3PT. This was done by first obtaining biocontrol agent strains of *Trichoderma* isolates that could be used against stem canker and black scurf disease caused by *R. solani* AG-3PT. The interaction between the resistance of potato varieties to Rhizoctonia disease and the effect of biological control agents was tested. Also, fertilizers and intercropping with *Brassica* plants were tested for their impact on potato growing and on antagonism between *Trichoderma* isolates and *R. solani* AG-3PT. The application of *Trichoderma* isolates was tested for the ability to eliminate Rhizoctonia stem canker and black scurf disease of potato plants within five experimental chapters. The severity of symptoms in all chapters was rated as mild or inconsistent, but the effect of *R. solani* AG-3PT on potato plant growth and yield was obviously high. Therefore, the disease systems was observed by comparing growth between treatments and control (pathogen only) in this study. The outcomes of this study illustrated that these techniques eliminated disease symptoms (canker and scurf), and drastically reduce the impact of the pathogen and disease on potato. on the stem and on the tubers. This final chapter presents a synopsis of the key findings, conclusions and recommendations for further studies.

8.1. Key findings

8.1.1. Chapter 3

Trichoderma strains were isolated from healthy tubers and soil from the root zone. The isolates were identified as a species of *Trichoderma* by DNA gene sequencing. Inoculating the tubers with 8 isolates of *Trichoderma* plus *R. solani* protected the potato plants from the pathogen (stem canker and black scurf disease) and enhanced plant growth. However, some isolates (*T. harzianum* T5 and *T. hamatum* T8) showed higher growth promotion effect on the plants, and may also induce systemic resistance. Although visual symptoms were rated as mild, *R. solani* AG-3PT had high effects on growth and yield. Therefore, the emphasis of this study was on the plant growth indicators to compare between treatments and control (pathogen only). Applying *R. solani* AG-3PT as inoculum on wheat grain reduced the shoot and root dry weights, number of stolons, number of tubers and tuber fresh weight.

This work confirmed in Chapter 3 that isolates of *Trichoderma* as biocontrol agents can eliminate the symptoms of canker and scurf of potato plants and at the same time promote plant growth. *T. hamatum* T8 generally gave more growth promotion than *T. harzianum* T5. Hicks et al. (2014) had previously shown that some isolates of *T. virens*, *T. harzianum* and *T. barbatum* from New Zealand soils were more effective at suppression of stem canker disease than other isolates, but that these isolates differed in which aspects of plant growth they promoted. Biocontrol strains can obviously be found in several species of *Trichoderma*, and variations in effectiveness are likely to be due to differences between isolates rather than between species. However, all *Trichoderma* isolates reduced or eliminated the pathogen growth and promoted potato plant growth in pot trials and in a repeated assay with vermiculite which gave the same indicators. Future work should also need to do further research to confirm the results, especially glasshouse trials.

8.1.2. Chapter 4

Inoculating potato varieties with *R. solani* AG-3PT showed different levels of effect of this fungus in the number of sclerotia, and effects on shoots, roots, stolons, tubers and tuber fresh weight, compared with un-inoculated plants. The results of pot tests with potato varieties demonstrated more sclerotia in Desiree and Sebago, compared with Sapphire and Royal Blue varieties, possibly because of the susceptibility of Desiree and Sebago to the pathogen. According to Yanar et al. (2005), some potato varieties had high levels of resistance; and other varieties had different levels of susceptibility to *R. solani* AG-3PT. Also, Wegener and Jansen (2007) reported that different coloured potatoes had different levels of resistance to the soft rot disease caused by *Pectobacterium carotovorum*.

There were small differences in the effects of plant tissues from peel and sprouts of different potato varieties on pathogen growth. *Trichoderma* isolates were more sensitive than *R. solani* to the effects of peel and sprouts of all potato varieties on growth. On the other hand, *Trichoderma* isolates gave higher growth on the extracts from peel than sprouts. Biocontrol agents did not grow on sprouts of Sebago. This could be due to chemical compounds that affect the growth of biocontrol agents. In dual culture, inhibition of the pathogen by *Trichoderma* isolates was greater in the presence of plant tissues from peel than from sprouts. T8 gave higher inhibition of the pathogen than T5 with sprouts of each variety. Although there was a prevention in the effects of the pathogen when using the biocontrol agent with three varieties

of potato, this did not differ between varieties even though there were large differences in resistance. Therefore, there was no interaction between resistance of varieties and biocontrol *Trichoderma*.

The results from this chapter suggest the need for future research on other potato varieties and more investigation of the effects of phenolics and other compounds in the tissues of potato varieties.

8.1.3. Chapter 5

In laboratory studies, the interaction between five different levels of nutrients and biocontrol agents plus pathogen showed different results. For example, in dual culture tests, the concentration of K, Mg, Mn, N or P and *Trichoderma* spp. affected the inhibition of pathogen growth; in contrast, concentrations of Ca or Fe with *Trichoderma* had no effect on inhibition. In antibiotic tests, the interaction between concentration of all nutrients and inhibition of the pathogen by biocontrol agents was significant.

In glasshouse studies, there were generally no significant interactions between nutrients and *Trichoderma* isolates plus *R. solani* inoculum in their effects on plant growth. On the other hand, biocontrol alone or nutrients alone significantly promoted plant growth. According to Rubio et al. (2017), shoot and dry weight of tomato plants were significantly increased by *T. harzianum* when treated with NPK fertilizer, but not in unfertilized plants.

In field studies, results indicate that the interaction between some low nutrient levels and biocontrol could significantly increase potato yield. This trial was repeated with the same treatments (Low K, Low N and NPK standard) to confirm the results, but it was done under glasshouse conditions. The results confirmed results of the field experiment that the interaction of NPK levels and biocontrol significantly increased the shoot dry weight of potato plants. Some of the growth promotion due to *Trichoderma* may have been due to disease control. However, *Trichoderma* species have been shown to increase nutrient use efficiency of plants for nitrogen, phosphorus and potassium (Mehetre and Mukherjee, 2015). This may explain why the *Trichoderma* treatment had similar effects to increasing the level of fertilizer. It is true that an experiment in Chapter 6 suggested that the nutrients in the bran carrier may also contribute to growth promotion, but it was also shown in Chapters 3 and 6 that spore suspensions of T5

and T8 promoted the growth of potato, cabbage and broccoli under conditions where the inoculum would not have supplied extra nutrients.

The activity of *Trichoderma* isolates was generally not adversely influenced by the nutrients. Thus, the application of biocontrol *Trichoderma* may reduce the level of fertilizer required, however more study is required in the future. This could be done by inoculating the tubers with *Trichoderma*, then after germination using a reduced rate of standard NPK fertilizer to give high protection against the pathogen and high potato yields. This kind of biological control is safe for the environment and it could cost less money compared with using fertilizers continuously. It would be a good to expand the study in pot trials and field trials to confirm the results.

8.1.4. Chapter 6

Studies in Chapter 6 provide the basic information on many factors in the laboratory assays required for the intercropping programs in the glasshouse and field trials. Growth of *Brassica* plants (broccoli and cabbage) was not affected by *R. solani* AG-3PT and their growth was promoted by inoculation with *Trichoderma* isolates in glasshouse tests.

Toxic volatile and non-volatile substances released by *Brassica* plant root tissues in Petri-dishes significantly reduced the radial growth of the pathogen and *T. harzianum* T5, compared with leaf and stem tissues. Generally, the growth of *T. hamatum* T8 was reduced less than that of T5. There was no growth of the pathogen with both volatile and non-volatile substances from root tissues of cabbage. Cabbage roots have high amounts of GSL (glucoerucin and gluconasrturtiin) and produce high amounts of ITCs compared with plants like broccoli and cauliflower (Bhandari et al., 2015). In dual culture, the combination of biocontrol agents with volatiles from root tissues of *Brassica* plants strongly inhibited the growth of the pathogen, compared with non-volatile substances which showed nearly the same rate of inhibition in all *Brassica* plant tissues. Data also showed that root tissues of *Brassica* and biocontrol *Trichoderma* are compatible and could interact for managing the stem canker disease.

Trichoderma biomass production increased on all *Brassica* plant tissues, while these tissues inhibited the growth of the pathogen, particularly root tissues which had higher inhibition of *R. solani* fungal biomass. Biocontrol agents were more tolerant than the pathogen

to the volatile or non-volatile compounds of *Brassica* crops in different tests (radial growth, dual culture and mycelial biomass growth).

This work was continued in the next chapter about the intercropping between *Brassica* plants and potato with inoculation of biocontrol in the glasshouse tests initially and then field trial.

8.1.5. Chapter 7

In a glasshouse trial either *T. hamatum* T8 or intercropping with cabbage or broccoli greatly inhibited expression of disease symptoms and prevented reduction in growth and yield caused by the pathogen. This protection is related to the isothiocyanates (ITCs) which are released into soil (Bhandari et al., 2015; Hanschen et al., 2015; Sarwar et al., 1998) in addition to biocontrol which is also responsible to control soilborne pathogens (Bastakoti et al., 2017; Hicks et al., 2014). This was confirmed in a field trial with cabbage. There are several studies for incorporation of *Brassica* plant tissues before cropping with potato plants in glasshouse and field experiments (Motisi et al., 2009; Njoroge et al., 2008; Taylor, 2013). Each study has its pros and cons. In this study, this is the first time that intercropping between cabbage plants and potato plants plus biocontrol has been tested in the field trial to manage Rhizoctonia stem canker and black scurf disease caused by *R. solani* AG-3PT. Combining T8 and intercropping did not give better disease control than either T8 or intercropping alone, but did give growth promotion of the cabbage. The result of this trial showed that cabbage plants protected the potato plants from the pathogenic disease. However, they also reduced the potato plant growth compared with potato treatment only. This could be because the early growth of the cabbage plants was faster than the potato or row spacing was narrow which led to competition for nutrients and space.

If cabbage-potato intercropping is to be used, further work is needed to test varieties and time of sowing to reduce competition but also to ensure that harvest maturity was coordinated so that the cabbages could be harvested at a time that was compatible with the growth and harvest of the potatoes. Also, future work could be repeated a field trial to confirm the results with more replicates and more row spacing, and also find a suitable machine to sow the brassica seedlings and potato tubers. Then, the possibility to apply this intercropping system between cabbage and potato tubers widely.

8.2. Conclusion

The findings of each of Chapter 3, 4, 5, 6 and 7 supported the goals of this study. In conclusion, *T. hamatum* T8 prevented the pathogen growth and promoted plant growth and this supports the aim that antagonistic strains of *Trichoderma* that can inhibit the activity of the pathogen. Potato varieties had different levels of resistance to the pathogen and tolerance to the biocontrol. The effects of *Trichoderma* on disease and growth promotion were the same for all varieties. Therefore, there was no interaction between biocontrol and potato variety resistance. This did not support the hypothesis that resistance could alter the relative effect of biocontrol. However, it does show that applying biocontrol agents is effective on all varieties. The effect of biocontrol was not altered by fertilizer application in the pot trials. Application of T8 reduced the need for fertilizer in the field trials. Therefore, there was no interaction between fertilizer level and the effect of biocontrol on disease. However, the growth promotion activity of *Trichoderma* species could enable fertilizers to be used more efficiently. The interaction between *Brassica* plant tissues and biocontrol reduced the pathogen growth in the laboratory, and intercropping between *Brassica* plants and potato plants protected the potato plants from Rhizoctonia stem canker and black scurf disease in the glasshouse and field trials. Rhizoctonia diseases can be managed by using either intercropping, or *Trichoderma* alone. However, there was no advantage regarding Rhizoctonia disease to using both at the same time, but there may be a growth promotion advantage to the intercropped species due to the presence of the *Trichoderma*. To conclude, the use of *Trichoderma* isolates alone or in combination with potato varieties or fertilizers or intercropping systems with *Brassica* plants can control the stem canker and black scurf disease caused by *R. solani* AG-3PT and at the same time promote potato plants growth.

8.3. Directions for future research

From the results in this thesis *T. hamatum* T8 was the best isolate for antagonism of *R. solani* AG-3PT and enhancing the plant growth and potato yields. Further study is required to develop it as a commercial product in the markets for use as a biofungicide, plant growth promoter and soil amendment. For example, a new formulation: concentration and stability, application strategy, the time of application, optimum inoculum dose and potential for using mixed *Trichoderma* isolates. Also, future work to determine the mechanisms of action, for example to confirm induced resistance, for example biochemistry or gene signalling in

response to *Trichoderma* inoculation. The promotion of plant growth by *Trichoderma* is difficult to separate from the effect of *Trichoderma* on disease. *Trichoderma* isolates also need to be tested under conditions of higher inoculum and disease levels.

Moreover, more studies are needed on potato varieties to investigate the mechanisms of resistance to the pathogen, and tolerance to the biocontrol agents. Further work is needed to study the content of bioactive phenols, anthocyanin and other compounds in tuber tissue for each variety, for example the extent of protective role of anthocyanin and phenols in Rhizoctonia disease and other soilborne pathogens on potato, and also for possible health effects in human diets.

Modifying of nutrients individually in hydroponic solution had no interaction with biocontrol agents when they were used by spraying/irrigating the soil surface in each pot. It would be useful to find a way that can interact between biocontrol agent and the nutrient concentrations for controlling Rhizoctonia disease and high growth promotion. Also, future work needs to be focused to use different levels of NPK as spread fertilizers on soil to see if application to the plant has more effect on growth than spraying and on the interaction with biocontrol agents. This may promote plant growth highly and interactions between *Trichoderma* and fertilizers to control the pathogen growth.

It will be good to study other *Brassica* crops such as mustard, radish, kale and Chinese cabbage for use in intercropping systems with potato plants, and what the effects on the pathogen and non-pathogen of their volatile and non-volatile compounds are. In addition, future work should be considered which incorporates the cabbage roots into soil, where potato tubers are planted at different times to ensure the activity of isothiocyanates in soil against soilborne pathogens such as *R. solani*. It would be a good idea to study the residues of cabbage roots in the field after harvesting the tops. That could give an opportunity to understand how long the glucosinolates can be effective against *R. solani* and also other soilborne pathogens. Also, study of the amount of ITCs in different stages of plant growth to see which stage of growth has high concentration of ITCs either in laboratory or glasshouse or field trials, and water management strategies to maximise ITCs release are warranted. Future work in a comprehensive study for testing more resistant potato varieties with intercropping brassica crops in the glasshouse and field trials to see if intercropping is more efficient to control soilborne pathogens. Moreover, further work is needed to find a standard and optimised procedure so that farmers can have more information about the best brassica species and

varieties, reliable growing methods, and suitable cultivation procedures in the wide field as intercropping with potato tubers.

Further work should also focus on isolates of *R. solani* from other anastomosis groups (AG) that are associated with stem canker and black scurf of potato by using IPM strategies for managing of Rhizoctonia diseases.

8.4. Concluding remark

If farmers are informed about these results and support them by adopting these strategies, whilst consumers are taught to understand not to be continually anticipating perfect produce, then alternative strategies and tactics of disease control might be greatly adopted so that we can start to create a sustainable agricultural future.

References

Abd-El-Khair, H. , Khalifa, R. K. M. and Haggag, K. 2010. Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. *Journal of American Science 6*(9), 486-497.

Abro, M. A. , Lecompte, F. , Bardin, M. and Nicot, P. C. 2014. Nitrogen fertilization impacts biocontrol of tomato gray mold. *Agronomy for Sustainable Development, 34*(3), 641-648. doi: 10.1007/s13593-013-0168-3

Ahmad, I. and Shah, M. H. 2012. *Trichoderma* and intercropping impact production, quality and corm rot disease of gladiolus grandiflorus. *Floriculture and Ornamental Biotechnology, 7*(1), 86-93.

Alamri, S. , Mostafa, Y. S. , Hashem, M. and Alrumman, S. 2016. Enhancing the biocontrol efficiency of *Trichoderma harzianum* jf419706 through cell wall degrading enzyme production. *International Journal of Agriculture and Biology, 18*(4), 765-772. doi: 10.17957/IJAB/15.0164

Alkooranee, J. T. , Aledan, T. R. , Xiang, J. , Lu, G. and Li, M. 2015. Induced systemic resistance in two genotypes of *Brassica napus* (aacc) and *Raphanus oleracea* (rrcc) by *Trichoderma* isolates against *Sclerotinia sclerotiorum*. *American Journal of Plant Sciences, 6*(10), 1662. doi: 10.4236/ajps.2015.610166

Altomare, C. , Norvell, W. , Björkman, T. and Harman, G. 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied and Environmental Microbiology, 65*(7), 2926-2933.

Anke, H. , Kinn, J. , Bergquist, K.-E. and Sterner, O. 1991. Production of siderophores by strains of the genus *Trichoderma*. *Biology of Metals, 4*(3), 176-180.

Aparna, K. and Girija, V. 2018. Effect of biofumigation with plant extracts on mycelial growth and sclerotial germination of *Rhizoctonia solani* causing collar rot and web blight of cowpea. *International Journal Current Microbiology Applied Sciences, 7*(3), 2990-2999. doi: org/10.20546/ijcmas.2018.703.346

Arora, D. and Dwivedi, R. 1980. Mycoparasitism of *Fusarium* spp. on *Rhizoctonia solani* Kühn. *Plant and Soil, 55*(1), 43-53.

Atanasova, L. , Jaklitsch, W. M. , Komoń-Zelazowska, M. , Kubicek, C. P. and Druzhinina, I. S. 2010. Clonal species *Trichoderma parareesei* sp. nov. likely resembles the ancestor of the cellulase producer *Hypocrea jecorina*/*T. reesei*. *Applied and Environmental Microbiology, 76*(21), 7259-7267. doi: 10.1128/AEM.01184-10

Atiq, M. , Karamat, A. , Sahi, S. T. , Habib, A. , Nawaz, A. and Hussain, W. 2013. Screening of potato varieties/cultivars against black scurf disease of potato. *Pakistan Journal of Phytopathology, 25*(1), 48-51.

Atkinson, D. , Thornton, M. K. and Miller, J. S. 2010. Development of *Rhizoctonia solani* on stems, stolons and tubers of potatoes. Effect of inoculum source. *American Journal of Potato Research, 87*(4), 374-381. doi: 10.1007/s12230-010-9143-6

Baijnath, S. (2012). *Effect of inoculum source, inoculum pressure and cultivar on development of black scurf on potatoes in South Africa.* (MSc), Citeseer.

Bailey, B. A. and Lumsden, R. D. (1998). Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In G. E. Harman & Kubicek, C. P. (Eds.), *Trichoderma & Gliocladium* (Vol. 2, pp. 185-200). Boca Raton CRC Press

Bailey, B. A. and Melnick, R. L. (2013). The endophytic *Trichoderma*. In B. A. H. P. K. Mukherjee, U. S. Singh, M. Mukherjee, and M. Schmoll (Ed.), *Trichoderma: Biology and Applications. CABI* (pp. 152-172). UK.

Bailey, K. and Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research, 72*(2), 169-180. doi: 10.1016/S0167-1987(03)00086-2

Bains, P. , Bennypaul, H. , Lynch, D. , Kawchuk, L. and Schaupmeyer, C. 2002. Rhizoctonia disease of potatoes *Rhizoctonia solani* fungicidal efficacy and cultivar susceptibility. *American Journal of Potato Research, 79*(2), 99-106. doi: 10.1007/BF02881518

Baker, R. 1988. *Trichoderma* spp. as plant-growth stimulants. *Critical Reviews in Biotechnology, 7*(2), 97-106. doi: 10.3109/07388558809150724

Balali, G. , Neate, S. , Scott, E. , Whisson, D. and Wicks, T. 1995. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. *Plant Pathology, 44*(6), 1050-1057. doi: 10.1111/j.1365-3059.1995.tb02664.x

Banani, H. , Roatti, B. , Ezzahi, B. , Giovannini, O. , Gessler, G. , Pertot, I. and Perazzolli, M. 2014. Characterization of resistance mechanisms activated by *Trichoderma harzianum* T39 and benzothiadiazole to downy mildew in different grapevine cultivars. *Plant Pathology, 63*(2), 334-343. doi: 10.1111/ppa.12 089

Barak, R. , Elad, Y. , Mirelman, D. and Chet, I. 1985. Lectins: a possible basis for specific recognition in the interaction of *Trichoderma* and *Sclerotium rolfsii*. *Phytopathology, 75*(4), 458-462.

Barak, R. and Sneh, B. 2001. Biological control of black scurf on potato under organic management. *Crop Protection, 20*(2), 145-150. doi: 10.1016/S0261-2194(00)00124-1

Bassi, D. , Menossi, M. and Mattiello, L. 2018. Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Scientific Reports, 8*(1), 2327.

Bastakoti, S. , Belbase, S. , Manandhar, S. and Arjyal, C. 2017. *Trichoderma* species as biocontrol agent against soilborne fungal pathogens. *Nepal Journal of Biotechnology, 5*(1), 39-45. doi: 10.3126/njb.v5i1.18492

Beagle-Ristaino, J. and Papavizas, G. 1985. Biological control of Rhizoctonia stem canker and black scurf of potato. *Phytopathology, 75*(5), 560-564.

Benitez, T. , Rincon, A. , Limón, M. and Codón, A. 2004. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology, 7*(4), 249-260.

Benson, D. M. and Baker, R. 1970. Rhizosphere competition in model soil systems. *Phytopathology, 60*(7), 1058-1061.

Berg, G. , Krechel, A. , Ditz, M. , Sikora, R. A. , Ulrich, A. and Hallmann, J. 2005. Endophytic and ectophytic potato associated bacterial communities differ in structure and antagonistic

function against plant pathogenic fungi. *Fems Microbiology Ecology, 51*(2), 215-229. doi: 10.1016/j.femsec.2004.08.006

Bernard, E. , Larkin, R. P. , Tavantzis, S. , Erich, M. S. , Alyokhin, A. and Gross, S. D. 2014. Rapeseed rotation, compost and biocontrol amendments reduce soilborne diseases and increase tuber yield in organic and conventional potato production systems. *Plant and Soil, 374*(1-2), 611-627. doi: 0.1007/s11104-013-1909-4

Bhandari, S. R. , Jo, J. S. and Lee, J. G. 2015. Comparison of glucosinolate profiles in different tissues of nine *Brassica* crops. *Molecules, 20*(9), 15827-15841. doi: 10.3390/molecules200915827

Bienkowski, D. (2012). *Biological control of Rhizoctonia diseases of potato.* (PhD), Lincoln University.

Blok, W. J. , Lamers, J. G. , Termorshuizen, A. J. and Bollen, G. J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology, 90*(3), 253-259. doi: 10.1094/PHYTO.2000.90.3.253

Booth, E. J. , Walker, K. C. and Sutherland, K. G. 2002. Natural bio-fumigation to control *Rhizoctoni*a in potatoes. *GCIRC Bulletin 19*, 4.

Borrero, C. , Trillas, M. , Delgado, A. and Avilés, M. 2012. Effect of ammonium/nitrate ratio in nutrient solution on control of Fusarium wilt of tomato by *Trichoderma asperellum* T34. *Plant Pathology, 61*(1), 132-139. doi: 10.1111/j.1365-3059.2011.02490.x

Brewer, M. T. and Larkin, R. P. 2005. Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. *Crop Protection, 24*(11), 939-950. doi: 10.1016/j.cropro.2005.01.012

Brierley, J. L. , Stewart, J. A. and Lees, A. K. 2009. Quantifying potato pathogen DNA in soil. *Applied Soil Ecology, 41*(2), 234-238. doi: DOI: 10.1016/j.apsoil.2008.11.004

Budge, G. , Shaw, M. , Lambourne, C. , Jennings, P. , Clayburn, R. , Boonham, N. and McPherson, M. 2009. Characterization and origin of infection of *Rhizoctonia solani* associated with *Brassica oleracea* crops in the UK. *Plant Pathology, 58*(6), 1059-1070. doi: 10.1111/j.1365-3059.2009.02138.x

Buskila, Y. , Tsror, L. , Sharon, M. , Teper-B., P. , Holczer-Erlich, O. , Warshavsky, S. , Ginzberg, I. , Burdman, S. and Eshel, D. 2011. Postharvest dark skin spots in potato tubers are an oversuberization response to *Rhizoctonia solani* infection. *Phytopathology, 101*(4), 436-444. doi: org/10.1094/PHYTO-09-10-0251

Bussan, A. J. , Mitchell, P. D. , Copas, M. E. and Drilias, M. J. 2007. Evaluation of the effect of density on potato yield and tuber size distribution. *Crop Science, 47*(6), 2462-2472.

Campion, C. , Chatot, C. , Perraton, B. and Andrivon, D. 2003. Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. *European Journal of Plant Pathology, 109*(9), 983-992. doi: 10.1023/B:EJPP.0000003829.83671.8f

Carisse, O. , El-Bassam, S. and Benhamou, N. 2001. Effect of *Microsphaeropsis* sp. strain P130A on germination and production of sclerotia of *Rhizoctonia solani* and interaction between the antagonist and the pathogen. *Phytopathology, 91*(8), 782-791. doi: 10.1094/PHYTO.2001.91.8.782

Carling, D. , Baird, R. , Gitaitis, R. , Brainard, K. and Kuninaga, S. 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology, 92*(8), 893-899. doi: 10.1094/PHYTO.2002.92.8.893

Carling, D. and Leiner, R. 1990. Effect of temperature on virulence of *Rhizoctonia solani* and other *Rhizoctonia* on potato. *Phytopathology, 80*(10), 930-934. doi: 10.1094/Phyto-80-930

Castagno, L. N. , Estrella, M. J. , Sannazzaro, A. I. , Grassano, A. E. and Ruiz, O. A. 2011. Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). *Journal of Applied Microbiology, 110*(5), 1151-1165. doi: 10.1111/j.1365-2672.2011.04968.x

Chand, T. and Logan, C. 1983. Cultural and pathogenic variation in potato isolates of *Rhizoctonia solani* in Northern Ireland. *Transactions of the British Mycological Society, 81*(3), 585-589. doi: org/10.1016/S0007-1536(83)80129-6

Chand, T. and Logan, C. 1984. Antagonists and parasites of *Rhizoctonia solani* and their efficacy in reducing stem canker of potato under controlled conditions. *Transactions of the British Mycological Society, 83*(1), 107-112. doi: 10.1016/S0007-1536(84)80250-8

Chang, D. C. and Lee, Y. B. 2016. Response of potatoes to different nutrient solution management in a closed hydroponic system. *Journal of Plant Nutrition, 39*(11), 1547-1555. doi: org/10.1080/01904167.2015.1109120

Chang, Y.-C. , Chang, Y.-C. , Baker, R. , Kleifeld, O. and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Disease, 70*(2), 145-148.

Charron, C. S. and Sams, C. E. 1999. Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by shredded leaves of *Brassica* species. *Journal of the American Society for Horticultural Science, 124*(5), 462-467.

Chaudhary, A. , Naz, F. , Ahmad, I. , Haque, I. and Riaz, A. 2015. Management of black scurf of potato with effective microbes (EM), biological potassium fertilizer (BPF) and *Trichoderma harzianum*. *International Journal of Agriculture and Biology, 17*(3). doi: 10.17957/IJAB/17.3.14.019

Chen, L. , Cui, Y. , Yang, X. , Zhao, D. and Shen, Q. 2012. An antifungal compound from *Trichoderma harzianum* SQR-T037 effectively controls Fusarium wilt of cucumber in continuously cropped soil. *Australasian Plant Pathology, 41*(3), 239-245. doi: 10.1007/s13313-012-0119-5

Chet, I. and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathology, 71*, 286-290.

Chet, I. , Harman, G. and Baker, R. 1981. *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microbial Ecology, 7*(1), 29-38.

Chet, I. and Inbar, J. 1994. Biological control of fungal pathogens. *Applied Biochemistry and Biotechnology, 48*(1), 37-43.

Chet, I. , Inbar, J. and Hadar, I. 1997. Fungal antagonists and mycoparasites. *The Mycota IV: Environmental and Microbial Relationships. Springer-Verlag, Berlin*, 165-184.

Chinta, Y. D. , Eguchi, Y. , Widiastuti, A. , Shinohara, M. and Sato, T. 2015. Organic hydroponics induces systemic resistance against the air-borne pathogen, *Botrytis cinerea* (gray mould). *Journal of Plant Interactions, 10*(1), 243-251. doi: org/10.1080/17429145.2015.1068959

Chow, Y. Y. , Rahman, S. and Ting, A. S. Y. 2018. Interaction dynamics between endophytic biocontrol agents and pathogen in the host plant studied via quantitative real-time polymerase chain reaction (qPCR) approach. *Biological Control, 125*, 44-49. doi: org/10.1016/j.biocontrol.2018.06.010

Clouston, A. , Hill, R. , Minchin, R. , Braithwaite, M. and Stewart, A. 2010. A bioassay screening *Trichoderma* isolates for enhancement of root development in *Impatiens walleriana* cuttings. *New Zealand Plant Protection, 63*, 33-38.

Cochran, K. A. and Rothrock, C. S. 2015. Brassica green manure amendments for management of *Rhizoctonia solani* in two annual ornamental crops in the field. *HortScience, 50*(4), 555-558.

Cohen, M. , Yamasaki, H. and Mazzola, M. 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of Rhizoctonia root rot. *Soil Biology and Biochemistry, 37*(7), 1215-1227. doi: 10.1016/j.soilbio.2004.11.027

Collins, H. , Alva, A. , Boydston, R. , Cochran, R. , Hamm, P. , McGuire, A. and Riga, E. 2006. Soil microbial, fungal, and nematode responses to soil fumigation and cover crops under potato production. *Biology and Fertility of Soils, 42*(3), 247-257. doi: 10.1007/s00374-005-0022-0

Contreras-Cornejo, H. A. , Macías-Rodríguez, L. , del-Val, E. and Larsen, J. 2016. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *Fems Microbiology Ecology, 92*(4), fiw036. doi: https://doi.org/10.1093/femsec/fiw036

Cook, R. J. 1986. Plant health and the sustainability of agriculture, with special reference to disease control by beneficial microorganisms. *Biological Agriculture & Horticulture, 3*(2-3), 211-232. doi: 10.1080/01448765.1986.9754472

Cook, R. J. and Baker, K. F. 1983. *The nature and practice of biological control of plant pathogens*. St. Paul, USA American Phytopathological Society.

Cooke, B. M. (2006). Disease assessment and yield loss. In B. Cooke, Jones, D., Kaye, B. (Ed.), (pp. 43-80). Netherlands: The Epidemiology of Plant Diseases.

Cripps-Guazzone, N. (2014). *Rhizosphere competence of selected Trichoderma species.* (PhD), Lincoln University.

Crozier, C. , Creamer, N. G. and Cubeta, M. 2000. Fertilizer management impacts on stand establishment, disease, and yield of Irish potato. *Potato Research, 43*(1), 49-59.

Cruz, R. and Russo, V. 1985. Inorganic nutrition of *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*. *Mycopathologia, 89*(2), 123-126.

Cumagun, C. 2012. Managing plant diseases and promoting sustainability and productivity with *Trichoderma*: the Philippine experience. *Journal of Agricultural Science and Technology, 14*(4), 699-714.

Cumagun, C. and Ilag, L. 1997. Enhancing the efficacy of *Trichoderma harzianum* Rifai by chitin amendment against sheath blight of rice. *Phillipine Phytopathology 33*, 72-86.

Czajkowski, R. , Perombelon, M. C. , van Veen, J. A. and van der Wolf, J. M. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species a review. *Plant Pathology, 60*(6), 999-1013. doi: 10.1111/j.1365-3059.2011.02470.x

da Silva, J. C. , Torres, D. B. , Lustosa, D. C. , de Filippi, M. C. C. and da Silva, G. B. 2012. Rice sheath blight biocontrol and growth promotion by *Trichoderma* isolates from the Amazon. *Revista de Ciências Agrárias/Amazonian Journal of Agricultural and Environmental Sciences, 55*(4), 243-250. doi: 10.4322/rca.2012.078

Daami-Remadi, M. , Zammouri, S. and El Mahjoub, M. 2008a. Effect of the level of seed tuber infection by *Rhizoctonia solani* at planting on potato growth and disease severity. *The African Journal of Plant Science and Biotechnology, 2*(1), 34-38.

Daami-Remadi, M. , Zammouri, S. and El-Mahjoub, M. 2008b. Relative susceptibility of nine potato (*Solanum tuberosum* L.) cultivars to artificial and natural infection by *Rhizoctonia solani* as measured by stem canker severity, black scurf and plant growth. *The African Journal of Plant Science and Biotechnology, 2*, 57-66.

Dababat, A. , Selim, M. , Saleh, A. and Sikora, R. 2008. Influence of Fusarium wilt resistant tomato cultivars on root colonization of the mutualistic endophyte *Fusarium oxysporum* strain 162 and its biological control efficacy toward the root-knot nematode *Meloidogyne incognita*. *Journal of Plant Diseases and Protection, 115*(6), 273-278.

Dahnke, W. , Nelson, D. , Swenson, L. , Johnson, A. and Thoreson, M. 1989. Time and method of fertilizer application for potatoes. *North Dakota Farm Research-North Dakota, Agricultural Experiment Station (USA)*.

Das, S. , Shah, F. , Butler, R. , Falloon, R. , Stewart, A. , Raikar, S. and Pitman, A. 2014. Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. *Plant Pathology, 63*(3), 651-666. doi: 10.1111/ppa.12139

Davis, J. , Huisman, O. , Everson, D. and Schneider, A. 2001. Verticillium wilt of potato: a model of key factors related to disease severity and tuber yield in southeastern Idaho. *American Journal of Potato Research, 78*(4), 291-300.

Daxenbichler, M. E. , Spencer, G. F. , Carlson, D. G. , Rose, G. B. , Brinker, A. M. and Powell, R. G. 1991. Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry, 30*(8), 2623-2638. doi: org/10.1016/0031-9422(91)85112-D

Dechassa, N. and Schenk, M. K. 2004. Exudation of organic anions by roots of cabbage, carrot, and potato as influenced by environmental factors and plant age. *Journal of Plant Nutrition and Soil Science, 167*(5), 623-629. doi: 10.1002/jpln.200420424

Dekker, J. 1976. Acquired resistance to fungicides. *Annual Review of Phytopathology, 14*(1), 405-428.

Demirci, E. , Eken, C. and Dane, E. 2009. Biological control of *Rhizoctonia solani* on potato by *Verticillium biguttatum*. *African Journal of Biotechnology, 8*(11), 2503-2507.

Deng, Z. and Cao, L. 2017. Fungal endophytes and their interactions with plants in phytoremediation: a review. *Chemosphere, 168*, 1100-1106.

Devi, R. N. , Sinha, B. and Devi, P. S. 2017. Native *Trichoderma* for the management of wire stem of mustard (*Brassica* spp) caused by *Rhizoctonia solani*. *International Journal Current Microbiology Applied Sciences, 6*(9), 2319-2328. doi: org/10.20546/ijcmas.2017.609.284

Dhingra, O. D. and Sinclair, J. B. (1985). Basic plant pathology methods (pp. 261). Florida: CRC Press, Boca Raton, Florida.

Dijst, G. (1989). *The effect of chemical haulm destruction and haulm pulling on potato black scurf caused by Rhizoctonia solani AG-3.* (PhD), Agriculture University, Wageningen.

Djébali, N. and Belhassen, T. 2010. Field study of the relative susceptibility of eleven potato (*Solanum tuberosum* L.) varieties and the efficacy of two fungicides against *Rhizoctonia solani* attack. *Crop Protection, 29*(9), 998-1002. doi: org/10.1016/j.cropro.2010.06.012

Domínguez, S. , Rubio, M. B. , Cardoza, R. E. , Gutiérrez, S. , Nicolás, C. , Bettiol, W. , Hermosa, R. and Monte, E. 2016. Nitrogen metabolism and growth enhancement in tomato plants challenged with *Trichoderma harzianum* expressing the *Aspergillus nidulans* acetamidase amdS gene. *Frontiers in Microbiology, 7*, 1182. doi: 10.3389/fmicb.2016.01182

Donzelli, B. G. G. and Harman, G. E. 2001. Interaction of ammonium, glucose, and chitin regulates the expression of cell wall-degrading enzymes in*Trichoderma atroviride* strain P1. *Applied and Environmental Microbiology, 67*(12), 5643-5647. doi: 10.1128/AEM.67.12.5643–5647.2001

dos Reis Almeida, F. B. , Cerqueira, F. M. , do Nascimento Silva, R. , Ulhoa, C. J. and Lima, A. L. 2007. Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letters, 29*(8), 1189-1193. doi: 10.1007/s10529-007-9372-z

Duffy, B. K. and Défago, G. 1997. Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology, 87*(12), 1250-1257. doi: org/10.1094/PHYTO.1997.87.12.1250

Durak, E. D. (2016). *Biological control of Rhizoctonia solani on potato by using indigenous Trichoderma spp.* Paper presented at the AIP Conference Proceedings.

Eisendle, M. , Oberegger, H. , Buttinger, R. , Illmer, P. and Haas, H. 2004. Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH regulatory system in *Aspergillus nidulans*. *Eukaryotic Cell, 3*(2), 561-563. doi: 10.1128/EC.3.2.561-563.2004

El-Aziz, A. R. A. , Mahmoud, M. A. , Al-Othman, M. R. , El-sherif, E. M. and El-Marzouky, H. 2013. Differential interaction between isolates of *Rhizoctonia solani* AG-3 and potato cultivars. *African Journal of Microbiology Research, 7*(12), 1045-1054. doi: 10.5897/AJMR12.2074

El-Fattah, D. A. A. , Eweda, W. E. , Zayed, M. S. and Hassanein, M. K. 2013. Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. *Annals of Agricultural Sciences, 58*(2), 111-118. doi: org/10.1016/j.aoas.2013.07.001

El-Mohamedy, R. S. R. 2012. Biological control of *Pythium* root rot of broccoli plants under greenhouse conditions. *Journal Agricultural Technology, 8*, 1017-1028.

El-Naggar, M. A. , Al Rajhi, A. M. , Abdelkareem, E. M. and Abdelgany, M. M. 2013. Biochemical defense mechanism in potato against stem canker and black scurf disease. *Asian Journal of Plant Sciences, 12*(4), 165-170. doi: 10.3923/ajps.2013.165.170

El-Khaldi, R. , Daami-Remadi, M. and Cherif, M. 2016. Biological control of stem canker and black scurf on potato by date palm compost and its associated fungi. *Journal of Phytopathology, 164*(1), 40-51. doi: 10.1111/jph.12423

Elad, Y. , Chet, I. , Boyle, P. and Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*-scanning electron microscopy and fluorescence microscopy. *Phytopathology, 73*(1), 85-88.

Elad, Y. , Chet, I. and Henis, Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Canadian Journal of Microbiology, 28*(7), 719-725. doi: org/10.1139/m82-110

Elad, Y. , Chet, I. and Katan, J. 1980a. *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology, 70*(2), 119-121. doi: 10.1094/Phyto-70-119

Elad, Y. , Katan, J. and Chet, I. 1980b. Physical, biological and chemical control integrated for soilborne diseases in potatoes. *Phytopathology, 70*(418-422), 222.

Ellouze, W. , Esmaeili Taheri, A. , Bainard, L. D. , Yang, C. , Bazghaleh, N. , Navarro-Borrell, A. , Hanson, K. and Hamel, C. 2014. Soil fungal resources in annual cropping systems and their potential for management. *Biomed Research International, 2014*, 531824. doi: http://dx.doi.org/10.1155/2014/531824

Engström, K. , Widmark, A. , Brishammar, S. and Helmersson, S. 1999. Antifungal activity to *Phytophthora infestans* of sesquiterpenoids from infected potato tubers. *Potato Research, 42*(1), 43-50.

Erper, I. , Turkkan, M. , Karaca, G. H. and Kilic, G. 2011. Evaluation of in vitro antifungal activity of potassium bicarbonate on *Rhizoctonia solani* AG 4 HG-I, *Sclerotinia sclerotiorum* and *Trichoderma* sp. *African Journal of Biotechnology, 10*(43), 8605-8612. doi: 10.5897/AJB11.411

Esna-Ashari, M. and Villiers, T. 1998. Plant regeneration from tuber discs of potato (*Solanum tuberosum* L.) using 6-benzylaminopurine (BAP). *Potato Research, 41*(4), 371-382.

Espinoza, N. , Estrada, R. , Tovar, P. , Bryan, J. and Dodds, J. H. 1984. *Tissue culture micropropagation conservation, and export of potato germplasm* Lima-Peru: International Potato Center

Fakhry, S. M. K. (2016). *Effect of mineral fertilization and some biological treatments on sesame.* (PhD), Cairo University

Fan, C. , Xiong, G. , Qi, P. , Ji, G. and He, Y. 2008. Potential biofumigation effects of *Brassica oleracea* var. *caulorapa* on growth of fungi. *Journal of phytopathology, 156*(6), 321-325.

Farooq, M. , Wahid, A. and Siddique, K. H. M. 2012. Micronutrient application through seed treatments: a review. *Journal of Soil Science and Plant Nutrition, 12*(1), 125-142. doi: org/10.4067/S0718-95162012000100011

Ferrucho, R. L. , Cifuentes, J. M. , Ceresini, P. and García-Domínguez, C. 2012. *Rhizoctonia solani* AG-3PT is the major pathogen associated with potato stem canker and black scurf in Colombia. *Agronomía Colombiana, 30*(2), 204-213.

Fiers, M. (2010). *Origins of the blemishes of potato tubers: from the soil microbiology to the pedoclimatic environment.* (PhD), Université de Bourgogne, France.

Fiorentino, N. , Ventorino, V. , Woo, S. L. , Pepe, O. , De Rosa, A. , Gioia, L. , Romano, I. , Lombardi, N. , Napolitano, M. and Colla, G. 2018. *Trichoderma*-based biostimulants modulate rhizosphere microbial populations and improve N uptake efficiency, yield, and nutritional quality of leafy legetables. *Frontiers in Plant Science, 9*, 1-15. doi: 10.3389/fpls.2018.00743

Fredrickson, A. and Stephanopoulos, G. 1981. Microbial competition. *Science, 213*(4511), 972-979.

Friberg, H. , Edel-Hermann, V. , Faivre, C. , Gautheron, N. , Fayolle, L. , Faloya, V. , Montfort, F. and Steinberg, C. 2009. Cause and duration of mustard incorporation effects on soil-borne plant pathogenic fungi. *Soil Biology and Biochemistry, 41*(10), 2075-2084. doi: 10.1016/j.soilbio.2009.07.017

Gajera, H. , Rakholiya, K. and Vakharia, D. 2011. Bioefficacy of *Trichoderma* isolates against *Aspergillus niger* Van Tieghem inciting collar rot in groundnut (*Arachis hypogaea* L.). *Journal of Plant Protection Research, 51*(3), 240-247. doi: org/10.2478/v10045-011-0040-x

Galletti, S. , Fornasier, F. , Cianchetta, S. and Lazzeri, L. 2015. Soil incorporation of brassica materials and seed treatment with *Trichoderma harzianum*: effects on melon growth and soil microbial activity. *Industrial Crops and Products, 75*, 73-78. doi: org/10.1016/j.indcrop.2015.04.030

Galletti, S. , Sala, E. , Leoni, O. , Burzi, P. L. and Cerato, C. 2008. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control, 45*(3), 319-327. doi: 10.1016/j.biocontrol.2008.01.014

Gallou, A. , Cranenbrouck, S. and Declerck, S. 2009. *Trichoderma harzianum* elicits defence response genes in roots of potato plantlets challenged by *Rhizoctonia solani*. *European Journal of Plant Pathology, 124*(2), 219-230. doi: 0.1007/s10658-008-9407-x

Gimsing, A. L. and Kirkegaard, J. 2006. Glucosinolate and isothiocyanate concentration in soil following incorporation of Brassica biofumigants. *Soil Biology and Biochemistry, 38*(8), 2255-2264.

Gimsing, A. L. , Sørensen, J. C. , Strobel, B. W. and Hansen, H. C. B. 2007. Adsorption of glucosinolates to metal oxides, clay minerals and humic acid. *Applied Clay Science, 35*(3), 212-217. doi: org/10.1016/j.clay.2006.08.008

Glazener, J. and Wouters, C. 1981. Detection of rishitin in tomato fruits after infection with *Botrytis cinerea*. *Physiological Plant Pathology, 19*(2), 243-248. doi: org/10.1016/S0048-4059(81)80027-6

Graham, R. D. and Webb, M. J. (1991). Micronutrients and disease resistance and tolerance in plants. In J. J. Mortredt (Ed.), *Micronutrients in Agriculture* (Second Edition ed., pp. 329-370). Madison, USA: Soil Science Society of American

Grosch, R. , Faltin, F. , Lottmann, J. , Kofoet, A. and Berg, G. 2005. Effectiveness of 3 antagonistic bacterial isolates to control *Rhizoctonia solani* Kühn on lettuce and potato. *Canadian Journal of Microbiology, 51*(4), 345-353. doi: 10.1139/W05-002

Grosch, R. , Scherwinski, K. , Lottmann, J. and Berg, G. 2006. Fungal antagonists of the plant pathogen *Rhizoctonia solani* selection, control efficacy and influence on the indigenous microbial community. *Mycological Research, 110*(Pt 12), 1464-1474. doi: 10.1016/j.mycres.2006.09.014

Guchi, E. 2015. Disease management practice on potato (*Solanum tuberosum* L.) in Ethiopia. *World Journal of Agricultural Research, 3*(1), 34-42. doi: 10.12691/wjar-3-1-8

Gudmestad, N. , Taylor, R. and Pasche, J. 2007. Management of soilborne diseases of potato. *Australasian Plant Pathology, 36*(2), 109-115.

Handiseni, M. , Jo, Y.-K. , Lee, K.-M. and Zhou, X.-G. 2016. Screening brassicaceous plants as biofumigants for management of *Rhizoctonia solani* ag1-ia. *Plant Disease, 100*(4), 758-763. doi: org/10.1094/PDIS-06-15-0667-RE

Hanschen, F. S. , Yim, B. , Winkelmann, T. , Smalla, K. and Schreiner, M. 2015. Degradation of biofumigant isothiocyanates and allyl glucosinolate in soil and their effects on the microbial community composition. *Plos One, 10*(7), e0132931. doi: org/10.1371/journal.pone.0132931

Harman, G. E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology, 96*(2), 190-194. doi: 10.1094/PHYTO-96-0190

Harman, G. E. , Howell, C. R. , Viterbo, A. , Chet, I. and Lorito, M. 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology, 2*(1), 43-56. doi: 10.1038/nrmicro797

Hayder, A. A. (2017). *Effect of environmetal and host factors on biological control of Fusarium wilt by non-pathogenic Fusarium oxysporum in tomato* (PhD), University of New England, Australia.

Hermosa, R. , Viterbo, A. , Chet, I. and Monte, E. 2012. Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology, 158*(1), 17-25. doi: 10.1099/mic.0.052274-0.

Hicks, E. , Bienkowski, D. , Braithwaite, M. , Mclean, K. , Falloon, R. and Stewart, A. 2014. *Trichoderma* strains suppress *Rhizoctonia* diseases and promote growth of potato. *Phytopathologia Mediterranea, 53*(3), 502-514. doi: 10.14601/Phytopathol_Mediterr-14476

Hide, G. A. , Read, P. J. and Hall, S. M. 1992. Stem canker (*Rhizoctonia solani*) on three early and three maincrop potato cultivars: effects of seed tuber size on growth and yield. *Annals of Applied Biology, 120*(3), 391-403.

Hohmann, P. , Jones, E. E. , Hill, R. A. and Stewart, A. 2011. Understanding *Trichoderma* in the root system of *Pinus radiata*: associations between rhizosphere colonisation and growth promotion for commercially grown seedlings. *Fungal Biology, 115*(8), 759-767. doi: 10.1016/j.funbio.2011.05.010

Hohmann, P. , Jones, E. E. , Hill, R. A. and Stewart, A. 2012. Ecological studies of the bio-inoculant *Trichoderma hamatum* LU592 in the root system of *Pinus radiata*. *FEMS Microbiology Ecology, 80*(3), 709-721.

Howell, C. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease, 87*(1), 4-10.

Hridya, A. , Byju, G. and Misra, R. S. 2013. Effect of biocontrol agents and biofertilizers on root rot, yield, harvest index and nutrient uptake of cassava (*Manihot esculanta* Crantz). *Archives of Agronomy and Soil Science, 59*(9), 1215-1227. doi: org/10.1080/03650340.2012.702896

Huang, X. , Chen, L. , Ran, W. , Shen, Q. and Yang, X. 2011. *Trichoderma harzianum* strain SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in cucumber seedlings mainly by the mycoparasitism. *Applied Microbiology and Biotechnology, 91*(3), 741-755. doi: 0.1007/s00253-011-3259-6

Huber, D. (1980). The role of mineral nutrition in defense. In J. G. Horsfall & Cowling, E. B. (Eds.), *Plant Disease, Advanced Treatise. How Plants Defend Themselves* (Vol. 5, pp. 381-406). London: Academic Press

Illmer, P. and Schinner, F. 1995. Phosphate solubilizing microorganisms under non-sterile conditions. *Bodenkultur, 46*, 197-197.

Inbar, J. , Abramsky, M. , Cohen, D. and Chet, I. 1994. Plant growth enhancement and disease control by*Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *European Journal of Plant Pathology, 100*(5), 337-346. doi: 10.1007/BF01876444

Jacobsen, B. J. , Zidack, N. K. and Larson, B. J. 2004. The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology, 94*(11), 1272-1275. doi: org/10.1094/PHYTO.2004.94.11.1272

Jager, G. and Velvis, H. 1988. Inactivation of sclerotia of *Rhizoctonia solani* on potato tubers by *Verticillium biguttatum*, a soil-borne mycoparasite. *Netherlands Journal of Plant Pathology, 94*(5), 225-231.

Jegathambigai, V. , Wijeratnam, R. W. and Wijesundera, R. 2009. *Trichoderma* as a seed treatment to control Helminthosporium leaf spot disease of *Chrysalidocarpus lutescens*. *World Journal of Agricultural Sciences, 5*(6), 720-728.

Jeromela, A. M. , Mikić, A. M. , Vujić, S. , Ćupina, B. , Krstić, Đ. , Dimitrijević, A. , Vasiljević, S. , Mihailović, V. , Cvejić, S. and Miladinović, D. 2017. Potential of legume–*Brassica* intercrops for forage production and green manure: encouragements from a temperate southeast european environment. *Frontiers in Plant Science, 8*, 312. doi: org/10.3389/fpls.2017.00312

Jin, C. W. , Ye, Y. Q. and Zheng, S. J. 2013. An underground tale: contribution of microbial activity to plant iron acquisition via ecological processes. *Annals of Botany, 113*(1), 7-18. doi: org/10.1093/aob/mct249

Jönsson, E. L. and Asp, H. 2013. Effects of pH and nitrogen on cadmium uptake in potato. *Biologia Plantarum, 57*(4), 788-792. doi: 10.1007/s10535-013-0354-9

Kamal, R. K. , Athisayam, V. , Gusain, Y. S. and Kumar, V. 2018. *Trichoderma*: a most common biofertilizer with multiple roles in agriculture. *Journal of Scientific and Technical Research, 4*(5), 1-3. doi: 10.26717/BJSTR.2018.04.001107

Kamil, D. , Kumar, R. and Sinha, A. 2009. Effect of green manuring of *Crotolaria juncea* L. on some soil-borne pathogens. *Indian Phytopathology, 62*(3), 304-309.

Kavoo-Mwangi, A. , Kahangi, E. , Ateka, E. , Onguso, J. , Mukhongo, R. , Mwangi, E. and Jefwa, J. 2013. Growth effects of microorganisms based commercial products inoculated to tissue cultured banana cultivated in three different soils in Kenya. *Applied Soil Ecology, 64*, 152-162. doi: rg/10.1016/j.apsoil.2012.12.002

Khallil, A. M. 2001. Phytofungitoxic properties in the aqueous extracts of some plants. *Pakistan Journal of Biological Sciences, 4*(4), 392-394.

Khandaker, M. M. , Khair, A. and Bhuiyan, M. K. A. 2011. Disease reaction of potato germplasms and true potato seeds against *Rhizoctonia Solani* Kuhn. *Bangladesh Journal of Botany, 40*(2), 193-196.

Khattabi, N. , Ezzahiri, B. , Louali, L. and Oihabi, A. 2004. Effect of nitrogen fertilizers and *Trichoderma harzianum* on *Sclerotium rolfsii*. *Agronomie, 24*(5), 281-288. doi: 10.1051/agro:2004026

Kim, D.-J. , Baek, J.-M. , Uribe, P. , Kenerley, C. M. and Cook, D. R. 2002. Cloning and characterization of multiple glycosyl hydrolase genes from *Trichoderma virens*. *Current Genetics, 40*(6), 374-384. doi: 10.1007/s00294-001-0267-6

Kirk, W. , Berry, D. and Snapp, S. 2007. Disease cycle of potato stem canker. *Michigan Agiculture Experiment Station and the Michigan Potato Industry Commission*.

Kirkegaard, J. and Sarwar, M. 1999. Glucosinolate profiles of Australian canola (*Brassica napus* annua L.) and Indian mustard (*Brassica juncea* L.) cultivars: implications for biofumigation. *Australian Journal of Agricultural Research, 50*(3), 315-324. doi: org/10.1071/A98124

Kirkegaard, J. , Wong, P. and Desmarchelier, J. 1996. In vitro suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology, 45*(3), 593-603.

Kleifeld, O. and Chet, I. 1992. *Trichoderma harzianum*—interaction with plants and effect on growth response. *Plant and Soil, 144*(2), 267-272. doi: 10.1007/BF00012884

Klikocka, H. , Haneklaus, S. , Bloem, E. and Schnug, E. 2005. Influence of sulfur fertilization on infection of potato tubers with *Rhizoctonia solani* and *Streptomyces scabies*. *Journal of Plant Nutrition, 28*(5), 819-833. doi: 10.1081/pln-200055547

Kloepper, J. W. , Leong, J. , Teintze, M. and Schroth, M. N. 1980. *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. *Current Microbiology, 4*(5), 317-320.

Kraft, J. M. and Papavizas, G. C. 1983. Use of host resistance, *Trichoderma*, and fungicides to control soilborne diseases and increase seed yields of peas. *Plant Disease, 67*(11), 1234-1237.

Kredics, L. , Antal, Z. , Szekeres, A. , Hatvani, L. , Manczinger, L. , Vágvölgyi, C. and Nagy, E. 2005. Extracellular proteases of *Trichoderma* species. *Acta Microbiologica Et Immunologica Hungarica, 52*(2), 169-184. doi: org/10.1556/AMicr.52.2005.2.3

Kumar, G. , Maharshi, A. , Patel, J. , Mukherjee, A. , Singh, H. and Sarma, B. 2016. *Trichoderma*: a potential fungal antagonist to control plant diseases. *SATSA Mukhapatra, 21*, 206-218.

Kumar, M. , Singh, J. K. , Kumar, S. and Kumar, A. 2017a. A comprehensive overview on black scurf of potato. *International Journal of Current Microbiology and Applied Sciences, 6*(10), 4981-4994. doi: org/10.20546/ijcmas.2017.610.472

Kumar, N. V. , Rajam, K. S. and Rani, M. E. 2017b. Plant growth promotion efficacy of indole acetic acid (IAA) produced by a mangrove associated fungi-*Trichoderma viride* VKF3. *International Journal of Current Microbiology and Applied Sciences, 6*(11), 2692-2701. doi: 10.20546/ijcmas.2017.611.xxx

Kumar, R. , Sinha, A. , Srivastava, S. and Mahajan, G. 2017c. Evaluation of biocontrol potential of some fungal decomposers of *Sesbania aculeata* L. green manure against some soil-borne plant pathogens. *Journal of Environmental Biology, 38*(1), 37.

Kuninaga, S. , Carling, D. E. , Takeuchi, T. and Yokosawa, R. 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology, 66*(1), 2-11. doi: 10.1007/PL00012917

Kushad, M. M. , Cloyd, R. and Babadoost, M. 2004. Distribution of glucosinolates in ornamental cabbage and kale cultivars. *Scientia Horticulturae, 101*(3), 215-221. doi: org/10.1016/j.scienta.2003.10.011

Lahlali, R. and Hijri, M. 2010. Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *Fems Microbiology Letters, 311*(2), 152-159. doi: :10.1111/j.1574-6968.2010.02084.x

Lakhesar, D. P. S. , Backhouse, D. and Kristiansen, P. 2010. Nutritional constraints on displacement of *Fusarium pseudograminearum* from cereal straw by antagonists. *Biological Control, 55*(3), 241-247. doi: 10.1016/j.biocontrol.2010.09.002

Lal, M. , Yadav, S. , Singh, V. and Nagesh, M. (2016). The use of bio-agents for management of potato diseases *Plant Growth*, 4-11. doi: 10.5772/64853.

Larkin, R. , Honeycutt, C. , Griffin, T. , Olanya, O. , He, Z. and Halloran, J. 2017. Cumulative and residual effects of different potato cropping system management strategies on soilborne diseases and soil microbial communities over time. *Plant Pathology, 66*(3), 437-449. doi: 10.1111/ppa.12584

Larkin, R. , Roberts, D. and Gracia-Garza, J. (1998). Biological control of fungal diseases. In D. H. Hutson & Miyamoto, J. (Eds.), *Fungicidal Activity, Chemical and Biological Approaches to Plant Protection* (pp. 149-191). New York: Wiley

Larkin, R. P. 2016. Impacts of biocontrol products on Rhizoctonia disease of potato and soil microbial communities, and their persistence in soil. *Crop Protection, 90*, 96-105. doi: org/10.1016/j.cropro.2016.08.012

Larkin, R. P. and Griffin, T. S. 2007. Control of soilborne potato diseases using *Brassica* green manures. *Crop Protection, 26*(7), 1067-1077. doi: 10.1016/j.cropro.2006.10.004

Larkin, R. P. and Halloran, J. M. 2014. Management effects of disease-suppressive rotation crops on potato yield and soilborne disease and their economic implications in potato production. *American Journal of Potato Research, 91*(5), 429-439. doi: 10.1007/s12230-014-9366-z

Larkin, R. P. and Honeycutt, C. W. 2006. Effects of different 3-year cropping systems on soil microbial communities and Rhizoctonia diseases of potato. *Phytopathology, 96*(1), 68-79. doi: 10.1094/PHYTO-96-0068

Lehtonen, M. , Ahvenniemi, P. , Wilson, P. , German-Kinnari, M. and Valkonen, J. 2008a. Biological diversity of *Rhizoctonia solani* (AG-3) in a northern potato-cultivation environment in Finland. *Plant Pathology, 57*(1), 141-151. doi: 10.1111/j.1365-3059.2007.01694.x

Lehtonen, M. , Somervuo, P. and Valkonen, J. 2008b. Infection with *Rhizoctonia solani* induces defense genes and systemic resistance in potato sprouts grown without light. *Phytopathology, 98*(11), 1190-1198. doi: 10.1094/PHYTO-98-11-1190

Lei, Z. and Ya-qing, Z. 2015. Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. *Journal of Integrative Agriculture, 14*(8), 1588-1597. doi: 10.1016/S2095-3119(14)60966-7

Li, R.-X. , Cai, F. , Pang, G. , Shen, Q.-R. , Li, R. and Chen, W. 2015. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *Plos One, 10*(6), e0130081. doi: 10.5061/dryad.p460c

Lo, C.-T. and Lin, C.-Y. 2002. Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. *Plant Pathology Bulletin, 11*(4), 215-220.

Mach, R. L. , Peterbauer, C. K. , Payer, K. , Jaksits, S. , Woo, S. L. , Zeilinger, S. , Kullnig, C. M. , Lorito, M. and Kubicek, C. P. 1999. Expression of two major chitinase genes of *Trichoderma atroviride* (*T. harzianum* P1) is triggered by different regulatory signals. *Applied and Environmental Microbiology, 65*(5), 1858-1863.

Mäck, G. and Schjoerring, J. 2002. Effect of NO3-supply on N metabolism of potato plants (*Solanum tuberosum* L.) with special focus on the tubers. *Plant, Cell & Environment, 25*(8), 999-1009. doi: 10.1046/j.1365-3040.2002.00892.x

Marfil, C. F. , Hidalgo, V. and Masuelli, R. W. 2015. In situ conservation of wild potato germplasm in Argentina: example and possibilities. *Global Ecology and Conservation, 3*, 461-476. doi: org/10.1016/j.gecco.2015.01.009

Martin, J. P. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science, 69*(3), 215-232.

Martínez-Medina, A. , Alguacil, M. D. M. , Pascual, J. A. and Van Wees, S. C. 2014. Phytohormone profiles induced by *Trichoderma* isolates correspond with their biocontrol and plant growth-promoting activity on melon plants. *Journal of Chemical Ecology, 40*(7), 804-815. doi: 10.1007/s10886-014-0478-1

Mathivanan, N. , Srinivasan, K. and Chelliah, S. 2000. Biological control of soil-borne diseases of cotton, eggplant, okra and sunflower by *Trichoderma viride*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 107*, 235-244.

Matthiessen, J. N. and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Sciences, 25*(3), 235-265. doi: org/10.1080/07352680600611543

Mayo, S. , Gutierrez, S. , Malmierca, M. G. , Lorenzana, A. , Campelo, M. P. , Hermosa, R. and Casquero, P. A. 2015. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. *Frontiers in Plant Science, 6*, 685. doi: 10.3389/fpls.2015.00685

Mayton, H. S. , Olivier, C. , Vaughn, S. F. and Loria, R. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology, 86*(3), 267-271.

McGuire, A. M. 2003. Mustard green manures replace fumigant and improve infiltration in potato cropping system. *Crop Management, 2*(1). doi: 10.1094/CM-2003-0822-01-RS

McGuire, R. G. and Kelman, A. 1986. Calcium in potato tuber cell walls in relation to tissue maceration by *Erwinia carotovora* pv. *atroseptica*. *Phytopathology, 76*(4), 401-406.

McQuilken, M. P. and Gemmell, J. 2004. Enzyme production by the mycoparasite *Verticillium biguttatum* against *Rhizoctonia solani*. *Mycopathologia, 157*(2), 201-205.

Mehetre, S. T. and Mukherjee, P. K. (2015). *Trichoderma* improves nutrient use efficiency in crop plants. In A. Rakshit, Singh, H. B. & Sen, A. (Eds.), *Nutrient Use Efficiency: from Basics to Advances* (pp. 173-180): Springer.

Mendoza-Mendoza, A. , Pozo, M. J. , Grzegorski, D. , Martínez, P. , García, J. M. , Olmedo-Monfil, V. , Cortés, C. , Kenerley, C. and Herrera-Estrella, A. 2003. Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. *Proceedings of the National Academy of Sciences, 100*(26), 15965-15970. doi: 10.1073/pnas.2136716100

Mihajlović, M. , Rekanović, E. , Hrustić, J. , Grahovac, M. and Tanović, B. 2017. Methods for management of soilborne plant pathogens. *PesticidiCIDES Fitomedicina, 32*(1), 9-24. doi: org/10.2298/PIF1701009M

Mihuta-Grimm, L. and Rowe, R. 1986. *Trichoderma* spp. as biocontrol agents of Rhizoctonia damping-off of radish in organic soil and comparison of four delivery systems. *Phytopathology, 76*(3), 306-312.

Millaleo, R. , Reyes-Díaz, M. , Ivanov, A. , Mora, M. and Alberdi, M. 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition, 10*(4), 470-481. doi: org/10.4067/S0718-95162010000200008

Mishra, B. , Mishra, R. K. , Mishra, R. , Tiwari, A. K. , Yadav, R. S. and Dikshit, A. 2011. Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. *Archives of Applied Science Research, 3*(2), 361-369.

Mohsan, M. , Niaz, M. Z. and Bashir, M. R. 2016. Reactions of 18 potato cultivars against black scurf disease caused by *Rhizoctonia solani* Kuhn. *International Journal of Advanced Research in Biological Sciences, 3*(8), 197-199.

Montfort, F. , Poggi, S. , Morliere, S. , Collin, F. , Lemarchand, E. and Bailey, D. J. (2011). Opportunities to reduce *Rhizoctonia solani* expression on carrots by biofumigation with Indian Mustard. In C. Hale (Ed.), *Xxviii International Horticultural Congress on Science and Horticulture for People* (Vol. 1, pp. 149-157). http://www.actahort.org/books/917/917_19.htm.

Morán-Diez, E. , Rubio, B. , Domínguez, S. , Hermosa, R. , Monte, E. and Nicolás, C. 2012. Transcriptomic response of *Arabidopsis thaliana* after 24h incubation with the biocontrol fungus *Trichoderma harzianum*. *Journal of Plant Physiology, 169*(6), 614-620. doi: org/10.1016/j.jplph.2011.12.016

Morandi, D. 1996. Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions, and their potential role in biological control. *Plant and Soil, 185*(2), 241-251.

Morra, M. and Kirkegaard, J. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissues. *Soil Biology and Biochemistry, 34*(11), 1683-1690. doi: 10.1016/S0038-0717(02)00153-0

Morris, R. A. , Ewing, D. F. , Whipps, J. M. and Coley-Smith, J. R. 1995. Antifungal hydroxymethyl-phenols from the mycoparasite *Verticillium biguttatum*. *Phytochemistry, 39*(5), 1043-1048. doi: 10.1016/0031-9422(95)00165-4

Mortvedt, J. J. , Berger, K. and Darling, H. 1963. Effect of manganese and copper on the growth of *Streptomyces scabies* and the incidence of potato scab. *American Potato Journal, 40*(3), 96-102.

Motisi, N. , Doré, T. , Lucas, P. and Montfort, F. 2010. Dealing with the variability in biofumigation efficacy through an epidemiological framework. *Soil Biology and Biochemistry, 42*(12), 2044-2057. doi: org/10.1016/j.soilbio.2010.08.016

Motisi, N. , Montfort, F. , Faloya, V. , Lucas, P. and Doré, T. 2009. Growing *Brassica juncea* as a cover crop, then incorporating its residues provide complementary control of Rhizoctonia root rot of sugar beet. *Field Crops Research, 113*(3), 238-245. doi: org/10.1016/j.fcr.2009.05.011

Mrabet, M. , Djebali, N. , Elkahoui, S. , Miloud, Y. , Saidi, S. , Tarhouni, B. and Mhamdi, R. 2013. Efficacy of selected *Pseudomonas* strains for biocontrol of *Rhizoctonia solani* in potato. *Phytopathologia Mediterranea*, 449-456. doi: 10.1111/jph.12423

Mucheru-Muna, M. , Pypers, P. , Mugendi, D. , Kung'u, J. , Mugwe, J. , Merckx, R. and Vanlauwe, B. 2010. A staggered maize–legume intercrop arrangement robustly increases crop yields and economic returns in the highlands of Central Kenya. *Field Crops Research, 115*(2), 132-139. doi: org/10.1016/j.fcr.2009.10.013

Murali, M. , Amruthesh, K. N. , Sudisha, J. , Niranjana, S. R. and Shetty, H. S. 2012. Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. *Journal of Phytology, 4*(5), 30-36.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497.

Muzhinji, N. , Woodhall, J. W. , Truter, M. and Van der Waals, J. E. 2018. Relative contribution of seed tuber-and soilborne inoculum to potato disease development and changes in the population genetic structure of *Rhizoctonia solani* AG 3-PT under field conditions in South Africa. *Plant Disease, 102*(1), 60-66. doi: org/10.1094/PDIS-03-17-0329-RE

Mwangi, M. W. , Monda, E. O. , Okoth, S. A. and Jefwa, J. M. 2011. Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal of Microbiology, 42*(2), 508-513.

Nagamani, P. , Bhagat, S. , Biswas, M. K. and Viswanath, K. 2017. Effect of volatile and non volatile compounds of *Trichoderma* spp. against soil borne diseases of chickpea. *International Journal of Current Microbiology and Applied Sciences 6*(7), 1486-1491. doi: org/10.20546/ijcmas.2017.607.177

Naseby, D. C. , Pascual, J. A. and Lynch, J. M. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. *Journal of Applied Microbiology, 88*(1), 161-169.

Nawrocka, J. , Małolepsza, U. , Szymczak, K. and Szczech, M. 2018. Involvement of metabolic components, volatile compounds, PR proteins, and mechanical strengthening in multilayer protection of cucumber plants against *Rhizoctonia solani* activated by *Trichoderma atroviride* TRS25. *Protoplasma, 255*(1), 359-373. doi: 10.1007/s00709-017-1157-1

Naz, F. , Rauf, C. A. , Abbasi, N. A. , Haque, I. and Ahmad, I. 2008. Influence of inoculum levels of *Rhizoctonia solani* and susceptibility on new potato germplasm. *Pakistan Journal of Botany 40*(5), 2199-2209.

Nedelnik, J. and Repkova, J. 1998. Plant selection in vitro for resistance to some pathogens using secondary toxic metabolites. *Genetika a Slechteni-UZPI; Plant Breeding Genetics, 34*(2), 69-76.

Nelson, E. B. (1991). Exudate molecules initiating fungal responses to seeds and roots. In D. L. Keister & Cregan, P. B. (Eds.), *The Rhizosphere and Plant Growth* (pp. 197-209). Dordrecht: Springer.

Ngadze, E. , Coutinho, T. A. , Icishahayo, D. and Van der Waals, J. E. 2014. Effect of calcium soil amendments on phenolic compounds and soft rot resistance in potato tubers. *Crop Protection, 62*, 40-45. doi: 10.1016/j.cropro.2014.04.009

Nieto-Jacobo, M. F. , Steyaert, J. M. , Salazar-Badillo, F. B. , Nguyen, D. V. , Rostás, M. , Braithwaite, M. , De Souza, J. T. , Jimenez-Bremont, J. F. , Ohkura, M. and Stewart, A. 2017. Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. *Frontiers in Plant Science, 8*. doi: 10.3389/fpls.2017.00102

Njoroge, S. M. , Riley, M. B. and Keinath, A. P. 2008. Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on damping-off and Fusarium wilt of watermelon. *Plant Disease, 92*(2), 287-294. doi: org/10.1094/PDIS-92-2-0287

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. *Annual Review of Phytopathology, 25*(1), 125-143.

Olanya, O. , Lambert, D. , Reeves §, A. and Porter, G. 2009. Evaluation of potato clones for resistance to stem canker and tuber black scurf in field studies following artificial inoculation with *Rhizoctonia solani* AG-3 in Maine. *Archives of Phytopathology and Plant Protection, 42*(5), 409-418. doi: org/10.1080/03235400601121570

Oliveira, C. 2000. Potato crop growth as affected by nitrogen and plant density. *Pesquisa Agropecuária Brasileira, 35*(5), 940-950. doi: org/10.1590/S0100-204X2000000500011

Olivier, C. , Vaughn, S. F. , Mizubuti, E. S. and Loria, R. 1999. Variation in allyl isothiocyanate production within *Brassica* species and correlation with fungicidal activity. *Journal of Chemical Ecology, 25*(12), 2687-2701.

Olmedo-Monfil, V. , Mendoza-Mendoza, A. , Gomez, I. , Cortes, C. and Herrera-Estrella, A. 2002. Multiple environmental signals determine the transcriptional activation of the mycoparasitism related gene prb1 in *Trichoderma atroviride*. *Molecular Genetics and Genomics, 267*(6), 703-712. doi: 0.1007/s00438-002-0703-4

Opoku-Ameyaw, K. and Harris, P. 2001. Intercropping potatoes in early spring in a temperate climate. 1. Yield and intercropping advantages. *Potato Research, 44*(1), 53-61.

Ordóñez-Valencia, C. , Alarcón, A. , Ferrera-Cerrato, R. and Hernández-Cuevas, L. V. 2009. In vitro antifungal effects of potassium bicarbonate on *Trichoderma* sp. and *Sclerotinia sclerotiorum*. *Mycoscience, 50*(5), 380-387. doi: org/10.1007/S10267-009-0495-Z

Ortega-Aguilar, B. L. , Alarcón, A. and Ferrera-Cerrato, R. 2011. Effect of potassium bicarbonate on fungal growth and sclerotia of *Sclerotium cepivorum* and its interaction with *Trichoderma*. *Revista Mexicana De Micología, 33*, 53-61.

Ozgonen, H. and Erkilic, A. 2013. Reactions of 11 potato cultivars against some important soil-borne pathogens. *European Journal of Plant Science and Biotechnology, 7*, 43-46.

Pal, K. K. and Gardener, B. M. 2006. Biological control of plant pathogens. *The Plant Health Instructor, 2*(5), 1117-1142. doi: 10.1094/PHI-A-2006-1117-02.

Pane, C. , Villecco, D. and Zaccardelli, M. 2017. Combined use of brassica carinata seed meal, thyme oil and a *bacillus amyloliquefaciens* strain for controlling three soil-borne fungal plant diseases. *Journal of Plant Pathology, 99*(1), 77-84.

Panique, E. , Kelling, K. , Schulte, E. , Hero, D. , Stevenson, W. and James, R. 1997. Potassium rate and source effects on potato yield, quality, and disease interaction. *American Potato Journal, 74*(6), 379-398.

Panjehkeh, N. , Backhouse, D. and Taji, A. 2010. Role of proanthocyanidins in resistance of the legume *Swainsona formosa* to *Phytophthora cinnamomi*. *Journal of Phytopathology, 158*(5), 365-371. doi: 10.1111/j.1439-0434.2009.01627.x

Pannecoucque, J. , Van Beneden, S. and Höfte, M. 2008. Characterization and pathogenicity of *Rhizoctonia* isolates associated with cauliflower in Belgium. *Plant Pathology, 57*(4), 737-746. doi: 10.1111/j.1365-3059.2007.01823.x

Papavizas, G. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology, 23*(1), 23-54. doi: org/10.1146/annurev.py.23.090185.000323

Platt, H. , Canale, F. and Gimenez, G. 1993. Effects of tuber-borne inoculum of *Rhizoctonia solani* and fungicidal seed potato treatment on plant growth and rhizoctonia disease in Canada and Uruguay. *American Journal of Potato Research, 70*(8), 553-559. doi: 10.1007/BF02850845

Qi, W. and Zhao, L. 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. *Journal of Basic Microbiology, 53*(4), 355-364. doi: 10.1002/jobm.201200031

Rabeendran, N. , Jones, E. , Moot, D. and Stewart, A. 2006. Biocontrol of *Sclerotinia* lettuce drop by *Coniothyrium minitans* and *Trichoderma hamatum*. *Biological Control, 39*(3), 352-362. doi: org/10.1016/j.biocontrol.2006.06.004

Rabeendran, N. , Moot, D. , Jones, E. and Stewart, A. 2000. Inconsistent growth promotion of cabbage and lettuce from *Trichoderma* isolates. *New Zealand Plant Protection 53*, 143-146.

Rahman, M. M. , Ali, M. E. , Khan, A. A. , Akanda, A. M. , Uddin, M. K. , Hashim, U. and Abd Hamid, S. B. 2012. Isolation, characterization, and identification of biological control agent for potato soft rot in Bangladesh. *Scientific World Journal, 2012*, 723293. doi: 10.1100/2012/723293

Rahmanpour, S. , Backhouse, D. and Nonhebel, H. 2009. Induced tolerance of *Sclerotinia sclerotiorum* to isothiocyanates and toxic volatiles from *Brassica* species. *Plant Pathology, 58*(3), 479-486. doi: 10.1111/j.1365-3059.2008.02015.

Rahmanpour, S. , Backhouse, D. and Nonhebel, H. M. 2010. Reaction of glucosinolate-myrosinase defence system in *Brassica* plants to pathogenicity factor of *Sclerotinia sclerotiorum*. *European Journal of Plant Pathology, 128*(4), 429-433.

Ramsay, A. J. and Bawden, A. 1983. Effects of sterilization and storage on respiration, nitrogen status and direct counts of soil bacteria using acridine orange. *Soil Biology and Biochemistry, 15*(3), 263-268. doi: 10.1016/0038-0717(83)90069-X

Rangkadilok, N. , Nicolas, M. E. , Bennett, R. N. , Premier, R. R. , Eagling, D. R. and Taylor, P. W. 2002. Determination of sinigrin and glucoraphanin in *Brassica* species using a simple extraction method combined with ion-pair HPLC analysis. *Scientia Horticulturae, 96*(1), 27-41. doi: org/10.1016/S0304-4238(02)00119-X

Rashid, M. , Khalil, S. , Ayub, N. , Alam, S. and Latif, F. 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pakistan Journal of Biological Sciences 7*(2), 187-196.

Rauf, C. A. , Naz, F. , Ahmad, I. , Haque, I. U. and Riaz, A. 2015. Management of Black Scurf of Potato with Effective Microbes (EM), Biological Potassium Fertilizer (BPF) and *Trichoderma harzianum*. *International Journal of Agriculture and Biology, 17*(3), 601-606. doi: 10.17957/IJAB/17.3.14.019

Ritchie, F. , Bain, R. and Mcquilken, M. 2013. Survival of sclerotia of *Rhizoctonia solani* AG-3PT and effect of soil-borne inoculum density on disease development on potato. *Journal of Phytopathology, 161*(3), 180-189.

Rodríguez-Falcón, M. , Bou, J. and Prat, S. 2006. Seasonal control of tuberization in potato: conserved elements with the flowering response. *Annual Review of Plant Biology, 57*, 151-180. doi: 10.1146/annurev.arplant.57.032905.105224

Ros, B. , Mohler, V. , Wenzel, G. and Thümmler, F. 2008. *Phytophthora infestans* triggered response of growth and defense related genes in potato cultivars with different levels of resistance under the influence of nitrogen availability. *Physiologia Plantarum, 133*(2), 386-396. doi: 10.1111/j.1399-3054.2008.01048.x

Rubio, M. , Hermosa, R. , Vicente, R. , Gómez-Acosta, F. A. , Morcuende, R. , Monte, E. and Bettiol, W. 2017. The combination of *Trichoderma harzianum* and chemical fertilization leads to the deregulation of phytohormone networking, preventing the adaptive responses of tomato plants to salt stress. *Frontiers in Plant Science, 8*. doi: 10.3389/fpls.2017.00294

Rubio, M. B. , Domínguez, S. , Monte, E. and Hermosa, R. 2012. Comparative study of *Trichoderma* gene expression in interactions with tomato plants using high-density oligonucleotide microarrays. *Microbiology, 158*(1), 119-128. doi: 10.1099/mic.0.052118-0

Rudakov, O. L. 1978. Physiological groups in mycophilic fungi. *Mycologia*, 150-159.

Saker, M. M. , Moussa, T. A. , Heikal, N. Z. , AboEllil, A. and Abdel-Rahman, R. 2012a. Selection of an efficient in vitro micropropagation and regeneration system for potato (*Solanum tuberosum* L.) cultivar Desiree. *African Journal of Biotechnology, 11*(98), 16388-16404. doi: 10.5897/AJB12.2313

Saker, M. M. , Moussa, T. A. , Heikal, N. Z. , Ellil, A. H. A. and Abdel-Rahman, R. M. 2012b. Selection of an efficient in vitro micropropagation and regeneration system for potato (*Solanum tuberosum* L.) cultivar Desiree. *African Journal of Biotechnology, 11*(98), 16388-16404. doi: org/10.5897/AJB12.2313

Santos, R. H. , Gliessman, S. R. and Cecon, P. R. 2002. Crop interactions in broccoli intercropping. *Biological Agriculture & Horticulture, 20*(1), 51-75. doi: i.org/10.1080/01448765.2002.9754948

Sarwar, M. , Kirkegaard, J. , Wong, P. and Desmarchelier, J. 1998. Biofumigation potential of brassicas. *Plant and Soil, 201*(1), 103-112.

Schirmböck, M. , Lorito, M. , Wang, Y.-L. , Hayes, C. K. , Arisan-Atac, I. , Scala, F. , Harman, G. E. and Kubicek, C. P. 1994. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Applied and Environmental Microbiology, 60*(12), 4364-4370.

Scholten, O. E. , Panella, L. W. , De Bock, T. S. and Lange, W. 2001. A greenhouse test for screening sugar beet (*Beta vulgaris*) for resistance to *Rhizoctonia solani*. *European Journal of Plant Pathology, 107*(2), 161-166. doi: 10.1023/A:1011208903344

Schreiner, P. R. and Koide, R. T. 1993. Antifungal compounds from the roots of mycotrophic and non-mycotrophic plant species. *New Phytologist, 123*(1), 99-105.

Shahid, M. , Singh, A. and Srivastava, M. 2014. Molecular characterization of *Trichoderma viride* isolated from rhizospheric soils of uttar pradesh based on rdna markers and analysis of their PCR-ISSR profiles. *Journal of Molecular Biomarkers & Diagnosis, 2014*. doi: 10.4172/2155-9929.1000169

Shalini, S. and Kotasthane, A. 2007. Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. *EJEAF Chemistry, 6*, 2272-2281. doi: 10.1007/s10658-007-9140-x

Shoresh, M. and Harman, G. E. 2008. The relationship between increased growth and resistance induced in plants by root colonizing microbes. *Plant Signaling & Behavior, 3*(9), 737-739. doi: org/10.4161/psb.3.9.6605

Shunka, E. , Chindi, A. , Gebremedhin, W. G. , Seid, E. and Tessema, L. 2017. Determination of optimum nitrogen and potassium levels for potato production in central high lands of Ethiopia. *Open Agriculture, 2*(1), 189-194. doi: 10.1515/opag-2017-0019

Šimkovič, M. , Kurucová, A. , Hunová, M. and Varečka, L. 2008. Induction of secretion of extracellular proteases from *Trichoderma viride*. *Acta Chimica Slovaca, 1*(1), 250-264.

Simons, S. and Gilligan, C. 1997. Relationships between stem canker, stolon canker, black scurf (*Rhizoctonia solani*) and yield of potato (*Solanum tuberosum*) under different agronomic conditions. *Plant Pathology, 46*(5), 651-658. doi: 10.1046/j.1365-3059.1997.d01-54.x

Singh, R. K. , Kumar, H. and Singh, A. K. 2010. Brassica based intercropping systems--a review. *Agricultural Reviews, 31*(4), 253-266.

Singleton, L. L. , Mihail, J. D. and Rush, C. M. 1992. *Methods for research on soilborne phytopathogenic fungi*. University of Alaska Fairbanks, USA: American Phytopathological Society.

Sivakumar, D. , Wijeratnam, R. W. , Wijesundera, R. , Marikar, F. and Abeyesekere, M. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of rambutan (*Nephelium lappaceum*). *Phytoparasitica, 28*(3), 240-247.

Sivan, A. and Chet, I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology, 79*(2), 198-203.

Sivasithamparam, K. and Ghisalberti, E. (1998). Secondary metabolism in *Trichoderma* and *Gliocladium*. In G. E. Harman & Kubicek, C. P. (Eds.), *Trichoderma and Gliocladium Basic Biology Taxonomy and Genetics* (Vol. 1, pp. 139-191). Boca Raton: CRC Press

Slininger, P. J. and Jackson, M. A. 1992. Nutritional factors regulating growth and accumulation of phenazine 1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *Applied Microbiology and Biotechnology, 37*(3), 388-392.

Smith, B. and Kirkegaard, J. 2002. In vitro inhibition of soil microorganisms by 2 phenylethyl isothiocyanate. *Plant Pathology, 51*(5), 585-593. doi: 10.1046/j.1365-3059.2002.00744.x

Smith, K. P. and Goodman, R. M. 1999. Host variation for interactions with beneficial plant-associated microbes. *Annual Review of Phytopathology, 37*(1), 473-491. doi: 10.1146/annurev.phyto.37.1.473

Smolinska, U. 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. *Journal of Phytopathology, 148*(6), 343-349. doi: 10.1046/j.1439-0434.2000.00519.x

Smolinska, U. and Horbowicz, M. 1999. Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soilborne fungal pathogens. *Journal of Phytopathology, 147*(2), 119-124. doi: 10.1046/j.1439-0434.1999.147002119.

Snapp, S. S. , Date, K. U. , Kirk, W. , O'Neil, K. , Kremen, A. and Bird, G. 2007. Root, shoot tissues of *Brassica juncea* and Cereal secale promote potato health. *Plant and Soil, 294*(1-2), 55-72. doi: 0.1007/s11104-007-9228-2

Somani, A. and Arora, R. 2010. Field efficacy of *Trichoderma viride*, *Bacillus subtilis* and *Bacillus cereus* in consortium for control of *Rhizoctonia solani* causing black scurf disease of potato. *Indian Phytopathology, 63*(1), 23.

Sonnewald, U. , Hajirezaei, M.-R. , Kossmann, J. , Heyer, A. , Trethewey, R. N. and Willmitzer, L. 1997. Increased potato tuber size resulting from apoplastic expression of a yeast invertase. *Nature Biotechnology, 15*(8), 794-797.

Srinivasa, N. , Devi, T. P. , Sudhirkumar, S. , Kamil, D. , Borah, J. L. and Prabhakaran, N. 2014. Bioefficacy of *Trichoderma* isolates against soil-borne pathogens. *African Journal of Microbiology Research, 8*(28), 2710-2723. doi: 10.5897/AJMR2013.6588

Stapleton, J. and Duncan, R. 1998. Soil disinfestation with cruciferous amendments and sublethal heating: effects on *Meloidogyne incognita*, *Sclerotium rolfsii* and *Pythium ultimum*. *Plant Pathology, 47*, 737-742.

Steyaert, J. , Ridgway, H. , Elad, Y. and Stewart, A. 2003. Genetic basis of mycoparasitism: a mechanism of biological control by species of *Trichoderma*. *New Zealand Journal of Crop and Horticultural Science, 31*(4), 281-291. doi: org/10.1080/01140671.2003.9514263

Szekeres, A. , Kredics, L. , Antal, Z. , Kevei, F. and Manczinger, L. 2004. Isolation and characterization of protease overproducing mutants of *Trichoderma harzianum*. *FEMS Microbiology Letters, 233*(2), 215-222. doi: 10.1016/j.femsle.2004.02.012

Tariq, M. , Yasmin, S. and Hafeez, Y. 2010. Biological control of potato black scurf by rhizosphere associated bacteria. *Environmental Microbiology, 41*(2), 439-451. doi: http://dx.doi.org/10.1590/S1517-83822010000200026

Taylor, F. I. (2013). *Control of soil borne potato pathogens using Brassica spp. mediated biofumigation.* (PhD), University of Glasgow.

Terna, T. , Odebode, A. , Okogbaa, J. and Waya, J. 2016. Invitro screening of *Trichoderma* isolates for biological control of a post-harvest rot fungus. *IOSR Journal of Agriculture and Veterinary Science 9*, 46-51. doi: 10.9790/2380-0904014651

Thornton, R. E. , Stevens, R. G. and Hammond, M. W. (1993). Selection the site and preparing it for planting. In R. C. Rowe (Ed.), *Potato Health Management* (pp. 11-18). USA.

Topolovec-Pintaric, S. , ka Zutic, I. and Dermic, E. 2013. Enhanced growth of cabbage and red beet by *Trichoderma viride*. *Acta Agriculturae Slovenica, 101*(1), 87. doi: 0.2478/acas-2013-0010

Truter, M. and Wehner, F. 2004. Anastomosis grouping of *Rhizoctonia solani* associated with black scurf and stem canker of potato in South Africa. *Plant Disease, 88*(1), 83-83. doi: org/10.1094/PDIS.2004.88.1.83B

Tsror, L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on potato. *Journal of Phytopathology, 158*(10), 649-658. doi: 10.1111/j.1439-0434.2010.01671.x

Tsror, L. and Peretz-Alon, I. 2005. The influence of the inoculum source of *Rhizoctonia solani* on development of black scurf on potato. *Journal of Phytopathology, 153*(4), 240-244. doi: org/10.1111/j.1439-0434.2005.00962.x

Ulrich, A. and Fong, K. H. 1973. Nitrogen nutrition of white rose potato in relation to vegetative growth and mineral content of leaves and roots. *Communications in Soil Science & Plant Analysis, 4*(6), 413-426. doi: 10.1080/00103627309366465

Van Bruggen, A. H. , Grünwald, N. J. and Bolda, M. (1996). Cultural methods and soil nutrient status in low and high input agricultural systems, as they affect *Rhizoctonia* species. In B. Sneh, Jabaji-Hare, S., Neate, S. & Dijst, G. (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 407-421). Dordrecht Springer.

Van den Boogert, P. and Luttikholt, A. 2004. Compatible biological and chemical control systems for *Rhizoctonia solani* in potato. *European Journal of Plant Pathology, 110*(2), 111-118.

Van den Brink, L. and Wustman, R. 2014. *Rhizoctonia solani* in potatoes and its control: Specific recommendations for seed production in Punjab (India). *Applied Plant Research*, 1-27.

Van Wees, S. C. , Van der Ent, S. and Pieterse, C. M. 2008. Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology, 11*(4), 443-448. doi: org/10.1016/j.pbi.2008.05.005

Verma, M. , Brar, S. K. , Tyagi, R. , Surampalli, R. and Valero, J. 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Biochemical Engineering Journal, 37*(1), 1-20. doi: org/10.1016/j.bej.2007.05.012

Vierheilig, H. and Ocampo, J. 1990. Effect of isothiocyanates on germination of spores of *G. mosseae*. *Soil Biology and Biochemistry, 22*(8), 1161-1162. doi: 10.1016/0038-0717(90)90044-Z

Villalta, O. , Wite, D. , Riches, D. A. , Guiano, J. , Chandolu, V. , Scoble, C. , Donald, C. , Porter, I. J. and Mattner, S. W. 2016. The concentration of 2-propenyl glucosinolate in biofumigant crops influences their anti-fungal activity (in-vitro) against soilborne pathogens. *Journal of Agricultural Chemistry and Environment, 5*(01), 38. doi: 10.4236/jacen.2016.51B006

Vinale, F. , Sivasithamparam, K. , Ghisalberti, E. L. , Marra, R. , Woo, S. L. and Lorito, M. 2008. *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry, 40*(1), 1-10. doi: org/10.1016/j.soilbio.2007.07.002

Virgen-Calleros, G. , Olalde-Portugal, V. and Carling, D. 2000. Anastomosis groups of *Rhizoctonia solani* on potato in central México and potential for biological and chemical control. *American Journal of Potato Research, 77*(4), 219-224. doi: 10.1007/BF02855789

Viterbo, A. , Montero, M. , Ramot, O. , Friesem, D. , Monte, E. , Llobell, A. and Chet, I. 2002. Expression regulation of the endochitinase chit36 from *Trichoderma asperellum* (*T. harzianum* T-203). *Current Genetics, 42*(2), 114-122. doi: 0.1007/s00294-002-0345-4

Wadas, W. and Dziugieł, T. 2015. Effect of complex fertilizers used in early crop potato culture on loamy sand soil. *Journal of Central European Agriculture, 16*(1), 23-40. doi: 10.5513/JCEA01/16.1.1536

Waghunde, R. R. , Shelake, R. M. and Sabalpara, A. N. 2016. *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research, 11*(22), 1952-1965. doi: 10.5897/AJAR2015.10584

Wegener, C. B. and Jansen, G. 2007. Soft-rot resistance of coloured potato cultivars (*Solanum tuberosum* L.): the role of anthocyanins. *Potato Research, 50*(1), 31-44. doi: 10.1007/s11540-007-9027-4

Weinding, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology 22*, 837-845

Westermann, D. T. (1993). Fertility management. In C. R. Randall (Ed.), *American Phytopathological Society* (pp. 77-86). USA: APS Press. (Reprinted from: Randall, C. Rowe).

Wharton, P. , Kirk, W. , Berry, D. and Snapp, S. 2007. *Rhizoctonia stem canker and black scurf of potato*: Michigan State University Extension.

Whipps, J. M. 1987. Effect of media on growth and interactions between a range of soilborne glasshouse pathogens and antagonistic fungi. *New Phytologist, 107*(1), 127-142. doi: 10.1111/j.1469-8137.1987.tb04887.x

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany, 52*(suppl_1), 487-511. doi: https://doi.org/10.1093/jexbot/52.suppl_1.487

White, T. J. , Bruns, T. , Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Chapter 38.* (PCR Protocols: a Guide to Methods and Applications (M. Innis, D. Gelfand, J. Sninsky and T. White, eds.).), Pages 315-322. .

Wicks, T. , Morgan, B. and Hall, B. 1995. Chemical and biological control of *Rhizoctonia solani* on potato seed tubers. *Australian Journal of Experimental Agriculture, 35*(5), 661-664. doi: org/10.1071/EA9950661

Williams, D. 1976. Improved likelihood ratio tests for complete contingency tables. *Biometrika, 63*(1), 33-37. doi: 10.2307/2335081

Wilson, P. , Ahvenniemi, P. , Lehtonen, M. , Kukkonen, M. , Rita, H. and Valkonen, J. 2008a. Biological and chemical control and their combined use to control different stages of the Rhizoctonia disease complex on potato through the growing season. *Annals of Applied Biology, 153*(3), 307-320. doi: 10.1111/j.1744-7348.2008.00292.x

Wilson, P. , Ketola, E. , Ahvenniemi, P. , Lehtonen, M. and Valkonen, J. 2008b. Dynamics of soilborne *Rhizoctonia solani* in the presence of *Trichoderma harzianum* effects on stem canker, black scurf and progeny tubers of potato. *Plant Pathology, 57*(1), 152-161. doi: 10.1111/j.1365-3059.2007.01706.x

Windham, M. , Elad, Y. and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology, 76*(5), 518-521.

Woodhall, J. , Lees, A. , Edwards, S. and Jenkinson, P. 2007. Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathology, 56*(2), 286-295. doi: 10.1111/j.1365-3059.2006.01545.x

Wu, F. , Wang, W. , Ma, Y. , Liu, Y. , Ma, X. , An, L. and Feng, H. 2013. Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture. *African Journal of Microbiology Research, 7*(20), 2150-2158.

Wyss, P. , Boller, T. and Wiemken, A. 1991. Phytoalexin response is elicited by a pathogen (*Rhizoctonia solani*) but not by a mycorrhizal fungus (*Glomus mosseae*) in soybean roots. *Experientia, 47*(4), 395-399.

Yadav, M. , Aravindan, S. , Ghritlahre, S. , Adak, T. and Berliner, J. 2015. Induced systemic resistance and plant responses to fungal biocontrol agents. *Popular Kheti, 3*(2), 63-67.

Yadav, S. L. , Mishra, A. K. , Dongre, P. N. and Singh, R. 2011. Assessment of fungitoxicity of phylloplane fungi against *Alternaria* brassicae causing leaf spot of mustard. *Journal of Agricultural Technology, 7*(6), 1823-1831.

Yanar, Y. , Yılmaz, G. , Cesmeli, I. and Coskun, S. 2005. Characterization of *Rhizoctonia solani* isolates from potatoes in Turkey and screening potato cultivars for resistance to AG-3 isolates. *Phytoparasitica, 33*(4), 370-376.

Yang, S. , Min, F. , Wang, W. , Wei, Q. , Guo, M. , Gao, Y. , Dong, X. and Lu, D. 2017. Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in Heilongjiang Province of China. *American Journal of Potato Research, 94*(2), 95-104. doi: 10.1007/s12230-016-9535-3

Yao, M. , Desilets, H. , Charles, M. , Boulanger, R. and Tweddell, R. 2003. Effect of *mycorrhization* on the accumulation of rishitin and solavetivone in potato plantlets challenged with *Rhizoctonia solani*. *Mycorrhiza, 13*(6), 333-336. doi: 10.1007/s00572-003-0267-0

Yao, M. , Tweddell, R. and Desilets, H. 2002. Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. *Mycorrhiza, 12*(5), 235-242. doi: 10.1007/s00572-002-0176-7

Yasumoto, S. , Suzuki, K. , Matsuzaki, M. , Hiradate, S. , Oose, K. , Hirokane, H. and Okada, K. 2011. Effects of plant residue, root exudate and juvenile plants of rapeseed (*Brassica napus* L.) on the germination, growth, yield, and quality of subsequent crops in successive and rotational cropping systems. *Plant Production Science, 14*(4), 339-348. doi: org/10.1626/pps.14.339

Yildirim, E. and Guvenc, I. 2005. Intercropping based on cauliflower: more productive, profitable and highly sustainable. *European Journal of Agronomy, 22*(1), 11-18. doi: 10.1016/j.eja.2003.11.003

Yousaf, M. , Li, J. , Lu, J. , Ren, T. , Cong, R. , Fahad, S. and Li, X. 2017. Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. *Scientific Reports, 7*, 1270. doi: 10.1038/s41598-017-01412-0

Zhang, F. , Yuan, J. , Yang, X. , Cui, Y. , Chen, L. , Ran, W. and Shen, Q. 2013. Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization. *Plant and Soil, 368*(1-2), 433-444. doi: 10.1007/s11104-012-1519-6

Zhang, X.-Y. , Yu, X.-X. , Yu, Z. , Xue, Y.-F. and Qi, L.-P. 2014. A simple method based on laboratory inoculum and field inoculum for evaluating potato resistance to black scurf caused by *Rhizoctonia solani*. *Breeding Science, 64*(2), 156-163. doi: 10.1270/jsbbs.64.156

Zhang, Z. , Mao, B. , Li, H. , Zhou, W. , Takeuchi, Y. and Yoneyama, K. 2005a. Effect of salinity on physiological characteristics, yield and quality of microtubers in vitro in potato. *Acta Physiologiae Plantarum, 27*(4), 481-489.

Zhang, Z. , Zhou, W. and Li, H. 2005b. The role of GA, IAA and BAP in the regulation of in vitro shoot growth and microtuberization in potato. *Acta Physiologiae Plantarum, 27*(3), 363-369.

Appendix

Appendix1. Results of statistical tests.

ANOVA tables are presented according the figure or table in which the data appear, ordered as they occur in the text of the thesis. A significance of zero indicates P < 0.001.

Chapter 3

Table3 -2. Colony interactions between *Rhizoctonia solani* and isolates of *Trichoderma* in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	9.303	8	1.163	29.902	0
Error	0.7	18	0.039		
Total	10.003	26			

Figure 3-2. Growth of *R. solani* hyphae in response to culture filtrate produced by *Trichoderma* spp.

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	16.592	9	1.844	19.204	0
Error	1.92	20	0.096		
Total	18.512	29			

Table 3-3. Effects of eight *Trichoderma* isolates (T1-T8) for suppression of Rhizoctonia disease on Desiree tubers grown in sand-potting mix

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.876	9	0.097	15.156	0
Error	0.128	20	0.006		
Total	1.005	29			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.496	9	0.055	7.424	0
Error	0.148	20	0.007		
Total	0.644	29			

Number of stolons (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.447	9	0.05	4.691	0.002
Error	0.212	20	0.011		
Total	0.659	29			

Number of tubers (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.552	9	0.061	2.823	0.026
Error	0.435	20	0.022		
Total	0.987	29			

Tuber fresh weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.307	9	0.034	4.294	0.003
Error	0.159	20	0.008		
Total	0.466	29			

Table 3-4. Effects of eight *Trichoderma* isolates (T1-T8) for suppression of Rhizoctonia disease on Desiree tubers grown in vermiculite

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.455	9	0.051	2.458	0.045
Error	0.411	20	0.021		
Total	0.867	29			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.583	9	0.065	3.289	0.013
Error	0.394	20	0.02		
Total	0.977	29			

Number of stolons (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.783	9	0.198	12.299	0
Error	0.322	20	0.016		
Total	2.105	29			

Number of tubers (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.206	9	0.134	11.598	0
Error	0.231	20	0.012		
Total	1.437	29			

Tuber fresh weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.801	9	0.089	6.464	0
Error	0.275	20	0.014		
Total	1.076	29			

Figure 3-4. Dry weight of potato plants (cv. Desiree) inoculated with *Trichoderma* strains (T5 + T8) and *R. solani* in sand-potting mix

Shoot dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0	3	6.13E-05	115.679	0
Error	4.24E-06	8	5.30E-07		
Total	0	11			

Root dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0	3	3.97E-05	221.425	0
Error	1.43E-06	8	1.79E-07		
Total	0	11			

Figure 3-5. Dry weight of potato plantlets inoculated with *Trichoderma* strains (T5 + T8)

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.631	2	0.316	502.221	0
Error	0.004	6	0.001		
Total	0.635	8			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.408	2	0.204	125.685	0
Error	0.01	6	0.002		
Total	0.417	8			

Figure 3-6. Dry weight of potato (cv. Desiree) after root inoculation with two isolates of *Trichoderma* (T5 + T8) and stem inoculation with *R. solani*

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.005	3	0.335	29.347	0
Error	0.091	8	0.011		
Total	1.096	11			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.658	3	0.219	24.235	0
Error	0.072	8	0.009		
Total	0.73	11			

Chapter 4

Table 4-1. Effect of *Rhizoctonia solani* AG-3PT on total number of sclerotia and the average number of tubers per plant of six varieties of potato.

Number of sclerotia

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	963.333	5	192.667	7.255	0.002
Error	318.667	12	26.556		
Total	1282	17			

Number of tubers

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	17.778	5	3.556	0.97	0.474
Error	44	12	3.667		
Total	61.778	17			

Figure 4-1. Number of sclerotia per tuber of six potato varieties infected with *Rhizoctonia solani* AG-3PT

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	59.98	5	11.996	30.466	0
Error	4.725	12	0.394		
Total	64.705	17			

Figure 4-2. Effect of *Rhizoctonia solani* AG-3PT on shoot dry weight of six potato varieties

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.834	5	0.167	67.177	0
Pathogen	0.312	1	0.312	125.739	0
Variety * Pathogen	0.075	5	0.015	6.028	0.001
Error	0.06	24	0.002		
Total	1.28	35			

Figure 4-3. Effect of*Rhizoctonia solani* AG-3PT on root dry weight of six potato varieties

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	28.978	5	5.796	26.241	0
Pathogen	17.126	1	17.126	77.54	0
Variety * Pathogen	3.997	5	0.799	3.62	0.014
Error	5.301	24	0.221		
Total	55.402	35			

Figure 4-4. Effect of *Rhizoctonia solani* AG-3PT on number of tubers of six potato varieties

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	97.472	5	19.494	9.357	0
Pathogen	1.361	1	1.361	0.653	0.427
Variety * Pathogen	32.139	5	6.428	3.085	0.027
Error	50	24	2.083		
Total	180.972	35			

Figure 4-5. Effect of *Rhizoctonia solani* AG-3PT on fresh weight of tubers of six potato varieties

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	8207.588	5	1641.518	13.781	0
Pathogen	2446.292	1	2446.292	20.538	0
Variety * Pathogen	2064.683	5	412.937	3.467	0.017
Error	2858.649	24	119.11		
Total	15577.212	35			

Figure 4-6. Effect of *Rhizoctonia solani* AG-3PT on the number of stolons of six potato varieties

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	181.222	5	36.244	9.665	0
Pathogen	40.111	1	40.111	10.696	0.003
Variety * Pathogen	16.556	5	3.311	0.883	0.508
Error	90	24	3.75		
Total	327.889	35			

Figure 4-7. The number of sclerotia on tubers of six potato varieties inoculated with *Rhizoctonia solani*

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	447.611	5	89.522	19.183	0
Error	56	12	4.667		
Total	503.611	17			

Figure 4-8. Growth rate of *Rhizoctonia solani* AG-3PT on water agar medium incorporating peel and sprout powder of six varieties of potatoes

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	2.722	5	0.544	19.029	0
Tissue	0.934	1	0.934	32.66	0
Variety * Tissue	3.992	5	0.798	27.907	0
Error	0.687	24	0.029		
Total	8.336	35			

Figure 4-9. Growth rate of *Trichoderma harzianum* T5 on water agar medium incorporating peel and sprout powder of six varieties of potatoes

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	9.469	5	1.894	74.107	0
Tissue	63.203	1	63.203	2473.141	0
Variety * Tissue	10.723	5	2.145	83.915	0
Error	0.613	24	0.026		
Total	84.007	35			

Figure 4-10. Growth rate of *Trichoderma hamatum* T8 on water agar medium incorporating peel and sprout powder of six varieties of potatoes

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	18.192	5	3.638	133.657	0
Tissue	103.361	1	103.361	3796.939	0
Variety * Tissue	14.876	5	2.975	109.29	0
Error	0.653	24	0.027		
Total	137.082	35			

Figure 4-11. Inhibition of growth of *Rhizoctonia solani* AG-3PT by *Trichoderma harzianum* T5 in dual-culture on water agar medium incorporating peel and sprout powder of six varieties of potatoes

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.127	5	0.025	57.693	0
Tissue	1.174	1	1.174	2666.283	0
Variety * Tissue	0.098	5	0.02	44.55	0
Error	0.011	24	0		
Total	1.41	35			

Figure 4-12. Inhibition of growth of *Rhizoctonia solani* AG-3PT by *Trichoderma hamatum* T8 in dual-culture on water agar medium incorporating peel and sprout powder of six varieties of potatoes

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.026	5	0.005	16.403	0
Tissue	0.344	1	0.344	1066.205	0
Variety * Tissue	0.103	5	0.021	63.702	0
Error	0.008	24	0		
Total	0.481	35			

Figure 4-13. Number of sclerotia per tuber of three potato varieties infected with *Rhizoctonia solani* AG-3PT

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	248	2	124	24.8	0.001
Error	30	6	5		
Total	278	8			

Tables 4-3 to 4-5. Effect of *Rhizoctonia solani* and two isolates of *Trichoderma* on growth and yield of 3 varieties of potato

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.149	2	0.074	6.096	0.008
Inoculation	0.738	3	0.246	20.178	0
Variety * Inoculation	0.167	6	0.028	2.278	0.072
Error	0.28	23	0.012		
Total	1.322	34			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.462	2	0.231	28.021	0
Inoculation	0.723	3	0.241	29.213	0
Variety * Inoculation	0.093	6	0.015	1.873	0.129
Error	0.19	23	0.008		
Total	1.478	34			

Number of stolons

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	36.821	2	18.41	11.143	0
Inoculation	145.852	3	48.617	29.426	0
Variety * Inoculation	4.533	6	0.756	0.457	0.832
Error	38	23	1.652		
Total	227.886	34			

Number of tubers

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	13.529	2	6.764	2.363	0.117
Inoculation	68.274	3	22.758	7.951	0.001
Variety * Inoculation	19.888	6	3.315	1.158	0.362
Error	65.833	23	2.862		
Total	162.686	34			

Tuber fresh weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	0.067	2	0.033	6.346	0.006
Inoculation	0.769	3	0.256	48.773	0
Variety * Inoculation	0.109	6	0.018	3.443	0.014
Error	0.121	23	0.005		
Total	1.083	34			

Chapter 5

Figure 5-1. Effect of calcium (Ca) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	0.008	4	0.002	1.095	0.386
Antagonist	0.009	1	0.009	4.939	0.038
Concentration * Antagonist	0.009	4	0.002	1.228	0.33
Error	0.037	20	0.002		
Total	0.063	29			

Figure 5-2. Effect of iron (Fe) concentration on inhibition of*Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	0.004	4	0.001	1.049	0.407
Antagonist	0.008	1	0.008	7.862	0.011
Concentration * Antagonist	0.003	4	0.001	0.72	0.589
Error	0.021	20	0.001		
Total	0.036	29			

Figure 5-3. Effect of potassium (K) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
ConcDual	461.59	4	115.397	8.945	0
FungDual	8.462	1	8.462	0.656	0.428
ConcDual * FungDual	316.022	4	79.006	6.124	0.002
Error	258.026	20	12.901		
Total	1044.1	29			

Figure 5-4. Effect of magnesium (Mg) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
ConcDual	0.023	4	0.006	4.298	0.011
FungDual	0.004	1	0.004	3.269	0.086
ConcDual * FungDual	0.006	4	0.001	1.083	0.391
Error	0.026	20	0.001		
Total	0.059	29			

Figure 5-5. Effect of manganese (Mn) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	0.021	4	0.005	10.828	0
Antagonist	0.012	1	0.012	24.713	0
Concentration * Antagonist	0.021	4	0.005	10.898	0
Error	0.01	20	0		
Total	0.064	29			

Figure 5-6. Effect of nitrogen (N) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
ConcDual	0.012	4	0.003	3.551	0.024
FungDual	0.026	1	0.026	29.836	0
ConcDual * FungDual	0.02	4	0.005	5.709	0.003
Error	0.017	20	0.001		
Total	0.075	29			

Figure 5-7. Effect of phosphorus (P) concentration on inhibition of *Rhizoctonia solani* colony growth by two *Trichoderma* isolates in dual culture

Source	Sum of Squares	df	Mean Square	F	Sig.
ConcDual	0.005	4	0.001	2.375	0.087
FungDual	0.008	1	0.008	17.113	0.001
ConcDual * FungDual	0.02	4	0.005	10.3	0
Error	0.009	20	0		
Total	0.042	29			

Figure 5-8. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of calcium

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	1.919	4	0.48	16.229	0
Fungus	11.426	2	5.713	193.293	0
Concentration * Fungus	4.192	8	0.524	17.729	0
Error	0.887	30	0.03		
Total	18.423	44			

Figure 5-9. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of iron

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	3.11	4	0.777	13.29	0
FungAnti	12.818	2	6.409	109.557	0
Concentration * FungAnti	5.447	8	0.681	11.639	0
Error	1.755	30	0.059		
Total	23.13	44			

Figure 5-10. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of potassium

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	4.796	4	1.199	10.706	0
FungAnti	20.934	2	10.467	93.454	0
Concentration * FungAnti	3.713	8	0.464	4.144	0.002
Error	3.36	30	0.112		
Total	32.803	44			

Figure 5-11. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of magnesium

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	3.724	4	0.931	8.089	0
Fungus	14.568	2	7.284	63.28	0
Concentration * Fungus	3.472	8	0.434	3.77	0.004
Error	3.453	30	0.115		
Total	25.218	44			

Figure 5-12. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of manganese

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	7.552	4	1.888	56.643	0
Fungus	7.116	2	3.558	106.747	0
Concentration * Fungus	6.877	8	0.86	25.788	0
Error	1	30	0.033		
Total	22.546	44			

Figure 5-13. Colony growth after5 days growth at20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of nitrogen

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	2.775	4	0.694	11.228	0
Fungus	14.88	2	7.44	120.435	0
Concentration * Fungus	3.451	8	0.431	6.982	0
Error	1.853	30	0.062		
Total	22.959	44			

Figure 5-14. Colony growth after5 days growthat 20ºC of *R. solani* on low sugar PDA amended with culture filtrate from *Trichoderma* isolates or *R. solani* grown in Hoagland solution amended with different concentrations of phosphorus

Source	Sum of Squares	df	Mean Square	F	Sig.
Concentration	5.148	4	1.287	20.038	0
FungAnti	10.288	2	5.144	80.097	0
Concentration * FungAnti	1.41	8	0.176	2.744	0.021
Error	1.927	30	0.064		
Total	18.772	44			

Figure 5-15. Effect of standard concentration of Hoagland solution on shoot dry weight of potato plants (cv. Desiree) with/without *R. solani* (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient	0.213	1	0.213	28.738	0
Rs	0.065	1	0.065	8.725	0.012
Nutrient * Rs	0	1	0	0.034	0.857
Error	0.089	12	0.007		
Total	0.366	15			

Figure 5-16. Effect of standard concentration of Hoagland solution on tuber fresh weight of potato plants (cv. Desiree) with/without *R. solani* (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient	0.109	1	0.109	10.52	0.007
Rs	0.122	1	0.122	11.754	0.005
Nutrient * Rs	0.064	1	0.064	6.14	0.029
Error	0.124	12	0.01		
Total	0.419	15			

Table 5-4. Effect of different concentrations of K by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production

Shoot dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	81.163	3	27.054	10.748	0
Potassium	21.914	2	10.957	4.353	0.021
Treatment * Potassium	29.833	6	4.972	1.975	0.096
Error	88.102	35	2.517		
Total	220.838	46			

Root dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	34.367	3	11.456	26.237	0
Potassium	0.879	2	0.439	1.006	0.376
Treatment * Potassium	20.878	6	3.48	7.969	0
Error	15.282	35	0.437		
Total	73.633	46			

Number of stolons

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	139.158	3	46.386	5.494	0.003
Potassium	14.225	2	7.113	0.842	0.439
Treatment * Potassium	41.875	6	6.979	0.827	0.557
Error	295.5	35	8.443		
Total	495.702	46			

Number of tubers

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	55.363	3	18.454	3.774	0.019
Potassium	5.689	2	2.844	0.582	0.564
Treatment * Potassium	66.29	6	11.048	2.259	0.06
Error	171.167	35	4.89		
Total	300.851	46			

Tuber fresh weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	23574.508	3	7858.169	3.162	0.037
Potassium	10629.569	2	5314.784	2.139	0.133
Treatment * Potassium	3943.213	6	657.202	0.264	0.95
Error	86979.44	35	2485.127		
Total	124233.947	46			

Plant height

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	3578.717	3	1192.906	27.729	0
Potassium	729.72	2	364.86	8.481	0.001
Treatment * Potassium	642.522	6	107.087	2.489	0.041
Error	1505.714	35	43.02		
Total	6543.137	46			

Table 5-5. Effect of different concentrations of N by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production

Shoot dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	72.08	3	24.027	6.975	0.002
Nitrogen	8.601	1	8.601	2.497	0.127
Treatment * Nitrogen	0.479	3	0.16	0.046	0.986
Error	82.673	24	3.445		
Total	163.833	31			

Root dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	17.561	3	5.854	9.811	0
Nitrogen	12.802	1	12.802	21.457	0
Treatment * Nitrogen	3.808	3	1.269	2.127	0.123
Error	14.319	24	0.597		
Total	48.49	31			

Number of stolons

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	76.844	3	25.615	2.237	0.11
Nitrogen	16.531	1	16.531	1.444	0.241
Treatment * Nitrogen	9.594	3	3.198	0.279	0.84
Error	274.75	24	11.448		
Total	377.719	31			

Number of tubers

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	36.25	3	12.083	2.248	0.109
Nitrogen	45.125	1	45.125	8.395	0.008
Treatment * Nitrogen	31.125	3	10.375	1.93	0.152
Error	129	24	5.375		
Total	241.5	31			

Tuber fresh weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	25426.681	3	8475.56	4.9	0.009
Nitrogen	828.042	1	828.042	0.479	0.496
Treatment * Nitrogen	5348.646	3	1782.882	1.031	0.397
Error	41516.848	24	1729.869		
Total	73120.217	31			

Plant height

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	2113.461	3	704.487	16.713	0
Nitrogen	185.281	1	185.281	4.396	0.047
Treatment * Nitrogen	150.591	3	50.197	1.191	0.334
Error	1011.635	24	42.151		
Total	3460.969	31			

Table 5-6. Effect of different concentrations of Mn by using Hoagland solution and activity of *Trichoderma* on plant growth and potato production

Shoot dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	53.913	3	17.971	8.828	0
Manganese	22.378	1	22.378	10.993	0.003
Treatment * Manganese	12.433	3	4.144	2.036	0.136
Error	48.857	24	2.036		
Total	137.582	31			

Root dry weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	13.677	3	4.559	7.188	0.001
Manganese	6.938	1	6.938	10.938	0.003
Treatment * Manganese	6.066	3	2.022	3.188	0.042
Error	15.222	24	0.634		
Total	41.903	31			

Number of stolons

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	42.344	3	14.115	1.481	0.245
Manganese	38.281	1	38.281	4.016	0.056
Treatment * Manganese	78.094	3	26.031	2.731	0.066
Error	228.75	24	9.531		
Total	387.469	31			

Number of tubers

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	48.625	3	16.208	1.87	0.162
Manganese	32	1	32	3.692	0.067
Treatment * Manganese	50.25	3	16.75	1.933	0.151
Error	208	24	8.667		
Total	338.875	31			

Tuber fresh weight

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	13299.059	3	4433.02	2.385	0.094
Manganese	11978.811	1	11978.811	6.446	0.018
Treatment * Manganese	5069.122	3	1689.707	0.909	0.451
Error	44601.036	24	1858.376		
Total	74948.028	31			

Plant height

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	3687.644	3	1229.215	31.099	0
Manganese	2.101	1	2.101	0.053	0.82
Treatment * Manganese	618.104	3	206.035	5.213	0.006
Error	948.63	24	39.526		
Total	5256.479	31			

Figure 5-17. Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on shoot dry weight of potato plants (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Trichoderma	1.501	1	1.501	56.619	0
Fertilizer	2.099	3	0.7	26.391	0
Trichoderma * Fertilizer	0.462	3	0.154	5.809	0.004
Error	0.636	24	0.027		
Total	4.698	31			

Figure 5-18. Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on number of tubers of potato plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Trichoderma	15.125	1	15.125	6.482	0.018
Fertilizer	34.5	3	11.5	4.929	0.008
Trichoderma * Fertilizer	6.375	3	2.125	0.911	0.451
Error	56	24	2.333		
Total	112	31			

Figure 5-19. Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on tuber fresh weight of potato plants (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Trichoderma	0.317	1	0.317	31.367	0
Fertilizer	0.522	3	0.174	17.215	0
Trichoderma * Fertilizer	0.195	3	0.065	6.439	0.002
Error	0.242	24	0.01		
Total	1.276	31			

Figure 5-20. Effect of application of *Trichoderma hamatum* T8 and different levels of K, N or NPK on shoot dry weight of potato plants (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	0.458	1	0.458	75.399	0
Fertilizer	0.22	3	0.073	12.058	0
Fungus * Fertilizer	0.062	3	0.021	3.383	0.044
Error	0.097	16	0.006		
Total	0.837	23			

Chapter 6

Figure 6-1. Dry weight of shoots and roots inoculated with *R. solani* AG-3PT on two-week-old broccoli and cabbage seedlings

Probability in t tests

	Shoot	Root
Cabbage	0.276921	0.228076
Broccoli	0.013363	0.073759

Figure 6-2. Dry weight of shoots and roots inoculated with *R. solani* AG-3PT on four-week-old broccoli and cabbage seedlings

Probability in t tests

	Root	Shoot
Cabbage	0.72402	0.872499
Broccoli	0.529357	0.307943

Figure 6-3. Ratio of dry weights of broccoli, cabbage and potato seedlings inoculated with *Trichoderma* strains to dry weights of control plants in potting mix

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	7.598	2	3.799	1079.259	0
Fungus	2.016	2	1.008	286.296	0
Plant * Fungus	0.171	4	0.043	12.149	0
Error	0.063	18	0.004		
Total	9.848	26			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	9.097	2	4.549	541.583	0
Fungus	1.137	2	0.569	67.707	0
Plant * Fungus	0.088	4	0.022	2.612	0.07
Error	0.151	18	0.008		
Total	10.473	26			

Figure 6-4. Ratio of dry weights of broccoli, cabbage and potato seedlings inoculated with *Trichoderma* strains to dry weights of control plants in Kirby soil

Shoot dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	8.685	2	4.342	1989.453	0
Fungus	0.977	2	0.489	223.848	0
Plant * Fungus	0.153	4	0.038	17.527	0
Error	0.033	15	0.002		
Total	9.782	23			

Root dry weight (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	11.311	2	5.656	1998.581	0
Fungus	0.857	2	0.428	151.375	0
Plant * Fungus	0.091	4	0.023	8.069	0.001
Error	0.042	15	0.003		
Total	12.278	23			

Figure 6-5. Mean radial growth of*R. solani* (Rs) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	1.371	2	0.685	89.384	0
Tissue	10.602	2	5.301	691.449	0
Plant * Tissue	1.852	4	0.463	60.399	0
Error	0.153	20	0.008		
Total	24.659	29			

Figure 6-6. Mean radial growth of *T. hamatum* (T8) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	1.772	2	0.886	24.214	0
Tissue	16.295	2	8.147	222.711	0
Plant * Tissue	2.493	4	0.623	17.039	0
Error	0.732	20	0.037		
Total	23.452	29			

Figure 6-7. Mean radial growth of *T. harzianum* (T5) exposed to volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	4.943	2	2.471	25.479	0
Tissue	63.503	2	31.751	327.335	0
Plant * Tissue	3.708	4	0.927	9.557	0
Error	1.94	20	0.097		
Total	80.879	29			

Figure 6-8. Mean radial growth of *R. solani* (Rs) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	2.677	2	1.339	144.715	0
Tissue	9.465	2	4.733	511.622	0
Plant * Tissue	1.984	4	0.496	53.634	0
Error	0.185	20	0.009		
Total	23.222	29			

Figure 6-9. Mean radial growth of *T. hamatum* (T8) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	4.88	2	2.44	155.745	0
Tissue	0.487	2	0.243	15.532	0
Plant * Tissue	2.153	4	0.538	34.362	0
Error	0.313	20	0.016		
Total	7.835	29			

Figure 6-10. Mean radial growth of *T. harzianum* (T5) exposed to non-volatiles derived from freeze-dried tissues of *Brassica* plants

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	1.407	2	0.704	301.587	0
Tissue	50.312	2	25.156	10781.11	0
Plant * Tissue	0.033	4	0.008	3.492	0.026
Error	0.047	20	0.002		
Total	60.155	29			

Figure 6-11. Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to volatiles from freeze-dried *Brassica* tissues

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	2634.694	2	1317.347	31.956	0
Part	4470.183	2	2235.091	54.218	0
Isolate	354.128	1	354.128	8.59	0.006
Plant * Part	5523.784	4	1380.946	33.499	0
Plant * Isolate	1529.434	2	764.717	18.55	0
Part * Isolate	1300.402	2	650.201	15.772	0
Plant * Part * Isolate	490.893	4	122.723	2.977	0.032
Error	1484.063	36	41.224		
Total	17787.58	53			

Figure 6-12. Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to non-volatiles from freeze-dried *Brassica* tissues

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	30.22	2	15.11	0.983	0.387
Part	214.176	2	107.088	6.967	0.004
Isolate	273.857	1	273.857	17.816	0
Plant * Part	1035.206	4	258.802	16.837	0
Plant * Isolate	29.869	2	14.935	0.972	0.391
Part * Isolate	44.973	2	22.486	1.463	0.249
Plant * Part * Isolate	37.404	4	9.351	0.608	0.66
Error	430.398	28	15.371		
Total	2143.709	45			

Figure 6-13. Inhibition of growth of *R. solani* in dual culture with *Trichoderma* isolates exposed to non-volatiles from cabbage and broccoli roots

Source	Sum of Squares	df	Mean Square	F	Sig.
Root	159.577	2	79.789	13.398	0.001
Fungus	2.11	1	2.11	0.354	0.563
Root * Fungus	71.343	2	35.672	5.99	0.016
Error	71.465	12	5.955		
Total	304.496	17			

Figure 6-14. Dry mycelial biomass from *T. hamatum* T8 after 10 days growth in a liquid medium supplemented with various parts of plant tissue

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	5.27E-05	2	2.63E-05	3.235	0.062
Tissue	0	2	9.54E-05	11.725	0
Plant * Tissue	7.84E-05	4	1.96E-05	2.409	0.085
Error	0	19	8.14E-06		
Total	0.001	28			

Figure 6-15. Dry mycelial biomass from *T. harzianum* T5 after 10 days growth in a liquid medium supplemented with various parts of plant tissue

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	0	2	6.69E-05	6.812	0.006
Tissue	0	2	5.49E-05	5.591	0.012
Plant * Tissue	0	4	0	11.907	0
Error	0	19	9.83E-06		
Total	0.001	28			

Figure 6-16. Dry mycelial biomass from *R. solani* AG-3PT after 10 days growth in a liquid medium supplemented with various parts of plant tissue

Source	Sum of Squares	df	Mean Square	F	Sig.
Plant	0.001	2	0.001	22.501	0
Tissue	0.005	2	0.003	106.049	0
Plant * Tissue	0	4	8.94E-05	3.584	0.024
Error	0	19	2.49E-05		
Total	0.009	28			

Figure 6-17. Inhibition of *R. solani* in dual culture by root-exudates derived from broccoli, cabbage and potato plants, and isolates of *Trichoderma*

Source	Sum of Squares	df	Mean Square	F	Sig.
Antagonist	2285.193	2	1142.596	162.854	0
Exudate	6658.263	2	3329.132	474.501	0
Antagonist * Exudate	73.219	4	18.305	2.609	0.07
Error	126.289	18	7.016		
Total	9142.964	26			

Figure 6-18. The effect of carrier materials on biocontrol agent of *T. hamatum* (T8) on shoot dry weight of cabbage seedlings

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	2143125	3	714375	23.902	0
Error	358650	12	29887.5		
Total	2501775	15			

Figure 6-19. The effect of carrier materials on biocontrol agent of *T. hamatum* (T8) on root dry weight of cabbage seedlings

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	5850	3	1950	22.286	0
Error	1050	12	87.5		
Total	6900	15			

Chapter 7

Table 7-1. Effects of intercropping with cabbage or broccoli and inoculation with*Rhizoctonia solani* AG-3PT and *Trichoderma hamatum* T8 on root dry weight and number of tubers of potato plants in two repeats of a glasshouse experiment

Root dry weight, experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	1.294	2	0.647	5.781	0.012
Crop	0.244	2	0.122	1.09	0.357
Fungus * Crop	0.152	4	0.038	0.34	0.847
Error	2.014	18	0.112		
Total	3.704	26			

Number of tubers, experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	2.113	2	1.056	0.412	0.668
Crop	0.255	2	0.128	0.05	0.952
Fungus * Crop	11.523	4	2.881	1.125	0.376
Error	46.111	18	2.562		
Total	60.001	26			

Root dry weight, experiment 2 (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Intercrop	0.103	2	0.051	10.182	0.001
Treatment	0.066	2	0.033	6.539	0.007
Intercrop * Treatment	0.061	4	0.015	3.01	0.046
Error	0.091	18	0.005		
Total	0.32	26			

Number of tubers, experiment 2

Source	Sum of Squares	df	Mean Square	F	Sig.
Intercrop	156.306	2	78.153	7.6	0.004
Treatment	22.28	2	11.14	1.083	0.36
Intercrop * Treatment	137.947	4	34.487	3.354	0.032
Error	185.108	18	10.284		
Total	501.641	26			

Figure 7-1. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato intercropped with broccoli and cabbage

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	63.543	2	31.772	4.735	0.022
Crop	110.193	2	55.096	8.211	0.003
Fungus * Crop	20.307	4	5.077	0.757	0.567
Error	120.778	18	6.71		
Total	314.821	26			

Figure 7-2. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato intercropped with broccoli and cabbage (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	0.12	2	0.06	4.06	0.035
Crop	0.137	2	0.068	4.643	0.024
Fungus * Crop	0.102	4	0.025	1.726	0.188
Error	0.265	18	0.015		
Total	0.623	26			

Figure 7-3. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage and broccoli intercropped with potato

Broccoli

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	1.688	2	0.844	0.039	0.962
Error	129.956	6	21.659		
Total	131.644	8			

Cabbage

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	21.724	2	10.862	6.226	0.034
Error	10.468	6	1.745		
Total	32.193	8			

Figure 7-4. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on root dry weight of cabbage and broccoli intercropped with potato

Broccoli

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	2.626	2	1.313	3.839	0.084
Error	2.052	6	0.342		
Total	4.678	8			

Cabbage

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	0.591	2	0.296	6.794	0.029
Error	0.261	6	0.044		
Total	0.852	8			

Figure 7-5. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato intercropped with broccoli and cabbage in a repeat assay (log transformed)

Source	Sum of Squares	df	Mean Square	F	Sig.
Intercrop	0.692	2	0.346	18.566	0
Treatment	0.155	2	0.077	4.151	0.033
Intercrop * Treatment	0.311	4	0.078	4.171	0.015
Error	0.335	18	0.019		
Total	1.493	26			

Figure 7-6. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato intercropped with broccoli and cabbage in a repeat assay

Source	Sum of Squares	df	Mean Square	F	Sig.
Intercrop	31.723	2	15.862	0.841	0.447
Treatment	361.632	2	180.816	9.592	0.001
Intercrop * Treatment	241.082	4	60.271	3.197	0.038
Error	339.311	18	18.851		
Total	973.748	26			

Figure 7-7. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage and broccoli intercropped with potato in a repeat assay

Broccoli

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	28	2	14	4.05	0.077
Error	20.74	6	3.457		
Total	48.74	8			

Cabbage

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	72.698	2	36.349	8.022	0.02
Error	27.188	6	4.531		
Total	99.886	8			

Figure 7-8. Effect of inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on root dry weight of cabbage and broccoli intercropped with potato in a repeat assay

Broccoli

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	0.354	2	0.177	2.3	0.181
Error	0.462	6	0.077		
Total	0.816	8			

Cabbage

Source	Sum of Squares	df	Mean Square	F	Sig.
Fungus	0.351	2	0.175	3.218	0.112
Error	0.327	6	0.054		
Total	0.678	8			

Figure 7-9. Effects of intercropping with cabbage and inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of potato plants in a field trial

Source	Sum of Squares	df	Mean Square	F	Sig.
Pathogen	1.829	1	1.829	0.156	0.696
T8	1893.74	1	1893.74	161.984	0
Intercrop	2714.924	1	2714.924	232.225	0
Pathogen * T8	315.319	1	315.319	26.971	0
Pathogen * Intercrop	26.993	1	26.993	2.309	0.142
T8 * Intercrop	166.486	1	166.486	14.241	0.001
Pathogen * T8 * Intercrop	521.887	1	521.887	44.64	0
Error	280.582	24	11.691		
Total	5921.759	31			

Figure 7-10. Effects of intercropping with cabbage and inoculation with *Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on number of tubers of potato plants in a field trial

Source	Sum of Squares	df	Mean Square	F	Sig.
Pathogen	22.781	1	22.781	6.856	0.015
T8	9.031	1	9.031	2.718	0.112
Intercrop	52.531	1	52.531	15.809	0.001
Pathogen * T8	19.531	1	19.531	5.878	0.023
Pathogen * Intercrop	7.031	1	7.031	2.116	0.159
T8 * Intercrop	0.031	1	0.031	0.009	0.924
Pathogen * T8 * Intercrop	19.531	1	19.531	5.878	0.023
Error	79.75	24	3.323		
Total	210.219	31			

Figure 7-11. Effects of intercropping with cabbage and inoculation with*Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on tuber fresh weight of potato plants in a field trial

Source	Sum of Squares	df	Mean Square	F	Sig.
Pathogen	83508.65	1	83508.65	28.401	0
T8	466025.129	1	466025.129	158.493	0
Intercrop	898225.5	1	898225.5	305.483	0
Pathogen * T8	58183.986	1	58183.986	19.788	0
Pathogen * Intercrop	95594.874	1	95594.874	32.511	0
T8 * Intercrop	145504.5	1	145504.5	49.486	0
Pathogen * T8 * Intercrop	143087.089	1	143087.089	48.663	0
Error	70568.246	24	2940.344		
Total	1960697.974	31			

Figure 7-12. Effect of inoculation with*Rhizoctonia solani* AG-3PT (Rs) and *Trichoderma hamatum* (T8) on shoot dry weight of cabbage intercropped with potato in a field trial

Source	Sum of Squares	df	Mean Square	F	Sig.
Tricho	2026673	1	2026673	55.102	0
Rhizoc	1619.459	1	1619.459	0.044	0.837
Tricho * Rhizoc	33.843	1	33.843	0.001	0.976
Error	441360.9	12	36780.07		
Total	2469687	15			