POTENTIAL OF *BRASSICA NAPUS* AND *TRICHODERMA HARZIANUM* IN CONTROL OF POWDERY SCAB (*Spongospora subterranea* f.sp.*subterranea.)* OF POTATO (*Solanum tuberosum* L.*)*

BY

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A dissertation submitted in partial fulfillment of the requirements of the degree of Masters of Science in Crop Science

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DECLARATION

I hereby declare that this dissertation prepared for the Masters of Science degree in Crop Science which I submitted to the Faculty of Agriculture and Natural Resources Management of Midlands State University of Zimbabwe December 2014 is my original work. All source of literature and materials used for this study has been duly acknowledged. I also agree that the Midlands State University has the sole right to the publication of this dissertation.

Signed on …….. at the Midlands State University, Gweru, Zimbabwe

Signature…….

Manditsvara Hilda

The undersigned certify that they have read and recommended for submission to the department of Agronomy, in partial fulfilment of the requirements of the Master of Science Degree in Crop Science, a dissertation by Manditsvara Hilda Tarisai entitled: Potential of *Brassica napus* and *Trichoderma harzianum* for the control of powdery scab (*Spongospora subterranea* f.sp. *subterranea*) in potato (*Solanum tuberosum* L.)

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ABSTRACT

Potato (*Solanum tuberosum* L.) is one of the most important crops grown in Zimbabwe owing to its nutritional and economic benefits. However, its production is often adversely affected by soilborne fungal pathogens such as *Spongospora subterranea* f.sp *subterranea*, the causal agent of powdery scab on potato tubers and the vector of Potato Mop Top Virus. The fungal pathogen forms root galls on roots and lesions on tubers. *Brassica* crops incorporated in crop rotations or applied as green manures have been associated with reduction in soilborne pathogens. Biological control of soilborne plant pathogens by the addition of antagonistic microorganisms to the soil such as *Trichoderma* spp. has also been suggested. In this study, two experiments were set up in a glasshouse with 34^0C (±3) and 21^0C (±3) day and night temperature respectively at Harare Research Station to determine the efficacy of *B. napus* and *T. harzianum*in controlling*S. subterranea* in potato. The first experiment was a 5 x 3 factorial in a CRD with three replications to investigate the effect of *B. napus* on the incidence and severity of powdery scab on potato. The first factor was *B. napus* at 10%, 20%, 30% rates with Mancozeb (positive control) and negative control where no control amendment was added. The second factor was potato variety and the levels were BP1, Diamond and Mondial. *B. napus* 20% reduced disease incidence by 31%, severity by 37% (root galls) and 67% (tubers). *B. napus* 20% on Mondial resulted inhigh proportion of 100% and 175% on marketable yield, extra-large and large tubers grade respectively compared to the positive control, Mancozeb on Mondial. The second experiment was a 5 x 3 factorial in a CRD with three replications to assess the effect of *T. harzianum* on the incidence and severity of powdery scab in potato. The first factor was *T. harzianum*rates were $1gl^{-1}$, $2g\ l^{-1}$, $3g\ l^{-1}$ at 1 x 10^7CFU$g^{-1}$with Mancozeb (positive control) and a negative control were no control amendment was applied. The second factor was potato variety and the levels were BP1, Diamond and Mondial.*T. harzianum* 1 x 10^7CFUg^{-1} $2g\ l^{-1}$ reduced disease incidence by 26% and severity by 38% (root galls) and 59% (tubers). *T. harzianum* 1 x 10^7CFUg^{-1} $2g\ l^{-1}$ on Mondial resulted in 343 % increase in proportion of extra-large tubers compared with the positive control, Mancozeb on Mondial.

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DEDICATION

I dedicate this dissertation:

- To my husband Onias, our sons, Anashe Ryan and Akatidaishe Keane. Thank you for your love and support.
- To my parents Isaac and Ethel. Thank you for being a pillar of strength, love and support

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ABBREVIATIONS

DAP	Days after planting
CFU	Colony forming unit
%	Percentage
ºC	Degrees Celsius
CRD	Completely Randomized Block Design
C.V	Coefficient of variation

CHAPTER 1

1.0INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth largest yielding crop in the world after wheat, rice and maize (FAOSTAT, 2010). Demand for potatoes in sub-Saharan Africa is projected to have a 250% increase between 1993 and 2020, with an annual growth in demand of 3.1% and the growth in area under production is estimated at 1.25% a year (Scott *et al*., 2000). It is also one of the most popular food crops grown in Zimbabwe as a substitute staple third after maize and rice (Chigumira wa Ngwerume, 2002). Potato is widely grown due to its varied uses which include chips (French fries), which are often ready prepared for catering, for crisps, as a vegetable relish/salad, for canning and for livestock feeding (from the surplus taken from normal market leftovers or the damaged, unmarketable tubers as a price support measure) (Manzira, 2010).

Although potatoes have high nutritional and economic benefits, they are prone to a wide range of pathogens that drastically reduce yield (Agrios, 2005). Powdery scab, a soilborne fungal disease caused by infection with *Spongospora subterranea* (Wallroth) Lagerheim f.sp. *subterranean* Tomlinson (*S. subterranea*) adversely affects potato.The fungal disease is expressed as unsightly pustules covering the tuber surface and renders stocks unsuitable for the pre-pack market. Infection of the root or stolon can sometimes lead to development of a gall which impairs normal root functions and hence reduce yield of potato (Falloon, 2008). The causal agent of powdery scab, *S. subterranea*, is also an important vector for Potato Mop-Top Virus(PMTV), a disease that causesspraing, a significant production limiting disease particularly in cool climatic zones (Xu *et al*., 2004).

The pathogen, *S. subterranea*, is difficult to control because once an area is infested, it will persist indefinitely (Larkin and Griffin, 2007). *S. subterranea* produces many resting spores which can remain dormant for at least twelve years and are highly resistant to environmental stresses and cultural practices (Merz and Falloon, 2009). Some limited reduction of the infection level of *S. subterranea* has been obtained by treating infested soils with organic pesticides and zinc-compounds (Wale, 2002). Although the chemicals registered against powdery scab can be effective, the cost of particularly soil applications is extremely high and control is not always consistent (Larkin and Griffin, 2006). Furthermore, use of synthetic pesticides can result in poisoning of farmers through inappropriate spraying methods, poisoning of consumers through high residual levels of these chemicals, elimination of non-target organisms, as well as selection of phytopathogens, pest and weed insensitive to certain active ingredients (Stangarlin *et al*., 2011). This has led to increased global awareness on the risks involved in use of pesticides which necessitates the consideration of alternative disease management methods (Fan *et al*., 2008).

One approach with potential to control *S. subterranea* is the use of *Brassica* spp. such as rape *(Brassica napus*), cabbage (*Brassica oleracea capitata*) and kale (*Brassica oleracea* var *acephala)* amongst others, as cover, rotation or green manure in a process termed biofumigation (Larkin and Griffin, 2007). Biofumigation is the process of using volatile chemicals (allelochemicals) released from decomposing plant tissues to suppress pests and diseases (Matthiessen and Shackleton, 2005). In this study, *Brassica napus* (*B. napus*), was considered for biofumigation of *S. subterranea* as it is readily available since it is grown for consumption in Zimbabwe and is non-polluting to the environment.

The use of biological control agents to control soilborne pathogens has been proven in other members of the Plasmodiphorids, to which *S. subterranea* belongs (Nielsen, 2003). The use of fungus in the genus *Trichoderma*, alone or in combination with other beneficial micro-organisms, is reported to be one of the most effective for disease control in various crops (Howell, 2003). Furthermore, Nielsen and Larsen, 2004, found out that biological control agents, particularly *Trichoderma* spp., have potential for reducing activity of *S. subterranea*. *Trichoderma harzianum* (*T harzianum*) biocontrol capacity is through competition, parasitism, production of inhibitor compounds and enzymes or inactivation of the pathogen's enzyme systems (Lester, 2009). The use of *T harzianum* was considered as it is readily available through isolation from the soil, effective in control and non-polluting to the environment.

In view of the current research in the use of *B. napus* and *T harzianum* in controlling soilborne pathogens, the rate at which these two control options are applied to achieve effective control is not yet clear. The research seeks to establish the optimum rates at which *B. napus* and *T harzianum* can be applied to achieve the highest level of efficacy against *S. subterranea*.

1.1OBJECTIVES

1.1.1 Overall Objective

To determine the efficacy of *T harzianum* and *B. napus* in controlling powdery scab (*S. subterranea*) in potato (*Solanum tuberosum L.*).

1.1.2 Specific objectives

Experiment 1

1. To evaluate the effect of *B. napus*and variety on the growth (stem diameter and stem length) of potato.

2. To evaluate the effect of *B. napus*and variety on the yield (fresh tuber weight) and proportion of marketable tubers (grades) of potato

3. To determine the effect of *B. napus* and variety on incidence and severity of powdery scab (*S. subterranea)* on potato.

Experiment 2

1. To evaluate the effect of *T. harzianum* and variety on the growth (stem diameter and stem length) and yield (tuber fresh weight) of potato.

2. To evaluate the effect of *T. harzianum* and variety on the yield (fresh tuber weight) and proportion of marketable tubers (grades) of potato.

3. To determine the effect of *T. harzianum* and variety on incidence and severity of powdery scab (*S. subterranea*) on potato.

1.1.3 Hypotheses

Experiment 1

1. *B. napus* and variety have an effect on the growth (stem diameter and stem length) and yield (tuber fresh weight) in potato

2. *B. napus* and variety have an effect on yield (fresh tuber weight) and proportion of marketable tubers (grades) of potato

3. *B. napus* and variety have an effect of reducing the incidence and severity of powdery scab (*S. subterranea*) in potato.

Experiment 2

1. *T. harzianum* and variety have an effect of reducing the incidence and severity of powdery scab (*S. subterranea*) in potato.

2. *T. harzianum* and variety have an effect on the growth (stem diameter and stem length) and yield (tuber fresh weight) in potato.

3. *T. harzianum* and variety have an effect on yield (fresh tuber weight) and proportion of marketable tubers (grades) of potato

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The economic value of potato

Irish potato (*S. tuberosum* L) is the world's number one non-grain food crop and the fourth main food crop grown in the world after maize, rice and wheat (Cunnington, 2008).It is also one of the most popular food crops grown in Zimbabwe as a substitute staple third after maize and rice (Chigumira wa Ngwerume, 2002). It is widely grown due to its varied uses which include chips (French fries), which are often ready prepared for catering; for crisps; as a vegetable relish/salad; for canning and for livestock feeding (from the surplus taken from normal market leftovers or the damaged, unmarketable tubers as a price support measure) (Manzira, 2010).

Although the economic value of potatoes is clear, potato crops are susceptible to more than 40 pests and diseases (Fiers *et al*.,2012). Powdery scab of potatoes is one such disease caused by the fungal soilborne pathogen *S. subterranea*.

2.2 Powdery scab in potatoes

Powdery scab was first described in local crops in Braunschweig, Germany in 1841 (Wallroth, 1842 in Wright, 2012).However, since then, the disease has been reported in many potato growing regions. The pathogen *S. subterranea* is thought to have originated from the Andes, where it spread to the rest of the world, presumably through trade and contaminated seed tubers (Harrison *et al*., 1997 in Wright, 2012). The increased use of certain agricultural practices has led to the development of micro-climates that favour powdery scab development (Nakayama *et al*., 2007; Merz, 2008). These practices include intensification of potato production leading to shorter

rotations and the build-up of inoculum in potato growing fields, increased use of irrigation which favours tuber infection and planting of potato varieties susceptible to the disease (Falloon *et al*., 2008).

2.3 Classification of *S. subterranea*

The classification of *S. subterranea* is complex as it was previously regarded as a distinct class of fungi, the Plasmodiophoromycetes, within the division Myxomycota (Clay and Walsh, 1997). Recent studies by Adl *et al*., 2012 classified Plasmodiophorids as Phytomyxea within the Cercozoa which is in super group known as the Rhizaria or Protozoa. There are three species of *Spongospora*, namely, *Spongospora subterranea* which is parasitic in Solanacea, *Spongospora capanulae* which is parasitic in Campanula and *Spongospora cotulae* which is parasitic in Cotula. Of the three species, *Spongospora subterranea*, the cause of powdery scab, is considered of economic importance (Merz and Falloon, 2009).

S. subterranea is an obligate parasite that cannot be cultured on any known media (Qu and Christ, 2007). One method of identifying this pathogen is by observation of the zoospore flagella under a microscope. Biflagellate zoospores develop from the resting spores and are unequal in length, apically attached and oppositely directed. The size of the individual zoospore is between 3 to 4 µm in diameter (Merz, 1992 in Wright, 2012).

There are however two distinct sub species of *S.subterranea* that are morphologically indistinguishable but are differentiated on the basis of their host range. *S. subterranea* fsp *nasturtii* Tomlinson infects watercress and not potato and tomato whereas *S. subterranea* f.sp. *subterranea* infects potato and tomato and not watercress (Spencer and Glassock, 1953 in Harrison *et al.*, 1997).

2.4 Symptoms caused by *S. subterranea* on potatoes

The initial visible symptom of powdery scab is the development of purple-brown pimple-like swellings at the rose end of the tubers (Harrison *et al*., 1997). Individual circular scab lesions develop to approximately 10 µm in size and the shapes may become irregular when the lesions become large and merge together (Genet *et al*., 2005). Mature lesions become hollow and filled with a powdery mass of sporeballs. Visual expression of *S. subterranea* is observed as powdery lesions on the surfaces of tubers, and thus the name powdery scab was derived for this disease (Falloon, 2008; Nitzan *et al*., 2009). The lesions are scab-like in appearance and contain sporeballs (Qu *et al*., 2006). Sporeballs, also known as cystosori, are sponge-like and are between 19-85 µm in size (van de Graaf *et al*., 2007). Other symptoms may include galls and cankers which develop on the roots and tubers resulting in their gross deformities (van de Graaf *et al*., 2003). Galls may also develop on potato stolons and roots becoming dark brown as they mature (van de Graaf *et al*., 2003).

Powdery scab symptoms are often confused with the symptoms of common scab (*Streptomycesscabies* (Thaxt.). These two diseases both cause quality limiting scab like lesions on tubers (de Haan and van den Bovenkamp, 2005). Therefore, it is necessary to correctly identify the causal pathogen (McCartney *et al*., 2003). The presence or absence of sporeballs in tuber lesions indicates powdery scab or common scab respectively. Current methods such as ELISA or PCR can be used to accurately identify powdery scab within a short space of time (McCartney *et al*., 2003; de Haan and van den Bovenkamp, 2005).

2.5 Life Cycle of *S. subterranea*

S. subterranea is able to survive for up to 18 years as resting spores (de Haan and van den Bovenkamp, 2005). These spores are so resistant that they can survive the passage through the gastrointestinal tract of animals (Merz and Falloon, 2009). Individual resting spores, 4 µm in diameter, are found clumped together within the sporeballs. When environmental conditions are favourable, that is, under high soil moisture and low soil temperature and only in the presence of free water, the resting spores germinate and release a single uninucleate zoospore (primary zoospore) (van de Graaf *et al*., 2003). The length of time that the primary zoospores will swim before they infect host tissue is dependent on the available free water in the soil and the temperature (Qu and Christ, 2006; Merz, 2008). According to Merz and Falloon (2009), zoospore release occurs at 5°C to 25°C and zoospores are most active at temperatures of 12°C to 13°C.

When a primary zoospore comes in contact with a susceptible root hair, the flagellae withdraw and the zoospore encysts (Harrison *et al*., 1997). A tube that contains a bullet shaped stylet (also called a rohr and stachel) develops within the zoospore cyst and the stylet is rapidly forced through the root hair cell wall (Merz, 2008). The pathogen is injected into the host cell and becomes an unwalled protoplast separated from the host by a single unit membrane (Harrison *et al*., 1997). This is known as a multinucleate plasmodium. The plasmodium develops and enlarges to form zoosporangia in the root hair(Qu and Christ, 2006; Falloon, 2008), which subsequently gives rise to between four and eight secondary zoospores (de Haan and van den Bovenkamp, 2005; van de Graaf *et al*., 2005; Merz, 2008). Secondary zoospores are indistinguishable from primary zoospores and have the ability to reinfect roots so that more

zoospores can be produced and released into the soil and therefore increasing inoculum levels (Figure 1).(Qu and Christ, 2006; Falloon, 2008).

Primary and secondary zoospores can infect tubers, roots and young shoots (Qu and Christ, 2006). This means that different plant tissues can become infected at the same time (Falloon, 2008). The infection of unsuberised lenticels leads to tuber scab development, thus indicating that the tubers will be most susceptible to infection during the first two to three weeks after their initiation (van de Graaf *et al*., 2005).

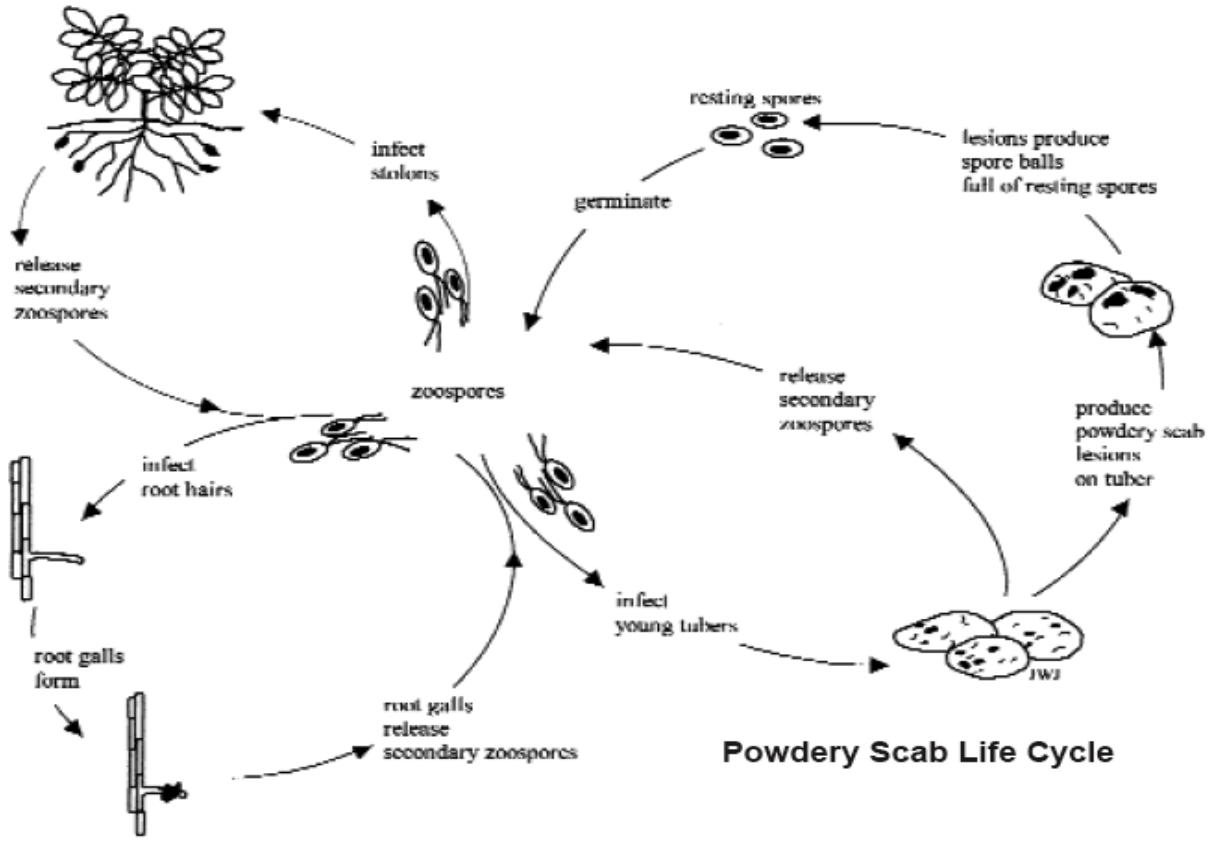


Figure 1:Life cycle of *Spongospora subterranea* f.sp. *subterranea* (Johnson, 2002)

2.6 Factors affecting development of *S. subterranea* in potato

Optimum conditions for powdery scab development include high soil moisture (free water) and low soil temperatures (van de Graaf *et al*., 2005; Merz, 2008). Powdery scab is a polycyclic disease with multiple infection cycles per season (Merz *et al*., 2004; Merz, 2008; Nitzan *et al*., 2009). This means that while environmental conditions are favourable for disease development, zoospores continually infect susceptible roots and new sporeballs will develop within root and tuber tissues (Montero-Astua *et al*., 2005; Nitzan *et al*., 2007). The factors that affect *S. subterranea* are as listed below.

2.6.1 Temperature

The occurrence of severe powdery scab has often been associated with plants grown at temperatures below 20°C (Harrison *et al*., 1997). It has been found that the optimum temperatures for disease development range between 12°C and 17 °C with the maximum temperature being 22°C to 25 °C and the minimum temperature of 11°C (Baldwin *et al*., 2008). Certain stages of infection and symptom development occur at different temperatures. As an example 5 to 25°C will promote zoospore release, 12°C to 13 °C influences zoospore activity and tuber infection and 17°C promotes root galling (van de Graaf *et al*., 2007; Merz and Falloon, 2009).

2.6.2 Soil Moisture

Soil that has a high water content (most soil pore spaces are filled with water) is associated with higher incidence of powdery scab (van de Graaf *et al*., 2007; Baldwin *et al*., 2008). High soil moisture encourages zoospore release and facilitates the movement of the zoospores towards susceptible roots or other susceptible host tissues (Baldwin *et al*., 2008; Merz, 2008; Merz and Falloon, 2009).

The soil moisture content also has an indirect effect on disease development as the oxygen concentration in the soil is reduced with higher soil moisture levels (Baldwin *et al*., 2008). Tuber development is thus slower and this increases the period of susceptibility to infection by *S. subterranea* (van de Graaf *et al*., 2005). Therefore, tubers grown in soils that are poorly drained or in soil types that have large pore spaces and high water holding capacities often have a higher incidence of powdery scab, whereas soils that have small pore spaces and lower oxygen levels will result in reduced infection and disease. Growing regions that have higher annual rainfall tend to favour powdery scab development, as rainfall influences the water content in the soil. The severity of powdery scab is determined by the intensity and duration of the rainfall (precipitation), as well as the rate at which water drains away from the zone of soil containing the developing tubers (Harrison *et al*., 1997).

2.6.3 pH of the soil

S. subterranea can be infectious across a wide pH range, 4.7 to 7.6 though Hughes (1980) and Wale (2004) state that its incidence is slightly less in acid soil conditions. The different stages of the disease cycle may be affected differently by pH. The zoospore release from sporeballs was not affected by a pH range of five to eight, but was reduced by pH below five and above eight. Van de Graaf *et al*. (2005) suggests that acid soils may prevent disease by inducing the release of zinc and manganese ions, which are toxic to *S. subterranea*.

2.6.4 Inoculum Level

The quantity of inoculum present in the field cannot be used to directly determine the severity of powdery scab that will occur, unless the environmental factors are taken into account (Merz, 2008). This is because low numbers of sporeballs can rapidly give rise to high numbers of zoospores under favourable conditions (van de Graaf *et al*., 2005; Baldwin *et al*., 2008; Merz and

Falloon, 2009). Yet if the conditions are less favourable for disease development, then the level of inoculum can be correlated with the disease severity. The initial amount of inoculum results from a number of factors, for example soil contaminated with sporeballs from the previous season, infected seed tubers, contaminated equipment and contaminated manure (Iftikhar *et al*., 2000).

2.6.5 Crop Rotation

S. subterranea can survive for many years in the soil (Merz *et al*., 2005; Merz, 2008). Falloon (2008) states that the pathogen is able to survive in the soil for over 50 years in the absence of a host. To prevent the amount of inoculum increasing in the soil before planting a potato crop, it is advisable to follow a crop rotation program (Agrios, 2005; Qu and Christ, 2006). A general crop rotation of three to five years with non *Solanum* species has been suggested (Qu and Christ, 2006) and in Japan four year crop intervals are practiced due to powdery scab (Nakayama *et al*., 2007).

2.7 Control Practices for powdery scab

The only reliable control of powdery scab disease is to plant disease-free seeds in uncontaminated fields (Genet *et al*., 2005; Qu *et al*., 2006; Merz, 2008). Several strategies are used to minimize the risk of powdery scab disease as there is no single effective way of controlling powdery scab (Iftikhar *et al*., 2007). The integration of management practices can reduce the potential of powdery scab becoming a serious problem (Iftikhar *et al*., 2007). The different methods for managing powdery scab include disease-free seed tubers, cultivar resistance, fungicides, cultural practices, antagonists and legislation (Gudmestad *et al*., 2007; Falloon, 2008).

2.7.1 Disease free seed tubers

There is a need to produce seed tubers that have a minimum of phytopathogenic infections and they must be true-to-type (Rolot and Seutin, 1999). This is because seed tubers tend to accumulate disease to the next generation. Seed tubers are the main source of inoculum in areas where powdery scab was not previously established (Jeger *et al*., 1996; Tsror *et al*., 1999). Seed potatoes with powdery scab are commercially unacceptable for establishment of new crops because the pathogen can be transmitted on infected and infested seed potatoes. Seed certification systems which allow low tolerance levels for powdery scab incidence lowers the market value of those seed tubers (Jeger *et al*., 1996).

2.7.2 Cultivar Resistance

The potato cultivar that is grown in the field is usually chosen by the producers for its marketability and/or processing characteristics, not purely on its ability to resist infection by *S. subterranea*. All potato cultivars are susceptible to powdery scab to some degree (Merz *et al*., 2005; Iftikhar *et al*., 2007; Falloon, 2008). The most cost effective and environmentally safe way of controlling powdery scab is to develop cultivars that are disease resistant (Merz *et al*., 2005; Iftikhar *et al*., 2007). Resistance may be achieved through genetic engineering. This means that the genes that convey resistance must be isolated from resistant species and introduced into the host plant's genome. These genes exist in several wild species such as *Solanum curtilobum* and *Solanum andigenum* (Harrison *et al*., 1997). Currently potato cultivars are being bred for resistance using conventional breeding methods. This process is lengthy as it may take more than three years to complete (Baldwin *et al*., 2008).

2.7.3 Use of fungicides

Many fungicides can be used to control plant diseases satisfactorily (Harrison *et al*., 1997). Yet, chemical control for soilborne pathogens like *S. subterranea* is difficult, due to the soil properties that reduce the efficiency of the fungicides, as well as the high inoculum levels in the soil (Wallace *et al*., 1995). This means that soil fungicide applications have a limited use and may not be cost effective. Falloon *et al*., (1996) demonstrated that there are a number of chemicals [chlorophenol (dichlorophen-Na), dithiocarbamate (mancozeb), and pyridinamine (Fluazinam) groups] that can be used to reduce the incidence and severity of powdery scab. The soil application of selected chemicals may lead to the reduction of powdery scab. Applications of sulphur or zinc salts have shown to reduce disease (Wale, 2004; Falloon, 2008). However soil applications are costly and may not be environmentally acceptable. Use of synthetic pesticides can result in poisoning of farmers through inappropriate spraying methods, poisoning of consumers through high residual levels of these chemicals, elimination of non-target organisms, as well as selection of phytopathogens, pest and weed insensitive to certain active ingredients (Stangarlin *et al*., 2011).

2.7.4 Cultural practices

Growers should consider avoiding fields that have a history of powdery scab (Harrison *et al*., 1997; Larkin and Griffin, 2007). The use of plough pans and irrigating with smaller quantities of water more frequently will reduce the chances of creating soil conditions that are conducive to disease development (Nakayama *et al*., 2007; Lees *et al*., 2008). It is possible to reduce powdery scab severity by reducing irrigation during the most susceptible stage of crop growth (during tuber initiation) (Merz, 2008; Merz and Falloon, 2009).

The most important methods of reducing the risk of contamination of powdery scab free soils are by using pathogen-free seed tubers, cleaning and disinfecting all equipment and containers that come in contact with the tubers (van de Graaf *et al*., 2005). Equipment can be disinfected with the use of chemicals such as formaldehyde and copper sulphate.

Growing trap crops to reduce the soil-borne inoculum directly before the potato crop is planted offers an alternative approach to managing *S. subterranea* (Qu and Christ, 2006). It has been shown that planting *Datura stramonium* (Jimsonweed) or *Raphanus sativus* (leafy daikon) in heavily infested soil before planting the potato crop can reduce the severity of powdery scab (Qu and Christ, 2006; Larkin and Griffin, 2007).

2.7.5 Legislation

Seed tuber inspections, strict quarantine and compulsory crop rotation have been implemented to reduce the problem caused by powdery scab in certain parts of the world (Harrison *et al*., 1997). These practices do not have a large effect on powdery scab control because of the widespread distribution of *S.subterranea* in soils, its ability to multiply rapidly in favourable conditions and the likelihood that it can survive for many years in the absence of potato crops.

2.7.6 Integrated Control

Singularly, management practices have minor effects in controlling powdery scab (Larkin and Griffin, 2007; Christ, 2008;Falloon, 2008). Combining the various management practices (crop rotation, cultural practices, host resistance and fungicide use) is the basis of integrated control of powdery scab (Gudmestad *et al*., 2007; Falloon *et al*., 2008). This will allow manipulation of various factors affecting the severity of powdery scab. The benefits resulting from optimizing each control practice are additive and therefore a reduction in powdery scab can be achieved.

2.8 Biofumigation as control for soil borne diseases

Biofumigation is an alternative control method for soilborne diseases which relies on the principle of exploiting the natural biocide compounds from glucosinolate containing plants (Kirkegaard *et al*., 1998, 1999, 2000; Matthiessen and Shackleton, 2005) to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes (Smolinska *et al*., 2003). The term was first used by Kirkegaard *et al*., 1993 who specifically described using glucosinolate hydrolysis products, notably isothiocyanates, to control soilborne pests and pathogens in horticulture and agriculture. Isothiocyanates are produced during glucosinolate hydrolysis which occurs when *Brassica* plant tissues are broken down allowing both glucosinolates and a myrosinase to come into contact with each other and hydrolysis occurs.

Brassicas are widely grown and consumed as relish. In Zimbabwe for example, cabbages (*Brassica oleracea* var *capitata*), broccoli (*Brassica olearceae* var *italica*), rape (*B. napus*), cauliflower (*Brassica oleracea* var *botrytis*), choumollier (*Brassica oleracea*), kale (*Brassica oleracea* var *acephala*), turnip (*Brassica campestris* var *rapa*), mustard (*Brassica juncea*) and radish (*Raphanus sativus*) are some of the commonly grown brassicas (Godfrey-Sam-Aggrey and Tekie, 1990 in Karavina and Mandumbu, 2012).These crops can be used in biofumigation against pest and diseases. It is now known that besides the *Brassicas*, plants in the Caricaceae, Moringaceae, Salvadoraceae and Tropaeolaceae families also have biofumigant properties (Gouws, 2004; van Dam *et al*., 2009).

Worldwide interest in biofumigation developed when it was hypothesized that the technique could be an effective alternative to the environmentally damaging chemical fumigants and sterilants (Gouws, 2004). It was realized that there is potential to use biofumigation as an alternative to methyl bromide in horticulture and broad agriculture to manage pests (Brown and

Morra,1997 in Karavina and Mandumbu, 2012). In most countries, methyl bromide and other synthetic pesticides perceived to be harmful to humans and the environment have been banned under the Montreal Protocol on Substances that Deplete the Ozone Layer, due to their negative effects on the environment (Ibekwe, 2004).Despite the ban, the demand for blemish-free produce is increasing. Biofumigation is a bio-pesticide that can be used to control soil-borne pests without causing harm to the environment (Karavina and Mandumbu, 2012).

2.8.1 The Biofumigation process

The biofumigation method exploits the glucosinolate hydrolysis products produced by *Brassica spp*. It has been well documented that *Brassica spp.* produce organic anion, secondary metabolites called glucosinolates (sulphur containing glucosides) within their tissues (Gardiner *et al*., 1999; Bianco *et al*., 2000; Gimsing and Kirkegaard, 2006). Furthermore, they also produce myrosinase enzymes intracellularly which are necessary for glucosinolate hydrolysis. Glucosinolates and myrosinases remain separated from each other while the plant tissues are intact, as they are compartmentalised within different cells. However upon tissue mastication, the cells are lysed and they will be brought into contact with others and resultantly glucosinolate hydrolysis occurs (Figure 2) (Bianco *et al.* 2000; Gimsing and Kirkegaard 2006, 2009; Fan *et al.* 2008).

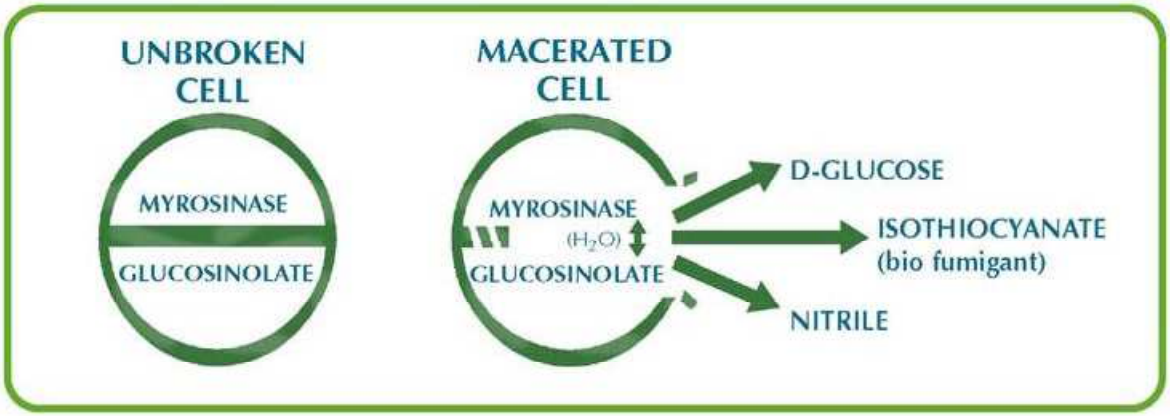


Figure 2: Basics of the biofumigation process.Image from http://serve-ag.com.au/services/seed-salesproduction/ biofumigation-seed/.

The enzymatic mechanism of myrosinase involves two steps namely the glycosylation step, in which the glycosyl-enzyme is formed and subsequently the a-glycone is released. This is followed by the deglycolyation step in which the glycosyl enzyme is hydrolysed by a water molecule (Burmeister *et al*. 1997). Glucosinolate hydrolysis can potentially release several different hydrolysis products, including nitriles, thiocyanates, however most commonly isothiocyanates are produced (Figure3) (Fahey *et al.* 2001; Gimsing and Kirkegaard 2009).

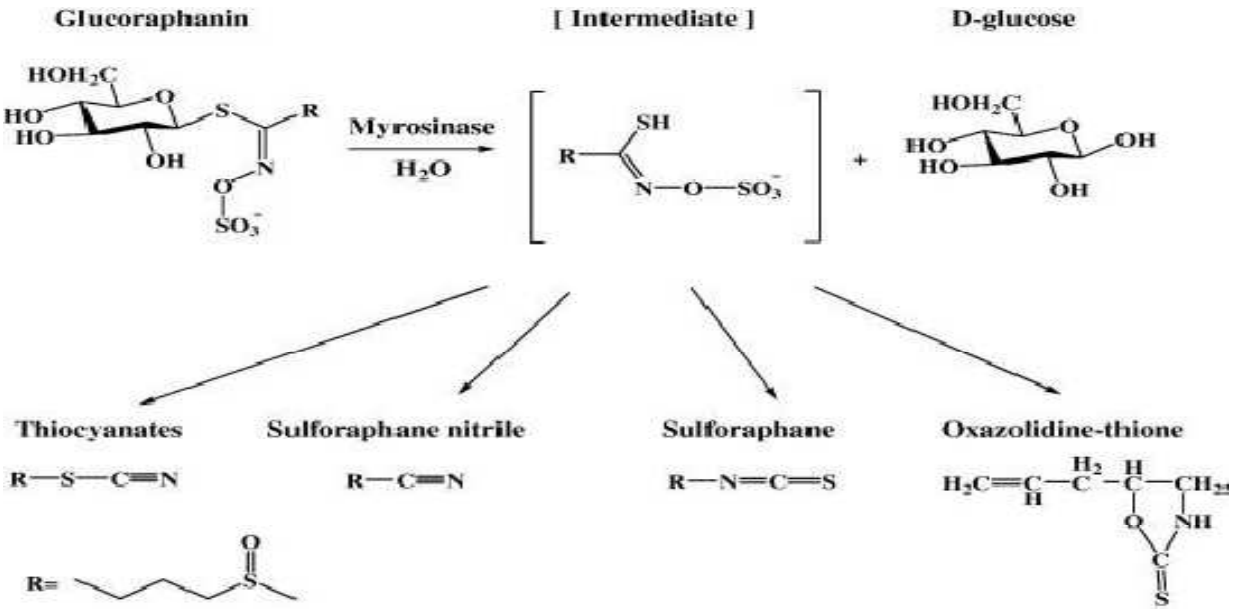


Figure 3: Glucosinolate hydrolysis – the diagram shows the different products that can be formed at different stages of the reaction. (Shen *et al*., 2010).

Glucosinolate content and concentration is known to vary between *Brassica* cultivars and throughout development (Al-Gencly and Lockwood 2003; Bellostas *et al.* 2007), it is well accepted that the efficacy of biofumigation is dependent on the specific glucosinolate hydrolysis products formed during tissue breakdown. It is understood that different biofumigant crops used will potentially have different biofumigation potential and produce different levels of pathogen control (Motisi *et al.* 2009). Therefore to achieve the most effective biofumigation results it appears that it is necessary to gain an understanding of glucosinolate hydrolysis products formed by different *Brassica* cultivars, and their interactions with different soilborne pathogens.

2.8.2 Characteristics of Glucosinates and factors that affect their concentration in plants

GSLs are derivative from α-amino acid precursors that are β-thioglycoside *N*-hydroxysulphates that possess a side chain 'R' and a sulphur linked β-D-glucopyranose oxime moiety (Verkerk *et al.* 2009). The side chain and sulphate group possess an anti stereochemical configuration across

the C=N double bond (Holst and Williamson 2004). The sulphate group is normally balanced by a (potassium) cation (Verkerk *et al.* 2009). To date more than 120 side chains have been identified, it is the side chain structure which largely determines the group each glucosinolate is assigned to, however chemical properties and biological activity also play their part. GSLs are divided into three groups – aliphatic, aromatic and indolyl (Figure. 4) (Dawson *et al.* 1993; Holst and Williamson 2004; Gimsing *et al.* 2005).

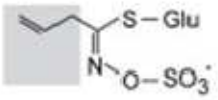
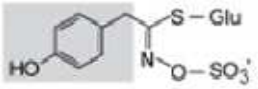
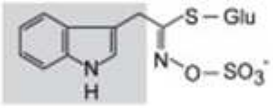
a)  b)  c) 

Figure 4: Different classes of glucosinolates a) aliphatic b) aromatic and c) indolyl (http://ars.els-cdn.com/content/image/1-s2.0981942808000363-gr1.jpg

The most abundantly produced GSLs in *Brassica* plant tissues are the aliphatic glucosinolates, derived from methionine. It is thought that the side chain elongation that is needed to develop aliphatic glucosinolates occurs early in the biosynthetic pathway (Figure 5). Before development of the glycine moiety through the single or multiple addition of the methyl carbon of acetate to methionine, after glycine moiety formation side chain modification will occur (Magrath *et al.* 1993). Generally GSLs are polar, highly water soluble compounds, but on contact with the enzyme myrosinase they will hydrolyse quickly, particularly if water is present (Gimsing *et al.* 2005).

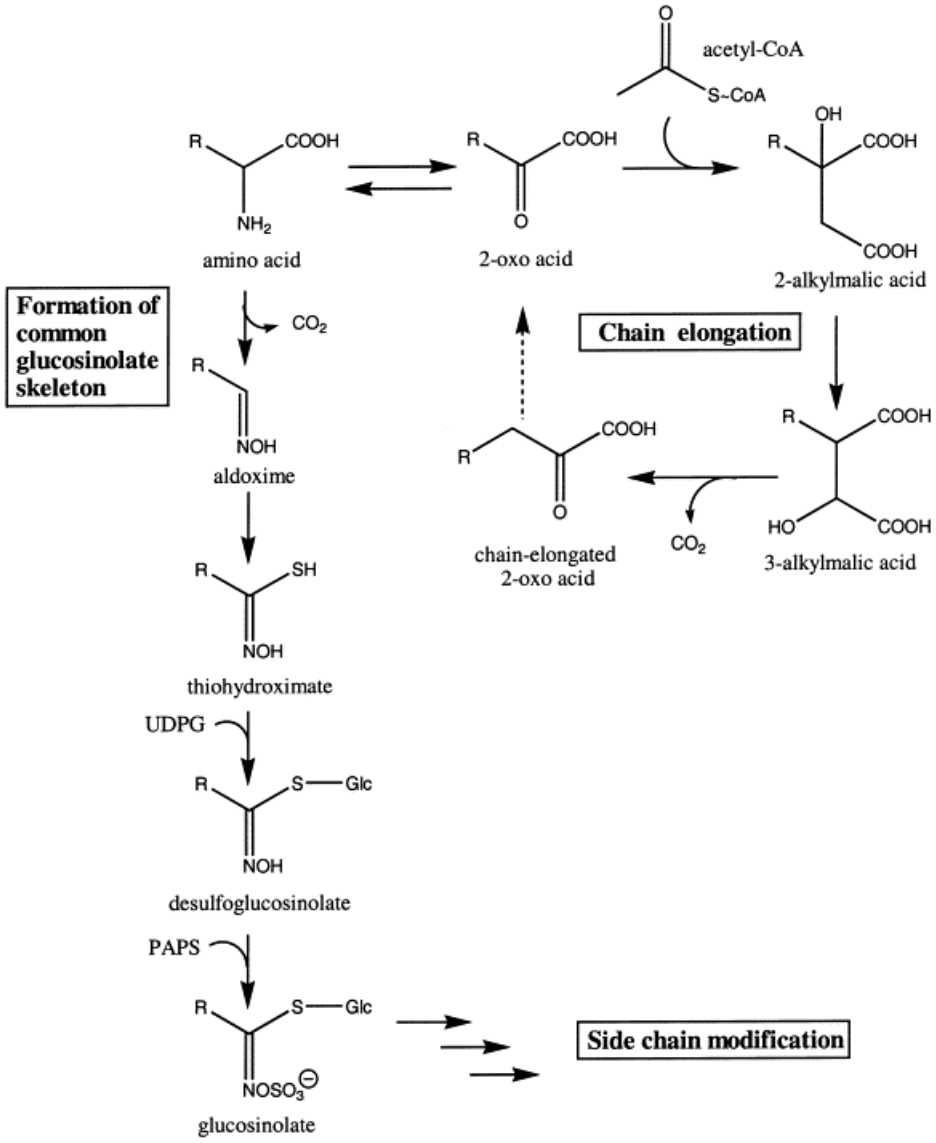


Figure 5: Glucosinolate biosynthesis process (http://ars.els -cdn.com/content/image/1-s2.0-S003194220000501X-gr1.gif.)

Glucosinolates (GSLs) are present within all parts of the plant, but differ in profiles and concentrations throughout the plants tissues (Velasco *et al.,* 2007). To date studies have shown that a single plant will most commonly contain approximately four differently structured GSLs in significant concentrations, however as many as 15 differently structured GSLs have been identified within the same plant (Verkerk *et al,.* 2009). GSLs are commonly found to most

readily accumulate in all vegetative and reproductive parts throughout plant development (Buskov *et al.*,2002). GSL concentration and composition are primarily affected by the plants genetics, however environmental and physiological factors, such as radiation, temperature and photoperiod, will also influence GSL expression and accumulation. Concentration and composition of GSLs are also known to change significantly throughout the plants development (Verkerk *et al.*,2009). GSL content within plants have also been shown to be affected by a number of agronomic factors, including; soil type, moisture and mineral nutrient availability (Velasco *et al.*,2007). Soil health has also been identified as having a significant influence on levels of GSLs in growing plants. Short days, cool temperatures and frost conditions during winter have been demonstrated to have a negative effect on GSL content (Velasco *et al.*,2007).

2.8.3 Factors that influence persistence of GSL-hydrolysis products in the soil

The soil persistence, stability and toxicity of GSL-derived ITCs are different from those of methyl- ITC (Borek *et al.,* 1995). Research has shown that GSL-hydrolysis products do not last long in the soil after they are formed (Brown and Morra, 2005). In a study to evaluate the transformation of glucosinolate-derived allelochemicals, it was observed that the half-lives of allyl-isothiocyanate and allyl-nitrile in soil ranged from 20-60 hours and 80-120 hours respectively (Borek *et al.,* 1995). The limited GSL-hydrolysis places limits on achieving effective pest control and may contribute to the variability observed in soilborne pest suppression by these compounds (Brown and Morra, 2005). A number of factors influence the persistence of GSLs in the soil after incorporation of GSL-containing plant biomass.

2.8.3.1 Moisture

The heterogeneity of ITC-distribution in the soil contributes to variations in its effectiveness. Generally, increasing soil-water content increases ITC longevity in the soil (Brown and Morra,

2005). Increasing soil-water content increased half-life of propenyl-ITC (Borek *et al.,* 1995). It has therefore been recommended to maintain wet conditions continually to improve pest inhibition resulting from longer exposure time periods (Brown and Morra, 2005).

2.8.3.2 Soil texture and organic matter content

Most fumigants, particularly ITCs, are more effective in sandy soil as compared to heavier textured soils like clay soils. For instance, phytotoxicity from *Brassica* extracts was generally greater when wheat was grown in sand than in clay soils (Mason-Sedun and Jessop, 1988). This is attributed to adsorption of ITCs to soil particles in heavy textured soils thereby reducing their effectiveness. Soil texture influences volatilization losses but is considered less important than organic matter content (Brown and Morra, 2005).

2.8.3.3 Soil temperature

It has been observed that greater concentrations of propenyl-ITC are found at elevated temperatures (Brown and Morra, 2005).

2.8.3.4 Additional allelochemicals

Sometimes, other compounds besides GSL-hydrolysis products may have biological activity contributing to pest suppression. For example the breakdown of sulphur-containing amino-acids like methionine and cysteine could produce inhibitory compounds (Monde *et al.,* 1991).

2.8.3.5 Soil pH

This influences the formation and disappearance of GSL-hydrolysis products. Amending the soil with lime has been shown to shorten the residence times of methyl-ITC (Ashley *et al.,* 1963). Generally, typical pH values of most agricultural soils are not expected to alter allelochemicals residence times (Brown and Morra, 2005).

2.8.3.6 Volatilization

Loss of ITCs occurs in many ways with volatile losses being a major route. Research has shown that unsealed bottles had much greater losses of methyl-ITC than sealed ones (Ashley *et al.,* 1963). In another experiment, the toxic effects of green manures were greater in polythene-covered than in open soil and toxicity had a correlation with the concentration of ITC-producing GSLs rather than total GSLs in the green manure (Lord *et al.,* 2011). Moreover, the wide variation in vapour-pressures of various ITCs generates huge differences in GSL-based fumigation (Brown and Morra, 2005).

2.8.4 Myrosinase enzyme system in *Brassica* plants

Myrosinase [thioglucoside (glucosinolate) glycohydrolase, EC 3.2.3.1] is understood to exist in all plants and plant organs that contain glucosinolates. Myrosinase activity has also been found in fungi, bacteria, mammals and insects (Tani *et al.*,1974; Rask *et al*,.2000). Myrosinase belongs to the large hydrolytic superfamily of enzymes, the *O* Glycosyl hydrolases or glycosidases. Due to its large number of members this family has been sub divided into, as it currently stands, 70 families, based on their amino acid sequence similarities. Myrosinase belongs to family 1 along with *O*-β-glucosidases, 6-phospho- β-glucosidases, 6-phosopho- β-galactosidases, β-galactosidases and lactase/phlorizin hydrolase (Rask *et al.* 2000). Glycosidases can also belong to one of two classes; retaining or inverting, based on the stereochemical outcome of the hydrolysis reaction they catalyse. Myrosinase has been identified as a retaining enzyme, which during hydrolysis undergoes a two-step mechanism, each involving an inversion therefore resulting in a net retention of stereochemistry, which is consistent with its sequence similarity with family 1 *O*-glycosidases (Bourderioux *et al.*,2005). Analysis of the three-dimensional structure of myrosinase identified several amino acid residues that are involved in binding the

glucose ring and the a-glycone they are also involved in the catalytic mechanism. Within the myrosinase sequences the a-glycone binding residues are conserved, however this is not the case for *O*- β-glucosidases.

Myrosinase activity within plants is dependent on several factors which include the species, cultivar and the specific plant organ studied. Most previous studies have identified that the highest levels of myrosinase activity occurred in seeds and seedlings. In addition to differences in myrosinase concentration levels different myrosinase isoenzymes have also been identified in different plant organs of the same plant. It should be noted that no direct correlation between levels of myrosinase activity and glucosinolate concentrations in plant tissues have yet been observed (Rask *et al.* 2000).

In 1884, Heinricher a special type of cell in *Brassica* crops which differed in both size and morphology from the adjacent cells was identified. These cells have been referred to as protein-accumulating idioblast, myrosin tubes and more recently, myrosin cells (Rask *et al.* 2000), it is myrosin cells which have been shown to contain myrosinase within the plant. Myrosin cells have been observed in seeds, parenchyma tissue, epidermis, and guard cells. The morphology of myrosin cells varies according to both the organ and tissue, and age of tissue in which they are present (Bones and Rossiter 1996) (Figure 6). The primary organelles in the myrosin cells are spherical myrosin grains, which appear to fuse during differentiation of the myrosin cells, the exact intracellular localisation of myrosinase has been greatly debated (Rask *et al.* 2000).

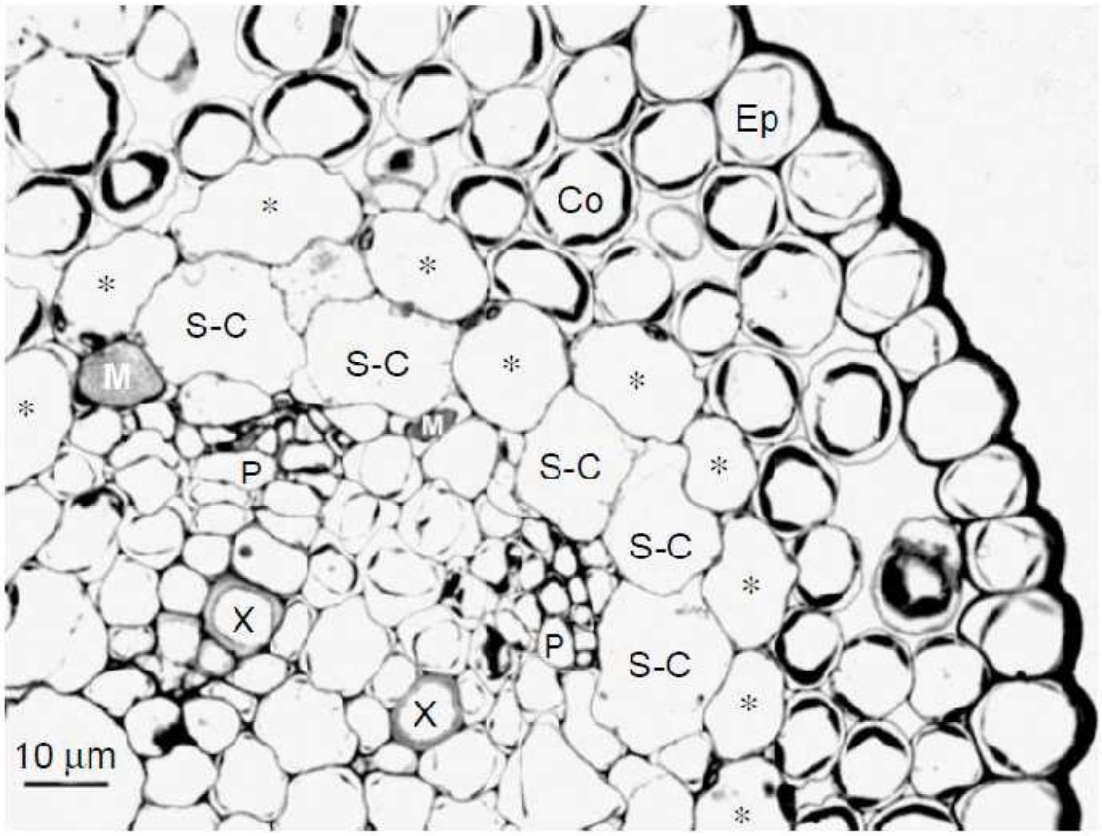


Figure 6: Compartmentalisation of glucosinolates and myrosinase within A. thaliana. Glucosinolates are thought to be present in sulphur rich cells (S-C) which are localised separated from myrosinase, stored in adjacent cells (M). Image shows a transverse section of a pedicle of A. thaliana is shown in which epidermis (Ep), cortex (Co), starch sheath (*), vascular bundles containing xylem (X) and phloem (P) can be seen. (Rask et al., 2000).

2.8.5 Glucosinolate-Myrosinase system

The majority of glucosinolates are both chemically and thermally stable and as a result hydrolysis has to be enzymatically driven. The process is initiated through the hydrolysis of the thioglucosidic bond which produces a glucose and unstable a-glycone, the thiohydoximate-*O*-sulphonate. The thiohydroximate-O-sulphonate then undergoes a spontaneous rearrangement, producing one of several possible products. The resultant product is dependent on the side chain

structure, the parent glucosinolate and the reaction conditions. At pH 6 to 7 the most common hydrolysis products formed are stable ITCs, unless the GSL possesses a β-hydroxylated side chain or an indole moiety; β-hydroxyl-ITCs are unstable and consequently will cyclise to oxazolidine-2-thiones, whereas indole ITCs will undergo lysis. Which will result in the corresponding alcohol, such as indol-3-carbinol being formed, this will condense into dimers, trimmers or tetramers. At pH 4 to 7 and when ascorbic acid is present during the reaction, thiocyanates and ascorbigen are the major products of indole GSL hydrolysis.

Glucosinolates possessing an aliphatic structured side chain are generally hydrolysed producing isothiocyanates at a neutral pH, yet in a more acidic pH or in the presence of Fe^{2+} ions it is more common that nitriles will be yielded (Holst and Williamson 2004). Generally glucosinolates will only yield a specific isothiocyanate, therefore examination of *Brassica* glucosinolate profiles can determine what potential isothiocyanates may be released (Table 1).

Table 3:Commonly found glucosinolates, their common name and corresponding isothiocyanate

Glucosinolate	Common Name	Isothiocyanate
Benzyl	Glucotropaeolin	Benzyl isothiocyante
Methyl	Glucocapparin	Methyl isothiocyanate
2-Phenylethyl	Gluconasturtiin; phenethyl	2-Phenylethyl isothiocyanate
2-Propenyl	Allyl, Sinigrin	Allyl isothiocyanate
3-Butenyl	3-Methylthiopropyl	3-Butenyl isothiocyanate
3-Methylthiopropyl	Glucoibervirin	3-Propyl isothiocyanate

2.8.6 Isothiocyanates in *Brassica* plants

Isothiocyanates have been shown as likely compounds for allelopathic activity as they have been shown to produce adverse effects on growth and survival of bacteria, fungi, mammals and insects (Choesin and Boerner 1991). Their broad range biocidal activity can be attributed to their ability to cause inhibitory effects through interaction with proteins, yet the mechanisms involved are not completely understood (Morra and Kirkegaard 2002). However three main hypotheses have been proposed as an explanation for observed reduction in microbial growth: 1) The intracellular enzymes may be inactivated through oxidative breakdown of the –S-S- bridges. 2) Inhibition of metabolic enzymes by a thiocyanate radical 3) Uncoupled action of oxidative phosphorylation as indicated by the inhibition of oxygen uptake of plant pathogenic *Pythium* fungal strains by several ITCs (Mancini *et al.*,1997).

Isothiocyanates are used in traditional control methods of soilborne pathogens, through the primary breakdown product of metamsodium – methyl isothiocyanate. Methyl isothiocyanate is the simplest structure of a large number of isothiocyanates that may be produced through *Brassica* tissue disruption, however methyl isothiocyanate is not commonly found to be produced in the Brassicaceae (Matthiessen and Shackleton 2005). One of the most ubiquitous and proportionally high identified and studied ITCs is Allyl isothiocyanate (AITC), it‟s parental glucosinolate, Allyl glucosinolate, has been readily found in *Brassica nigra*, *Brassica carinata* and *Brassica juncea* all of which have been shown to produce AITC through glucosinolate hydrolysis at a pH of 4.0 or greater (Mayton *et al.* 1996). The high levels of allyl glucosinolate which have been found within plant tissues and the fact that studies have shown AITC to be as toxic to a range of fungi as MITC *(*Mayton *et al.* 1996) have prompted a number of studies for its use a pathogen control agent.

It was originally thought that AITC was the only commonly found ITC to provide a level of disease control or pathogen suppression due to a number of studies investigating its effectiveness. Reports from Dhingra *et al*., (2004) also show that decreases in fungal growth were directly related to the concentration of AITC the fungal species were exposed to. However with an increasing number of studies on biofumigation, it is now understood that AITC is not the only ITC that has suppressive effects on microorganisms.

2.8.7 Biofumigant incorporation methods

As interest in the use of biofumigation as a sustainable agricultural practice increases, several methods to incorporate the biocidal isothiocyanates have been practiced. To date the most common method is the incorporation of green manures. In green manuring, the *Brassica* crops are grown on the land that is to be fumigated, prior to planting of the susceptible crop the *Brassicas* are chopped, mulched and pulverised and ploughed into the soil (Matthiessen and Kirkegaard 2002). The green manuring process will disrupt the *Brassica* tissues, allowing glucosinolate hydrolysis to take place, releasing isothiocyanates into the soil. Further options also exist which allow growers to avoid growing the *Brassica* crops. One such method results from a by-product from canola oil production, the process involves the extraction of oil from canola seeds, the seeds are then dried and crushed, and the resultant seed meal can then be ploughed into soil. This method has been seen to be an attractive option, as early studies of glucosinolate profiles indicate that seeds may contain high concentrations of the parental glucosinolates for isothiocyanate formation, (Borek and Morra 2005).

Additionally the use of dried *Brassica* plant material has also been described as an option for Biofumigation practice. Dried green manures known to contain high concentrations of both glucosinolates and myrosinase can be supplied to growers. The plant material can then be

ploughed into the ground, and with the addition of water the toxic alleochemicals are formed (Lazzeri *et al.* 2004). In order for the most effective biofumigation to occur, research must also be carried out to assess which methods achieve the highest levels of glucosinolate hydrolysis, in turn releasing the highest concentration of isothiocyanates.

2.8.8 Physiological response of fungi to isothiocyanates

The inhibition of fungal pathogens using isothiocyanates has highlighted the different types of responses that can be exhibited by fungi when exposed to various individual isothiocyanates. Predominantly two terms are used to describe fungal responses to toxic compounds: fungistatic and fungitoxic. Fungistatic describes the instance when the initial point of fungal growth is delayed in responses to the presence of the toxic compound. Fungitoxic describes the fungi being killed and therefore unable to grow and develop, in response to the presence of a toxic compound. Studies using *Fusarium oxysporum* by Smolinska *et al*., 2003 displayed both fungistatic and fungitoxic responses. In this instance fungistatic responses were attributed to the concentration the fungus was exposed to. Inyang *et al.*, (1999) showed using *in vitro* methods that isothiocyanates can also inhibit conidial germination and mycelial growth of the insect pathogenic fungus *Metarhizium anisopliae.* Fungitoxic effects on the mycelial growth of *Alternaria* spp. through exposure to allyl and benzyl isothiocyanate have also been observed (Sellam *et al.*,2006).

2.8.9 Influence of Biofumigation on soil microbial communities

To date little research has been carried out investigating how the practice of biofumigation affects naturally occurring soil microbial communities. It is believed that through the stimulation of microbial growth of antagonistic bacteria most organic matter amendments will achieve inhibition of soilborne pathogens. However in studying the use of a biofumigation system, there

is the added component of the release of biotoxic compounds. Although the use of green manures may encourage the development of antagonistic bacteria and aid in microbial suppression, it is unknown what the release of isothiocyanates will have on the naturally existent and beneficial bacterial groups. It could be assumed that if the addition of glucosinolate myrosinase products has a suppressive effect on soilborne pathogens, then the diversity of soil microorganisms may also be altered and again the effects of specific crops should be understood to achieve the maximum efficiency within a biofumigation system (Larkin *et al.* 2010).

While biofumigation holds a lot of promise as a crop protection tool, its broad spectrum toxicity might harm non-target beneficial soil biota such as biocontrol agents or other pest antagonists (Ramirez *et al*., 2009). This means the switch to *brassica* biofumigants might not eliminate all the harmful non-target effects associated with synthetic chemicals, thus potentially complicating the integration of cultural and biological control. Henderson *et al*., (2009) reported that biofumigation interfered with the biological control of *Meloidogyne chitwoodi* by the entomopathogenic nematodes *Steinernema feltiae* and *Steinernema riobrave*. Biofumigants are also non-persistent. Thus, it does not provide a long term control option for pests. Farmers might have to complement biofumigation with other crop protection tools.

2.9 Potential of Biological control of *S. subterranea* with *T harzianum*

Biological control involves the use of beneficial microorganisms, such as specialized fungi and bacteria to attack and control plant pathogens and the diseases they cause. The use of antagonistic microbes to control *S. subterranea* is attractive because antagonists will act specifically against the disease and this approach is more environmentally friendly than chemical and certain cultural practices. A variety of soil microorganisms have demonstrated activity in the

control of various soilborne plant pathogens with species from *Trichoderma* receiving the most attention.*Trichoderma* spp. have been tested against other members of the *Plasmodiphorales* for example, have been reported to control *Polymyxa betae* (Nowakowska 1994) and *Pseudomonas fluoresecens, Pseudomonas putida, Streptomyces* spp. and *Trichoderma* spp. have been successfully used to control *Plasmodiophora brassicae* (Cheah *et al*. 2000; Kim *et al*. 2002). Furthermore, the observation of D'Ambra and Mutto (1986) that cystosori of *P. betae* were parasitized and degraded by *T harzianum*is of special interest. It has also been shown that *T harzianum* has the ability to parasitize sporeballs of *S. subterranea* under controlled experimental growing conditions (Merz and Falloon, 2009). Further testing is required to assess the same phenomena under field conditions.

2.9.1 Classification and characteristics of *Trichoderma harzianum*.

Members of the *Trichoderma* genus are known as imperfect fungi with teleomorphs belonging to the *Hypocreales* order of the Ascomycota division (Howell, 2003; Kredics *et al*., 2003). These occur worldwide and are commonly associated with root, soil and plant debris (Howell, 2003).They are classified as imperfect fungi, in that they have no known sexual stage. Rapid growth rate in culture and the production of numerous spores (conidia) that are varying shades of green characterize fungi in this genus (Howell *et al*., 2003). The reverse side of colonies is often uncoloured, buff, yellow, amber, or yellow-green, and many species produce prodigious quantities of thick- walled spores (chlamydospores) in submerged mycelium (Gams and Bisset, 1998). These fungi colonize woody and herbaceous plant materials, in which the sexual telemorph (genus *Hypocrea*) has most often been found (Harman *et al*., 2004). However, many strains, including most biocontrol strains, have no known sexual stage. In nature, the asexual

forms of the fungi persist as clonal, often heterokaryotic, individuals and populations that probably evolve independently in the asexual stage (Harman *et al*., 2004).

2.9.2 Mechanisms of action of *Trichoderma harzianum*

Trichoderma species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development (Harman *et al*., 2004). The ecological role of this genus is that *Trichoderma* strains take part in the decomposition of plant residues in the soil. Some *Trichoderma* species are very good cellulose producers and therefore they are important for the biotechnological industry (Reczey *et al*., 1996). Antagonism is based on different mechanisms, like that of antifungal metabolites by *Trichoderma* spp., competition for production space and nutrients and mycoparasitism. Mycoparasitic *Trichoderma* strains are able to recognize the host hyphae, to coil around them, develop haustoria, penetrate the cell wall of the host with cell- wall degrading enzymes like chitinases, glucanases and proteases, and utilize the contents of the host hyphae as nutrient source (Kredics *et al*., 2003).

Weindling (1932) described in detail the mycoparasitism of a fungal pathogen causing damping off disease (*Rhizoctonia solani*) by the hyphae of *Trichoderma spp.*, including coiling around the hyphae, penetration, and subsequent dissolution of the host cytoplasm. He also described an antibiotic which was toxic to both *R. solani* and *Sclerotinia americana*, and named it gliotoxin. In the year following this study, many similar results were reported by other plant pathologists. The mechanism of antibiosis was demonstrated in several studies. An antibiotic, gliovirin, from *Trichoderma virens* demonstrated strong inhibition of *Pythium ultimum* and the *Phytophthora* species (Howell and Stipanovic, 1995).

Research has indicated that certain strains of *Trichoderma spp.* can induce systemic and localized resistance to several plant pathogens. Plants treated with *Trichoderma spp.* in the root zone can produce higher levels of peroxidase, chitinase activity, deposition of callose-enriched wall appositions on the inner surface of cell walls and pathogenesis-related proteins. Moreover, some strains may enhance plant growth and development. These phenomena where observed by several researchers who treated plants with *T harzianum*resulting in large increases in root area and cumulative root length, as well as significant increases in dry weight, shoot length, and leaf area over that of the untreated control (Howell, 2003). Due to effective control of plant diseases, several commercial biological products based on *Trichoderma* spp. are manufactured and marketed in Asia, Europe and USA for use on a wide range of crops. These can be efficiently used as conidia, mycelium and chlamydospores which are produced in either solid state or liquid fermentation (Harman *et al*, 2004).

2.9.3 Factors affecting effectiveness of *Trichoderma*spp.as a biological control agent

Abiotic and biotic environmental parameters may have negative influence on the biocontrol efficacy of *Trichoderma* strains, therefore it is very important to collect information about the effects of environmental factors on the different activities of *Trichoderma* strains with biocontrol (Kredics *et al*., 2003). A series of abiotic and biotic environmental parameters has an influence on the biocontrol efficacy of *Trichoderma*. Some important parameters to be considered are the effects of temperature, water potential and pH, and the presence of pesticides, metal ions and antagonistic bacteria in the soil (Kredics *et al*., 2003).

2.9.3.1 Temperature

Most of the *Trichoderma* strains are mesophilic. Low temperatures in winter may cause a problem during biological control by influencing the activity of the biocontrol agent (Kredics *et al*., 2003)

2.9.3.2 Water potential

Another problem emerging during the application of *Trichoderma* strains as biocontrol agents is that they cannot tolerate dry conditions, however, we may need biocontrol agents against plant pathogenic fungi which are able to grow and cause disease even in dry soils (Kredics *et al*., 2003)

2.9.3.3 pH

The pH characteristics of the soil also belong to the most important environmental parameters affecting the activities of mycoparasitic *Trichoderma spp*.

2.9.3.4 Pesticides and metal compounds

Trichoderma strains within the frames of a complex integrated plant protection strategy, we may have to combine *Trichoderma* strains with chemical pesticides or metal compounds, therefore it is important to collect information about the effects of pesticides and metal ions on the biocontrol strains (Kredics *et al*., 2003).

2.9.3.5 Antagonistic bacteria

Antagonistic soil bacteria may also have negative effects on the biocontrol abilities of *Trichoderma* strains, therefore it may be advantageous if a biocontrol strain possesses bacterium-degrading abilities as well (Kredics *et al*., 2003).

CHAPTER 3

EFFECT OF *B. NAPUS*ON THE INCIDENCE AND SEVERITY OF POWDERY SCAB (*S. SUBTERRANEA*) IN POTATO.

ABSTRACT

Powdery scab of potato caused by *S. subterranea* is a major disease of potato in Zimbabwe. An experiment was set up in a glasshouse with 34^0C (±3) and 21^0C (±3) day and night temperatures respectively to determine whether the use of biofumigation with *B. napus* and variety can significantly reduce the incidence and severity of *S. subterranea* in potato, The experiment was laid out in a 5 x 3 factorial in a CRD with three replications. *B. napus* was incorporated into plastic pots at 3 levels, 10%, 20% and 30% (m/v) with Mancozeb as a positive control and a negative control where no control amendment was made. Potato varieties BP1, Diamond and Mondial were used. *B. napus* 20% reduced disease incidence by 31%, severity by 37% (root galls) and 67% (tubers). *B. napus* 20% on Mondial resulted in high proportion of 100% and 175% on marketable yield, extra-large and large tubers grade respectively compared to the positive control, Mancozeb on Mondial. Biofumigation with *B.napus* was observed to be a potential control option for *S. subterranea*.

3.1 Introduction

Powdery scab of potato (*Solanum tuberosum* L.) is caused by the pathogen *Spongospora subterranea* (Wallroth) Langerheim f.sp. *subterranea* Tomlinson. The pathogen belongs to the Plasmodiophorids (Braselton, 1995) and was first described in Germany in 1841 (Morse, 1913). The pathogen causes powdery scabs that form on potato tubers contain masses of resting structures known as sporeballs or cystosori. These cystosori have the ability to survive in the soil for many years (Merz and Falloon, 2009). To date, there are no effective control measures for

powdery scab with disease management strategies such as cultivar resistance, disease free seed tubers, fungicides, cultural practices, antagonists and legislation being employed to try to curb the disease (Wright, 2012).

Members of the Brassicaceae family from which *B. napus* belongs to, are gaining attention as potential biofumigants for soil pest suppression because of their ability to release biologically active isothiocyanates (ITCs) and other compounds from hydrolysis of glucosinolates.It has been well documented that *B. napus*produces secondary metabolites called glucosinolate (sulphur containing glucosides) within their tissues that have a biocidal effect against soil borne pathogens in a process termed biofumigation (Gardiner *et al*., 1999; Bianco *et al*., 2000; Gimsing and Kirkegaard, 2006). Biofumigation has great potential to be an alternative control method to suppress soil borne diseases caused by fungi, bacteria and nematodes (Bianco *et al*., 2000; Smolinska *et al*., 2003).

B. napus have myrosinase enzymes intracellularly which are necessary for glucosinolate hydrolysis. Glucosinolates and myrosinases remain separated from each other while the plant tissues are intact, as they are compartmentalised within different cells. However upon tissue mastication, the cells are lysed and they will be brought into contact with others and resultantly glucosinolate hydrolysis occurs (Bianco *et al.* 2000; Gimsing and Kirkegaard 2006, 2009; Fan *et al.* 2008).

3.2 Methodology

3.2.1 Site description

The research was carried out at Harare Research Station (31^0 03′E and 17^0 48′S). It is located within agro ecological region IIa with an altitude of 1506m above sea level. The research station receives annual rainfall of 820mm. The experiment was conducted in a glasshouse and the

temperatures in the glasshouse ranged from 21 – 35^0C with a relative humidity of 60% over the duration of the experiment.

3.2.2 Experimental Design and Treatments

The experiment was laid out as a 5 x 3 factorial in a CRD replicated 3 times.The first factor was *B. napus*and the second factor was variety with the levels and treatment structure as shown in Table 2 below.

Table 4: Treatment structure for effect of *B. napus* on the incidence and severity of *S. subterranea* on potato

	Varieties		
Control Option	**BP1**	**Amethyst**	**Mondial**
***B. napus* 10%**	T1	T2	T3
***B. napus* 20%**	T5	T6	T7
***B. napus* 30%**	T8	T9	T10
Mancozeb	T11	T12	T13
No control amendment	T14	T15	T16

3.2.3 Preparation of soil

Red clayey soil with a pH of 7.1 ($CaCl_2$) was used as potting media. Soil was sterilized by oven incubation overnight at 100℃. A total number of 225 plastic pots were disinfected with 1 % NaOCl. Soil was dispensed into the plastic pots which measured 50cm x 40cm with a holding capacity of 20 L.

3.2.4 Preparation of *B. napus*

B. napus seedlings, variety English Giant Rape, were raised in a seedbed for 6 weeks. The crop residue was cut into 10mm pieces, incorporated into the soil and left to decompose for a month with 45 pots at 100 g kg^{-1} soil (10% mass of crop residue/mass of soil), 45 pots at 200 g kg^{-1} (20% mass of crop residue/mass of soil) and 45 pots with 300 g kg^{-1} (30% mass of crop residue/mass of soil).

3.2.5 Preparation of *S. subterranea* inoculum.

Powdery scab lesions were cut from infected tubers of variety KY20 using a sterile scapel. Microscopic slides from the scabbed tubers of variety KY20 were made and viewed under the light microscope. Diagnostic features of *S. subterrane* sporeballs were observed confirming its presence on the tubers. Powdery scab lesions were peeled off from the skin of infected tubers, they were air dried and ground to a fine powder using a pestle and mortar. The inoculum was stored under dark and dry conditions at room temperature. On the inoculation day, 5g of the powder were added per pot and mixed with the soil.

3.2.6 Planting and fertilization

Potato varieties BP1, Diamond and Mondial were allowed to sprout for a month. Tubers were planted when they had an average of four sprouts per tuber. Potato is a heavy feeder and an equivalent of 2000 kg/ha Compound S with N. P. K ratio of 7: 21: 7 was placed at 2.5 cm below the seed at a rate of

27g/pot. Top dressing, Ammonium Nitrate, was applied at 28DAP after emergence before filling up (ridging), at a rate of 200 kg /ha which amounts to 5g/pot. The second filling up (ridging) was done at week 63DAP. A plot consisted of 5 pots. One tuber was planted per pot.

3.2.7 Irrigation

Irish potato is shallow rooted and requires adequate moisture from reproduction (tuber initiation) to physiological maturity. Irrigation scheduling of 7 days was followed and the pots were irrigated to field capacity.

3.2.8 Weeding

Potatoes develop larger and more extensive root systems in response to proper weeding. Loose, friable soil improved tuber set and development of smooth, well-shaped and even coloured potatoes. Weeding was done when necessary to control weeds, keep soil hilled up and aid water penetration as well as improving aeration.

3.3 Data Collection

The following measurements were taken:

3.3.1 Stem diameter (cm)

The diameter of stems was measured using a Vernier calliper. Two plants per plot were sampled for measuring diameter and the diameter was measured at the middle of the stem. Stem diameter was measured at 28, 42, 56, 70 and 84DAP.

3.3.2 Stem length (cm)

Two pots per plot were randomly sampled for measuring of stem length. A 1m ruler was used for measuring stem length and was measured from the base of the stem to the apex of the plant. Measurements for stem length were done at 28, 42, 56, 70 and 84 DAP.

3.3.3 Fresh tuber yield per plant (kg)

All pots were harvested for each plot and the tubers weighed on a digital scale. The average yield per plant was calculated by dividing the total harvested yield for the plot by the total number of plants.

3.3.4 Grades of tubers (Seed Potato Growers Association, 2005):

The harvested tubers per plot were graded using the standards below. The number of tubers per each grade was recorded for each experimental unit.Diameter of tubers (max distance across tuber shoulders – cm) categorized as follows:

Table 3: Grading of potato according to size

Size	**Diameter of tuber**
Small	56-63.9mm
Medium	64-75.9mm
Large	76-83.9mm
Extra large	>84mm

Source: Seed Potato Growers Association Standards (2005)

3.3.5 Disease incidence scoring

The incidence of powdery scab was measured at112DAP using the following formula:

$$\% \text{ tubers infected} = \frac{\text{No. of tubers infected}}{\text{Total number of tubers counted}} \times 100$$

3.3.6 Disease severity

The severity was measured for root galls and powdery scabs as below:

3.3.6.1Root galls scale

Two potato plants per plot were randomly sampled to evaluate root galls at 84 DAP. The following severity scale was used in rating the root galling:

Table 4:Severity scale for root galls on potato roots

Score	Description
0	Healthy, no gall on the potato
1	Less than 10% hairy roots infected or fewer than 10 galls
2	(10~25%) hairy roots infected or 10~25 root galls
3	(25~ 50%) hairy roots infected or 25~50 root galls
4	(50% or more) hairy roots infected or no fewer than 50 root galls

Source: van de Graaf *et al*., 2007

3.3.7 Tubers scoring scale

Two potato tubers were randomly sampled at harvest and their tubers visually rated for the severity of powdery scab. The severity scale used for rating powdery scab was:

Table 5: Severity scale for rating powdery scab on potato tubers

Score	Description
0	Healthy, no lesions on the potato
1	Less than 1% area covered by scab lesions or no more than 5 scab lesions
2	(1~10%) area covered by scab lesions or 6-25 scab lesions
3	(10~ 25%) area covered by scab lesions
4	(25~50%) area covered by scab lesions
5	(50% or more) area covered by scab lesions

Source: Falloon *et al.,*2003

3.4 Data analysis

Analysis of variance was done using statistical package GENSTAT 14th Edition. Separation of means was done on significant treatments using the Duncan test at 5% significant level.

3.5 Results

3.5.1 Effect of *B. napus* on stem diameter (cm)

There were no interaction ($p>0.05$) between *B. napus* and variety on stem diameter. However, *B. napus* was observed to have a significant effect ($p<0.05$) on stem diameter at 84 DAP (Table 6). At 84 DAP, *B. napus* at 20 % had the highest stem diameter which were significantly different from the positive control, Mancozeb by 13% (Table 6).

Table 6: Effect of *B. napus* on the stem diameter of potato at 84DAP

Treatment	84DAP
B. napus 10%	1.12[b]
B. napus 20%	1.67[d]
B. napus 30%	1.42[c]
Mancozeb	1.48[c]
No control amendment	0.89[a]
P value	<0.001
sed	0.068
CV%	10.9%

*Means with the same letter in the column are not significantly different at P<0.05

Sed – Standard error of the difference between means

CV% - Coefficient of variation

3.5.2 Effect of variety on stem diameter (cm)

There were no interaction (p>0.05) between *B. napus* and variety on stem diameter. The effect of variety was significant (p<0.05) on stem diameter (Figure 7). Diamond and Mondial produced stems with the highest stem diameter and their means where not significantly different from each other, compared to BP1.

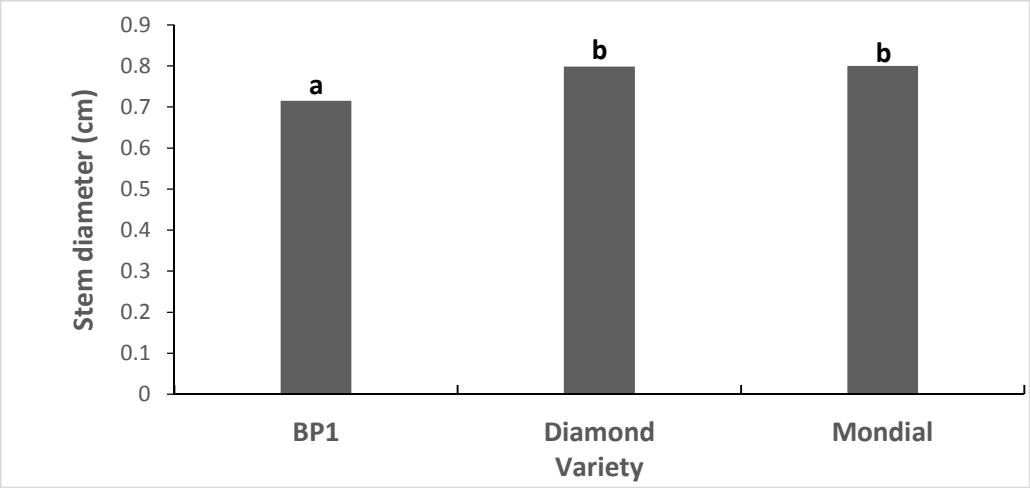


Figure 77: Effect of variety on stem diameter of potato

3.5.3. Effect of *B. napus* on stem length (cm)

There was no interaction between *B. napus* and variety on stem length of potato. However, *B. napus* had a significant effect ($p<0.05$) on stem length. *B. napus* 20%, *B. napus* 30% and the positive Mancozeb produced stems with the highest length that were not significantly different from each other. *B. napus* 10% and the negative control were no control amendment was applied produced stems with the lowest length that were not significantly different from each (Table 7). The effect of variety on stem length was not significant ($p>0.05$).

T**able 7: Effect of B. napus on stem length of potato**

Treatment	42DAP	56DAP	70DAP	84DAP
B. napus 10%	27.52[a]	34.11[a]	35.89[a]	37.07[a]
B. napus 20% 35.28[b]	42.59[bc]	47.84[b]	50.31[b]	
B. napus 30%	36.63[b]	44.16[c]	49.08[b]	50.89[b]
Mancozeb	38.78[b]	48.33[c]	53.97[b]	55.99[b]
No control amendment	30.33[a]	35.78[ab]	38.19[a]	38.93[a]
P value <0.001	<0.001	<0.001	<0.001	
sed	1.843	3.37	3.57	3.89
CV%	11.6	17.5	16.8	17.7

*Means with the same letter in the column are not significantly different at P<0.05

Sed – Standard error of the difference between means

CV% - Coefficient of variation

3.5.4 Effect of *B. napus* on potato yield (kg/plant)

There was no interaction (p>0.05) between *B. napus* and variety on potato tuber yield per plant. However, *B. napus* had a significant (p<0.05) effect on potato tuber yield per plant. *B. napus* 20% and *B. napus* 30% had plants with the highest yield that were not significantly different from each other compared to the positive control, Mancozeb (Figure 8). *B napus* 20% had plants with the highest yield per plant by 55% compared to the positive control, Mancozeb.

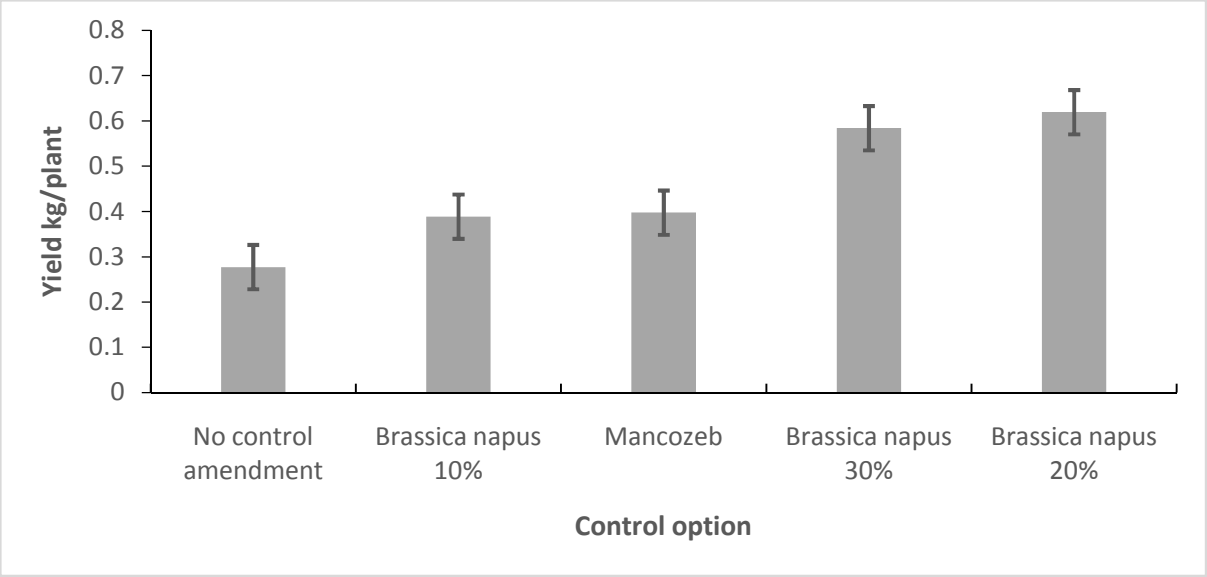


Figure 8: Effect *B. napus* rate on potato tuber yield per plant (kg)

3.5.5 Interaction of *B. napus* and variety on number of extra-large potato tuber

There was an interaction ($p<0.05$) between *B. napus* and variety on extra-large tuber grade. Mondial under *B. napus* 20% had the highest number of extra-large tubers which was not significantly different from the same variety under *B. napus* 30% compared to the same variety under the positive control, Mancozeb (Figure 9). BP1 under *B. napus* 20% and 30% produced tubers with the highest number of extra-large grade tubers which were not significantly different from each other compared to the positive control Mancozeb on the same variety. Diamond under all control options produced very low numbers of tubers with extra-large grade that were not significantly different from each other. *B. napus* 20% on Mondial produced the highest number of extra-large grade tubers by 100% compared to the positive control, Mancozeb on the same variety, Mondial (Figure 9).

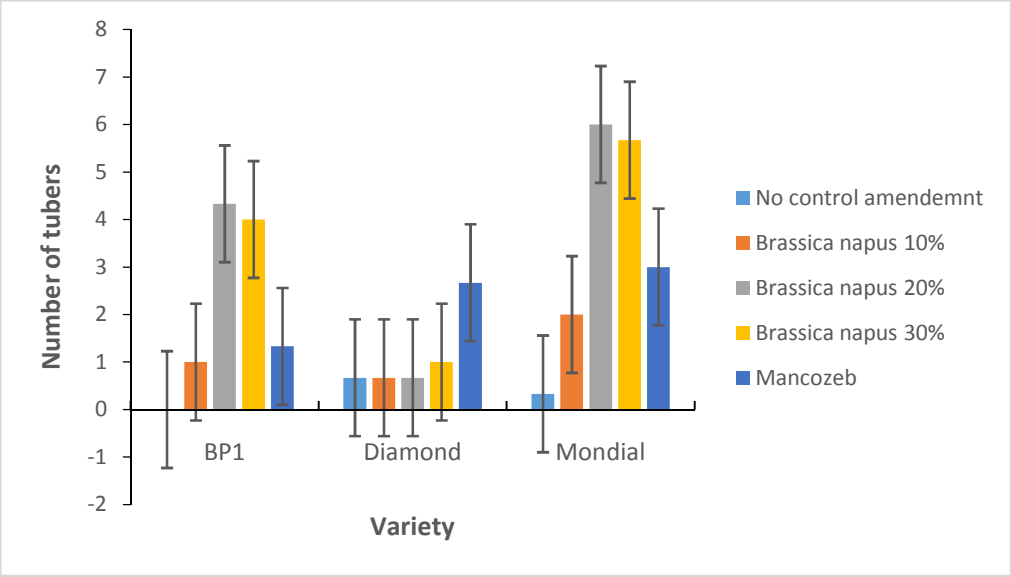


Figure 9: Interaction of *B. napus* and variety on number of extra-large size potato tubers

3.5.6 Interaction of *B. napus* and variety on large graded tuber of potato

There was an interaction ($p<0.05$) between *B. napus* and variety on large tuber grade of potato. Mondial under *B. napus* 20% had the highest number of large tubers which was not significantly different from the same variety under *B. napus* 30% compared to the positive control Mancozeb. *B. napus* regardless of concentration on BP1 resulted in number of large tubers that were not significantly different from both the negative and positive control, Mancozeb. *B. napus* 20% produced large graded tubers that were not significantly different to the positive and negative control in number. *B. napus* 20% on Mondial had the highest number of large graded tubers by 175% compared to the positive control, Mancozeb on Mondial (Figure 10).

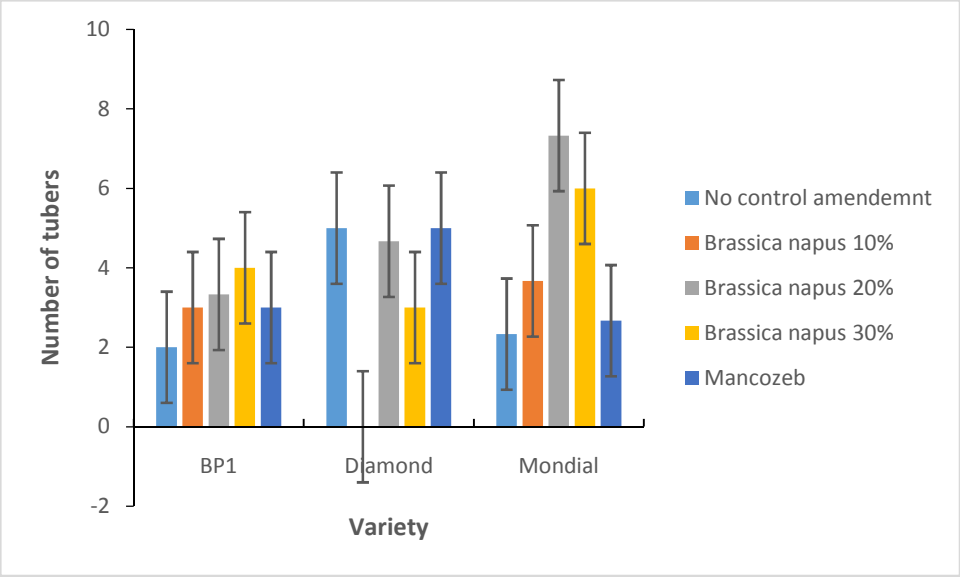


Figure 80: Interaction effects of *B. napus* and variety on large tuber grade of potato

3.5.7 Effects of *B. napus* on number of medium size potato tubers

There was no interaction ($p>0.05$) between *B. napus* and variety on medium grade of tubers. However, *B. napus* had a significant effect on medium grade of tubers. *B. napus* 10% had the highest number of medium sized tubers though not significantly different from the negative control where no control amendment was applied. *B. napus* 20%, B napus 30% and Mancozeb produced the least number of medium graded tubers that were not significantly different from each other (Figure 11).

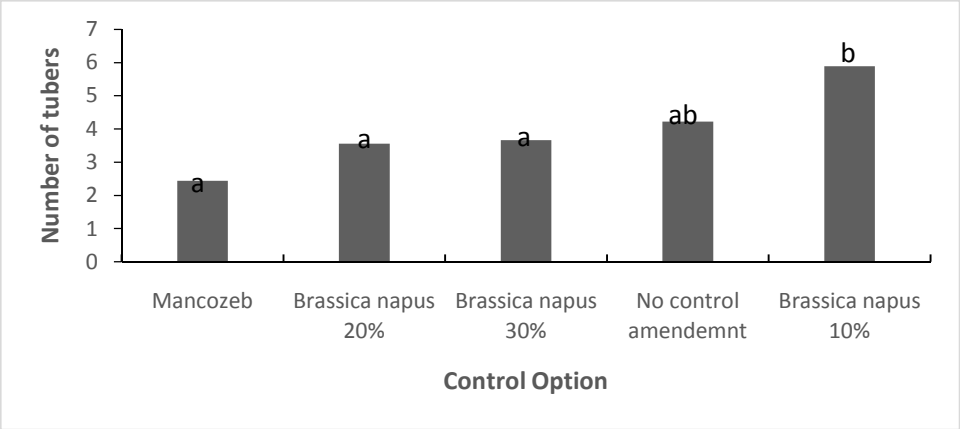


Figure 91: Effect of *B. napus* on number of medium size potato tubers

3.5.8 Effects of *B. napus* on number of medium size potato tubers

There was no interaction ($p>0.05$) between *B. napus* and variety on number of medium grade of tubers. Variety was significantly different ($p<0.05$) on number of medium size potato tubers. BP1 had the highest number of medium graded tubers by 124% compared to both Diamond and Mondial who had the least number of medium size tubers which were not significantly different from each other (Figure 12).

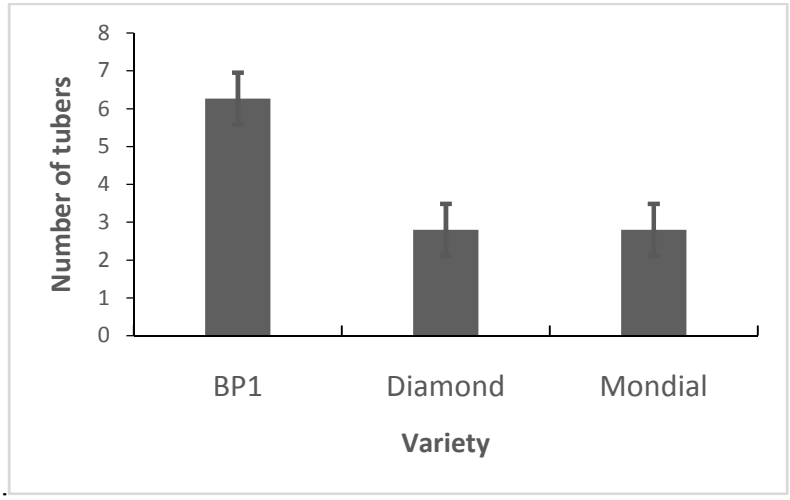


Figure 102: Effect of variety on number of medium size potato tubers

3.5.9 Effects of variety on number of small grade potato tubers

There was no interaction ($p>0.05$) between *B. napus* and variety on number of small size potato

tubers. Variety had a significant effect ($p<0.05$) on number of small size potato tubers. Mondial had the least number of tubers graded as small by 57% compared to Diamond and BP1 who had number of small graded tubers that were not significantly different from each other (Figure 13).

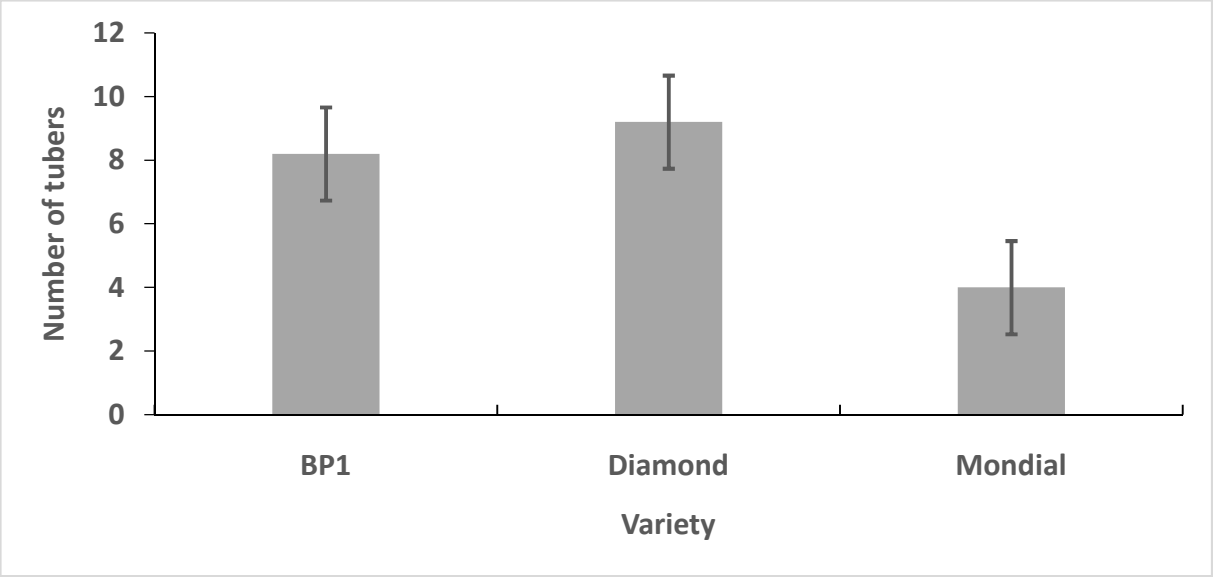


Figure 113: Effect of variety on number of small size potato tubers

3.5.10 Effect of B. napus on disease incidence on potato tubers

There was no interaction ($p>0.05$) between *B. napus* and variety on disease incidence on potato tubers. However, *B. napus* rate had a significant ($p<0.05$) effect on disease incidence on potato tubers (Figure 14). *B. napus* 20% had the lowest disease incidence of 33% which was significantly different ($p<0.05$) from the positive control, Mancozeb, which had 48% (Figure 14). Application of *B. napus 20%* amendment resulted in a 31 % reduction in disease incidence on potato tubers compared to the positive control, Mancozeb. The effect of *B. napus* 30% on disease incidence was comparable to Mancozeb (positive control) (Figure 14).

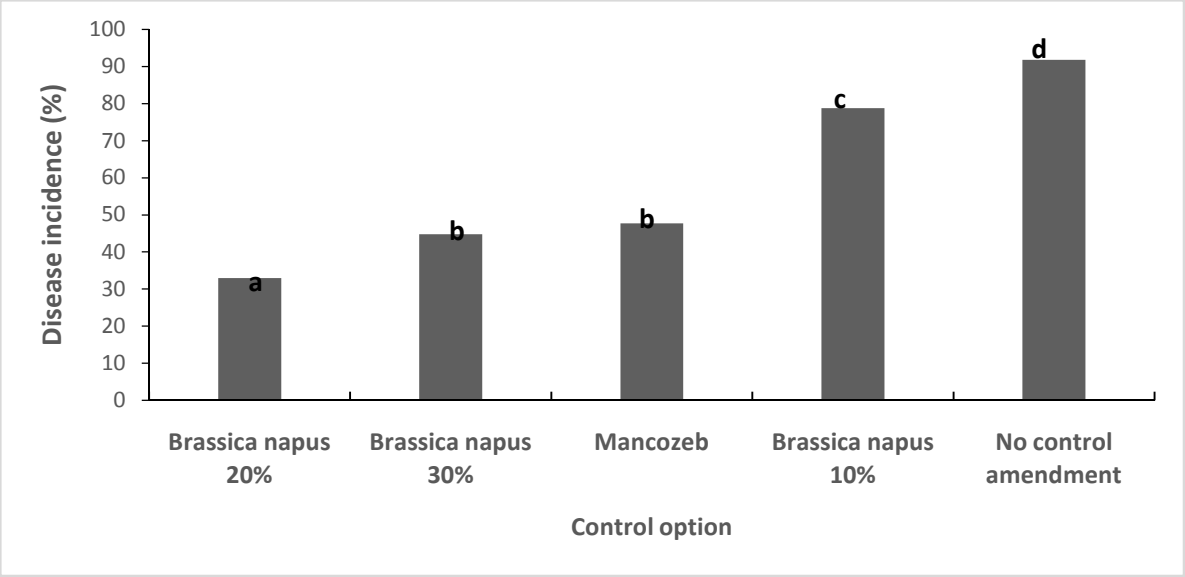


Figure 14: Effect of *B. napus* on disease incidence on potato tubers

3.5.11 Effect of *B napus* on severity of root galls on potato roots

There was no interaction ($p>0.05$) between B. napus and variety on root gall severity on potato. However, *B. napus* had a significant ($p<0.05$) effect on root gall severity on potato. B. napus 20% and 30% had roots with the least number of root galls which were not significantly ($p<0.05$) different from each other compared to the positive control, Mancozeb (positive control) (Figure 15). *B. napus* 20% reduced the severity of root galls on potato by 37 % compared to the positive control, Mancozeb. B. napus 10% was comparable to Mancozeb (positive control) on severity of root galls on potato roots.

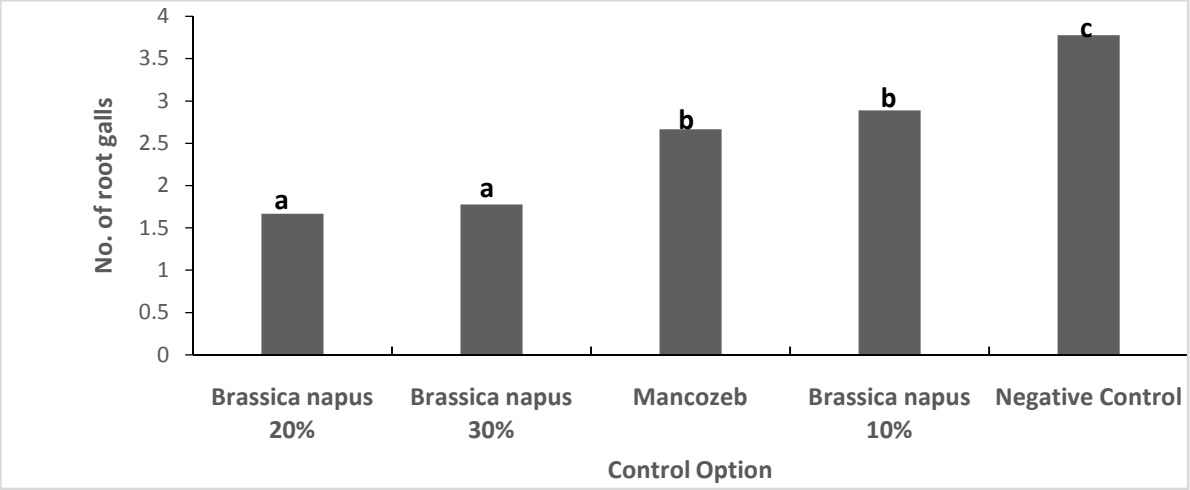


Figure 15: Effect of *B. napus* on root galls severity on potato

3.5.12 Effect of *B. napus* on powdery scab on potato tubers

There was no interaction ($p>0.05$) between *B. napus* and variety on powdery scab severity on potato. Significant differences ($p<0.05$) were noted on effect of *B. napus* on severity of powdery scab on potato. *B. napus* 30%, *B. napus* 20% and Mancozeb, the positive control, had tubers with the lowest severity of powdery scabs on potato tubers and their means were not significantly different from each other (Figure 16). *B. napus* 20% reduced severity of powdery scab on potato tubers by 67% compared to those under the negative control where no control amendment was applied (Figure 16).

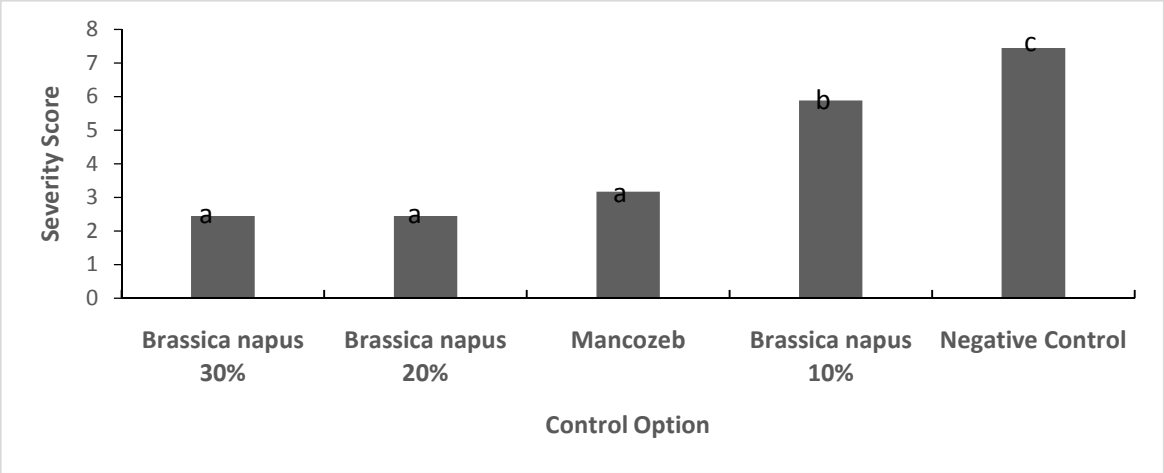


Figure 16: Effect of *B. napus* on powdery scab severity on potato tubers

3.5.12 Effect of variety on powdery scab on potato tubers

There was no interaction between B. napus and variety on appearance of powdery scab on potato tubers. Significant differences ($p<0.05$) were noted on the effect of variety on the severity of powdery scab on potato tubers. Mondial and Diamond had the lowest severity of powdery scab by 20% and they were not significantly different from each other compared to BP1. (Figure 17).

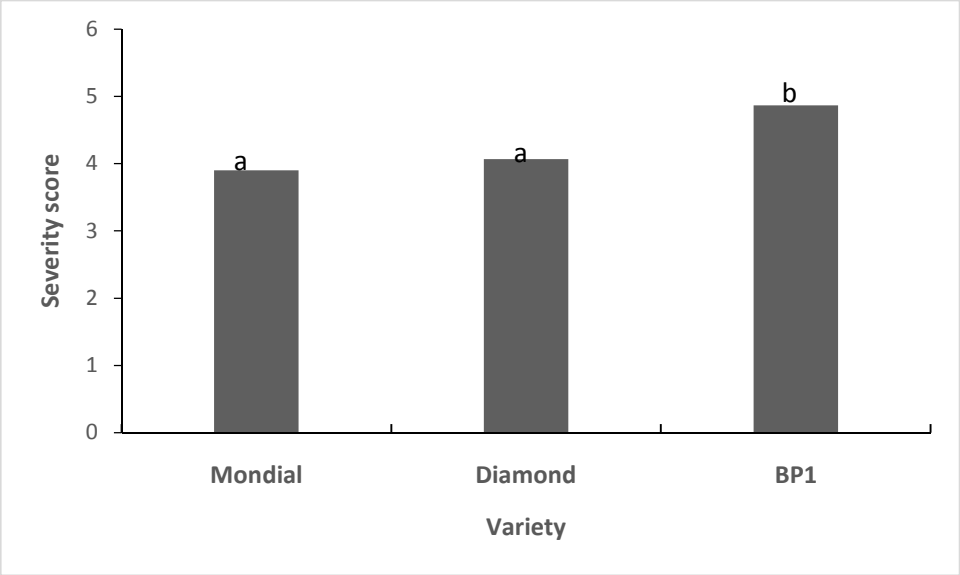


Figure 127: Effect of variety on powdery scab severity on potato tubers

3.6 DISCUSSION

3.6.1 Effect of *B. napus* and variety on growth parameters (stem diameter, stem length, yield) and proportion of marketable tubers (grades) of potato

There was no interaction between *B. napus* and variety on stem diameter. However, *B. napus* and variety had an effect on stem diameter independently. The effect of *B. napus* was rate dependent as stem diameter increased with increasing *B. napus* concentration. The significant increased stem diameter at *B. napus* 20% by 13% compared to the positive control, Mancozeb, could be attributed to *B. napus* acting as a green manure hence having a positive effect on soil physical and chemical characteristics. This concurs with the observations by Kirkegaard *et al*., 2008 that suggest that apart from soilborne biocidal properties, green manure amendment with *Brassica* crops could provide some agronomic benefits to the soil. Cher *et al*., 2006 is also of the same school of thought that suggests that green manure approaches may also result in increases of soil organic matter and microbial biomass hence further improving the nutrient retention and nitrogen uptake by plants. Nicholls and Altieri, 2001 also agrees that green manuring ensures a habitat and resources for beneficial organisms hence improving the soil physical and chemical characteristics. The improved soil physical and chemical characteristics avail an optimum growth environment for potato hence the probable resultant increase in stem diameter.

Effect of variety was also significant with Mondial and Diamond having thicker stems which were not significantly different from each other compared to BP1 which was probably due to differences in their genetic make-up as some varieties are more predisposed to high yielding capabilities than others. This could be attributed to the varietal differences in the absorption and efficient utilization of soil nutrients.

B. napus and variety did not interact at 42DAP, 56DAP, 70DAP and 84DAP on stem length. However, *B. napus* had a significant effect on stem length at 42DAP, 56DAP, 70DAP and 84DAP. *B. napus* 30% had the highest stem length which was not significantly different from *B. napus* 20% and the positive control, Mancozeb. Kirkegaard *et al*., 2008 suggested that apart from soilborne biocidal properties, green manure amendment with *Brassica* crops could provide some agronomic benefits to the soil resulting in improved soil physical and chemical properties hence the high stem length recorded here. *B. napus* 10% had the shortest stems which were not significantly different from the negative control were no control amendment was applied. This could probably be due to reduced green manuring effect at *B. napus* 10% compared to *B. napus* 20% hence the advantages of green manuring where not fully realized as the amount of biomass was not sufficient.

B. napus and variety did not interact on yield of potato tubers. *B. napus* was however significant on potato yield. The highest yield of potato tubers at was obtained at *B. napus* 30% which was not significantly different from *B. napus* 20%. This could have been due to the advantages of green manuring effect of *B. napus* on the soil physical and chemical characteristics (Kirkegaard *et al*., 2008). This might have allowed optimum root functions of water and nutrient uptake to occur hence high leaf assimilation rate and subsequent accumulation of starch in potato tubers. There was no advantage realized in increasing the *B. napus* concentration from 20% to 30% as the same amount of green manuring effect resulted.

B. napus and variety interacted on number of extra-large (> 84mm)and large grade (76 – 83.9mm) tubers. The significant high number of extra-large and large graded tubers in Mondial could be due to the contributory effect of the genetic makeup of the variety which predisposes it to having more extra-large and large tubers as opposed to medium and small tubers under the

high *B. napus*30% which was not significantly different from *B. napus* 20% . This could have been due to the high concentrations of isothiocyanates at *B. napus* 20% which suppressed *S. subterranea*.. The suppression of *S. subterranea* by isothiocyanates in *B. napus* and the genetic makeup of Mondial resulted in optimum potato root absorptive capacity of nutrients resulting in extra large (> 84mm) and large (76 – 83.9mm) tubers.

These findings show the great potential of use of Brassica crops as biofumigants to control soilborne pathogens in potato hence therefore could be considered as an alternative to chemical fumigation with methyl bromide

3.6.2 Effect of *B. napus* and variety on incidence and severity of *S. subterranea*

B. napus and variety did not interact on incidence and severity of *S. subterranea* in potato. B napus was however significant on incidence and severity of *S. subterranea*. Application of *B. napus* resulted in lower incidence of *S. subterranea* regardless of the variety used. The reduced disease incidence at higher concentrations *B napus* 20% and *B. napus* 30% could be due to the biocidal effect of GSL hydrolysis products isothiocyanates at high concentrations to suppress *S. subterranea*. This is in line with the findings of Mari *et al*., 2008 that to obtain better disease control, the largest amount of isothiocyanates should be applied. Possible mode of action of isothiocyanates on *S. subterranea* is based on the ability of the isothiocyanates to cause inhibitory effects on *S. subterranea* through interaction with proteins hence causing the pathogen's intracellular enzymes to be inactivated through oxidative breakdown of the –S-S bridges, inhibition of metabolic enzymes by a thiocyanate radical and uncoupled action of oxidative phosphorylation (Mancini *et al.*,1997; Taylor, 2013).

The reduced severity of root galls and powdery scabs on potato roots and tubers respectively, could be explained by the same phenomenon where the toxic action of isothiocyanates on *S.*

subterranea resulted in the pathogen's reduced severity on potato roots and tubers. The reduced severity of powdery scab on Mondial and Diamond could be due to differences in susceptibility to *S. subterranea* by different potato cultivars, breeding lines and germplasm accessions (de Boer, 1991). No cultivars yet have been found that are completely immune to *S. subterranea* (Anon, 1997:In Iftikhar et al., 2007).

Furthermore, results of this present study suggests that the efficacy of biofumigation on *S. subterranea* is rate dependent as was observed when greater reduction of 56% and 64% in severity of powdery scab on tubers of potato was obtained under *B. napus* 20% and *B. napus* 30% respectively compared to 21% reduction obtained under *B. napus* 10%. This is similar to what was observed by Gouws, 2006 who found out that there was greater reduction of infection by common scab, caused by *Streptomyces scabiei* by 90% with an application rate of 0.33% dry tissue than 41% reduction achieved with application rate of 0.1% dry tissue. *B. napus* 10% was observed to have the same effect on *S. subterranea* as the negative control which suggests that the concentration of isothiocyanates at this rate was not sufficient to cause biocidal effects to the fungal pathogen.

The varieties Mondial and Diamond presented the least visual expression of *S. subterranea* and performed better in terms of growth and yield compared to BP1. One likely reason for this is the difference in varietal resistance towards the fungal pathogen. Mondial and Diamond showed

This study confirms the inhibitory effect of isothiocyanates as observed by Smolinska *et al*., 2003 where isothiocyanates inhibited the growth of *Fusarium oxysporum* mycelia successfully. Furthermore, in a research conduct by Larkin and Griffin in 2006, *Brassica* crops inhibited the growth of *Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum,* and *Fusarium sambucinam*. On the contrary, Hartz *et al*., 2005 reported that the

use of *B. juncea* as a cover crop against soil populations of *Verticillum dahlia* Kleb and *Fusarium* spp. was not effective as there was no evidence of suppression on subsequent tomato crops. This was attributed to insufficient glucosinolate content in the residues, incomplete conversion of glucosinolates to isothiocyanates or inefficient field management practices that reduced biofumigation effectiveness

3.7 CONCLUSION

- *B. napus* 20% reduces disease incidence by 31% compared to the positive control, Mancozeb.
- *B. napus* 20% reduces severity of root galls by 37% compared to the positive control, Mancozeb
- *B. napus* 20% reduces severity of powdery scab by 67% compared to the negative control were no control amendment was applied and both Mondial and Diamond had 20% reduction in severity of powdery scab compared to BP1
- *B. napus* 20% had higher stem diameter by 13% compared to the positive control, Mancozeb and both Mondial and Diamond had higher stem diameter by 14% compared to BP1
- B napus 20% had higher stem lengths by 29% compared to the negative control
- *B. napus* 20% increased yield of potato by 55%
- *B. napus* 20% on Mondial resulted in 100% increase in extra-large graded tubers compared to Mondial under the positive control
- *B. napus* 20% on Mondial resulted in 175% increase in large graded tubers compared to the positive control, Mancozeb
- BP1 had the highest number by 124% of medium graded tubers compared to Diamond

and Mondial

- Mondial had the least number of small graded tubers by 57%

CHAPTER 4

EFFECT OF *T. HARZIANUM ON* THE INCIDENCE AND SEVERITY OF POWDERY SCAB (*S. SUBTERRANEA*) IN POTATO.

ABSTRACT

Powdery scab of potato caused by *S. subterranea* is a major disease of potato in Zimbabwe. To determine whether the use of biological control agent *T harzianum* can reduce incidence and severity coupled with increase in growth and yield of potato, a glasshouse experiment was set up. The glasshouse had an average relative humidity of 60% with 34 ^{0}C(±3) and 21 ^{0}C(±3) day and night temperatures respectively. The experiment was laid out in a 5 x 3 factorial in a CRD. The first factor was *T. harzianum* rates with 1gl^{-1}, 2g l^{-1} , 3g l^{-1} at 1 x 10^{7}CFUg^{-1}with Mancozeb (positive control) and a negative control were no control amendment was applied. The second factor was potato variety and the levels were BP1, Diamond and Mondial.*T harzianum* 1 x 10^{7}CFUg^{-1} 2g l^{-1} reduced disease incidence by 26% and severity by 38% (root galls) and 59% (tubers). *T harzianum* 1 x 10^{7}CFUg^{-1} 2g l^{-1} on Mondial resulted in 343 % increase in proportion of extra-large tubers compared with the positive control, Mancozeb on Mondial.

4.1 Introduction

Powdery scab of potato (*Solanum tuberosum* L.) is caused by the pathogen *Spongosporasubterranea* (Wallroth) Langerheim f.sp. *subterranea* Tomlinson. The pathogen belongs to the Plasmodiophorids (Braselton, 1995) and was first described in Germany in 1841 (Morse, 1913). The pathogen causes powdery scabs that form on potato tubers contain masses of resting structures known as sporeballs or cystosori. These cystosori have the ability to survive in the soil for many years (Harrison *et al*., 1997; Merz and Falloon, 2009). Under favourable environmental conditions of high moisture and cool temperatures, these sporeballs release

primary zoospores, which infect the potato host plants. To date, there are no effective control measures for powdery scab with disease management strategies such as cultivar resistance, disease free seed tubers, fungicides, cultural practices, antagonists and legislation being employed to try to curb the disease (Wright, 2012).

Biological control methods have been employed in agriculture as an alternative to chemical fungicides to control diseases caused by fungal plant pathogens (Quintana-Jones, 2011). Fungi in the genus *Trichoderma* have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens (Harman, 2005). Members of the *Trichoderma* genus are known as imperfect fungi with teleomorphs belonging to the *Hypocreales* order of the Ascomycota division (Howell, 2003; Kredics *et al*., 2003). Rapid growth rate and the production of numerous spores (conidia) that are varying shades of green characterize fungi in this genus (Howell *et al*., 2003). This high reproductive capacity, ability to survive under harsh conditions, efficiency in utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness towards phytopathogenic fungi and efficiency in promoting plant growth and defence mechanisms contribute to *Trichoderma* spp. success as a biocontrol agent. It has been shown that *T harzianum* has the ability to parasitize sporeballs of *S.subterranea* under controlled experimental growing conditions (Merz and Falloon, 2009).

4.2 Methodology

4.2.1 Site description

The research was carried out at Harare Research Station (31^0 03′E and 17^0 48′S). It is located within agro ecological region IIa with an altitude of 1506m above sea level. The research station receives annual rainfall of 820mm. The experiment was conducted in a glasshouse and the

temperatures in the glasshouse ranged from 21 – 35^0C with an average relative humidity of 60% during the time of the experiment.

4.2.2 Experimental Design and treatments

The experiment was laid out as a 5 x 3 factorial in a CRD replicated 3 times. The first factor was *T. harzianum* and the second factor was variety with the levels and treatment structure as shown in Table 8 below.

Table 8:Treatment structure for effect of *T. harzianum* on the incidence and severity of *S. subterranea* on potato

	Varieties		
Control Option	**BP1**	**Diamond**	**Mondial**
***T harzianum* 1g l^{-1}(1 x 10^7 CFU g^{-1})**	T1	T2	T3
***T harzianum* 2g l^{-1}(1 x 10^7 CFU g^{-1})**	T5	T6	T7
***T harzianum* 3g l^{-1}(1 x 10^7 CFU g^{-1})**	T8	T9	T10
Mancozeb	T11	T12	T13
No control amendment	T14	T15	T16

4.2.3 Preparation of soil

Red clayey soil was used as a potting media with a pH of 7.1 ($CaCl_2$). Oven incubation overnight at 100℃ was used as the soil sterilization method. A total number of 225 plastic pots were disinfected with 1 % NaOCl. Soil was dispensed into the plastic pots which measured 50cm x 40cm with a holding capacity of 20 L.

4.2.4 Preparation of *T harzianum*

T harzianum inoculum isolate T.77 was obtained from Kutsaga Research Station (Plant Protection Division). It was isolated from culture collection to bottles with slop media (Potato Dextrose Agar) and reinoculated and grown on Potato Dextrose Agar (PDA) in plates at 28-30°C for five days. The isolate of *T harzianum,* T77 was selected among a collection of other isolates. The liquid media was autoclaved at 121^0C for 25 minutes at 15-pounds/square inch (psi).The fungus was scrapped onto liquid media and grown in 750ml glass round neck bottles containing 100ml of a synthetic medium (Czapeks medium). Three discs of *T harzianum* from the plates were added in each round neck glass bottle. The bottles were then plugged with cotton wool and incubated for ten days at room temperature in a dark room. The mycelium and spores were harvested by pouring the synthetic media with the *T harzianum* on a perforated aluminium foil paper, capturing the mycelium and spores and the synthetic media drained away. The captured mycelium and spores were then oven dried at 28-30°Cfor two days. The dried mycelia and spores were refined by grinding using the paste and mortar method. On the inoculation day, 45 pots were inoculated with *T harzianum* at (1 x 10^7 CFU g^{-1})1g l^{-1},45 pots with *T harzianum* at (1 x 10^7 CFU g^{-1+})2gl^{-1} and 45 pots with *T harzianum* at (1 x 10^7 CFU g^{-1}) 3gl^{-1}.

4.2.5 *S. subterranea* inoculum preparation

A sterile scalpel was used to cut powdery scab lesions from infected tubers of variety KY20. Microscopic slides from the scabbed tubers of variety KY20 were made and viewed under the light microscope. Diagnostic features of lesions filled with masses of cystori of *S. subterranea* were identified confirming its presence on the tubers. The powdery scab lesions were peeled off from the skin of infected tubers, air dried and ground to a fine powder using a pestle and mortar. The inoculum was stored under dark and dry conditions at room temperature. On the inoculation day, 5g of the powder were added per pot and thoroughly mixed with the soil.

4.2.6 Plant Culture

Three potato varieties namely, BP1, Diamond and Mondial, were allowed to sprout for a month whilst spread on grass in a dark room. Once hardened off for a week in a shed area with no direct sunlight the tubers were planted out with an average of four sprouts per tuber. The potting media was made up of red clayey soil with each pot being 50cm x 40cm with a holding capacity of 20 L of soil. Each pot was half filled with soil. One tuber was planted per pot. Potato is a heavy feeder and an equivalent of 2000 kg/ha Compound S with N. P. K ratio of 7: 21: 7 was placed at 2.5 cm below the seed at a rate of 27g/pot. Top dressing, Ammonium Nitrate, was applied at 28 DAP weeks after emergence before filling up of pots, at a rate of 200 kg /ha which amounts to 5g/pot. The second filling up was done at week 63 DAP. A plot consisted of 5 pots. Irrigation scheduling of 7 days was followed and the pots were irrigated to field capacity. Weeding was done when necessary to control weeds, keep soil hilled up and aid water penetration as well as improving aeration.

4.3 Data Collection

The following measurements were taken:

4.3.1 Stem length (cm)

Two pots per plot were randomly sampled for measuring of stem length. A 1m ruler was used for measuring stem length and was measured from the base of the stem to the apex of the plant. Measurements for stem length were done at 28, 42, 56, 70 and 84 DAP.

4.3.2 Stem diameter (cm)

The diameter of stems was measured using a Vernier calliper. Two plants per plot were sampled for measuring diameter and the diameter was measured at the middle of the stem. Stem diameter was measured at 28, 42, 56, 70 and 84DAP.

4.3.3 Fresh tuber yield per plant (kg)

All pots were harvested for each plot and the tubers weighed on a digital scale. The average yield per plant was calculated by dividing the total harvested yield for the plot by the total number of plants.

4.3.4 Grades of tubers (Seed Potato Growers Association, 2005):

The harvested tubers per plot were graded using the standards below. The number of tubers per each grade was recorded for each experimental unit.Diameter of tubers (max distance across tuber shoulders – cm) categorized as follows:

Table 9: Grading of potato according to size

Size	Diameter of tuber
Small	56-63.9mm
Medium	64-75.9mm
Large	76-83.9mm
Extra large	>84mm

Source: Seed Potato Growers Association Standards (2005)

4.3.5 Disease incidence scoring

The incidence of powdery scab was measured at 112 DAP using the following formula:

$$\% \text{ tubers infected} = \frac{\text{No. of tubers infected}}{\text{Total number of tubers counted}} \times 100$$

4.3.5 Disease severity

4.3.5.1 Root galls scale

Two potato plants per plot were randomly sampled to evaluate root galls at 84 DAP. The following severity scale was used in rating the root galling:

Table 10: Severity scale for root galls on potato roots

Score	Description
0	Healthy, no gall on the potato
1	Less than 10% hairy roots infected or fewer than 10 galls
2	(10~25%) hairy roots infected or 10~25 root galls
3	(25~ 50%) hairy roots infected or 25~50 root galls
4	(50% or more) hairy roots infected or no fewer than 50 root galls

Source: van de Graaf *et al*., 2007

4.3.5.2 Tubers scoring scale

Two potato tubers were randomly sampled at harvest and their tubers visually rated for the severity of powdery scab. The severity scale used for rating powdery scab was:

Table 11: Severity scale for rating powdery scab on potato tubers

Score	Description
0	Healthy, no lesions on the potato
1	Less than 1% area covered by scab lesions or no more than 5 scab lesions
2	(1~10%) area covered by scab lesions or 6-25 scab lesions
3	(10~ 25%) area covered by scab lesions
4	(25~50%) area covered by scab lesions
5	(50% or more) area covered by scab lesions

Source: Falloon *et al.,*2003

4.4 Data analysis

Analysis of variance was done using statistical package GENSTAT 14th Edition. Separation of means was done on significant treatments using the Duncan test at 5% significant level.

4.5 Results

4.5.1 Effect of *T harzianum* and variety on stem diameter (cm)

There was an interaction ($p<0.05$) effect between *T harzianum* and variety on stem diameter at 84 DAP. *T harzianum* 2g on BP1 resulted in higher stem diameter by 77% which was not significantly different from both Diamond and Mondial under *T harzianum* 2g compared to the positive control, Mancozeb on BP1, Diamond and Mondial (Figure 18).

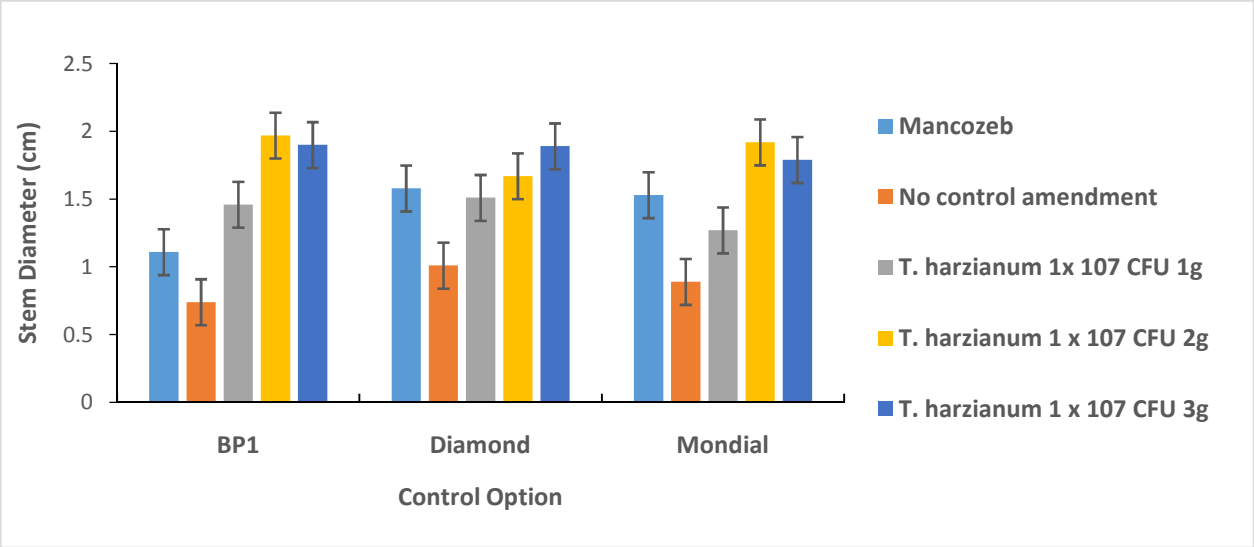


Figure 18: Interaction effects of *T harzianum* and variety on stem diameter at 84DAP

4.5.2 Effect of *T harzianum* and variety on stem length (cm)

There was an interaction ($p<0.05$) between *T harzianum* and variety at 84 DAP. Application of *T harzianum* 2g on Mondial resulted in higher stem length by 32% compared to the positive control, Mancozeb on Mondial (Figure 19).

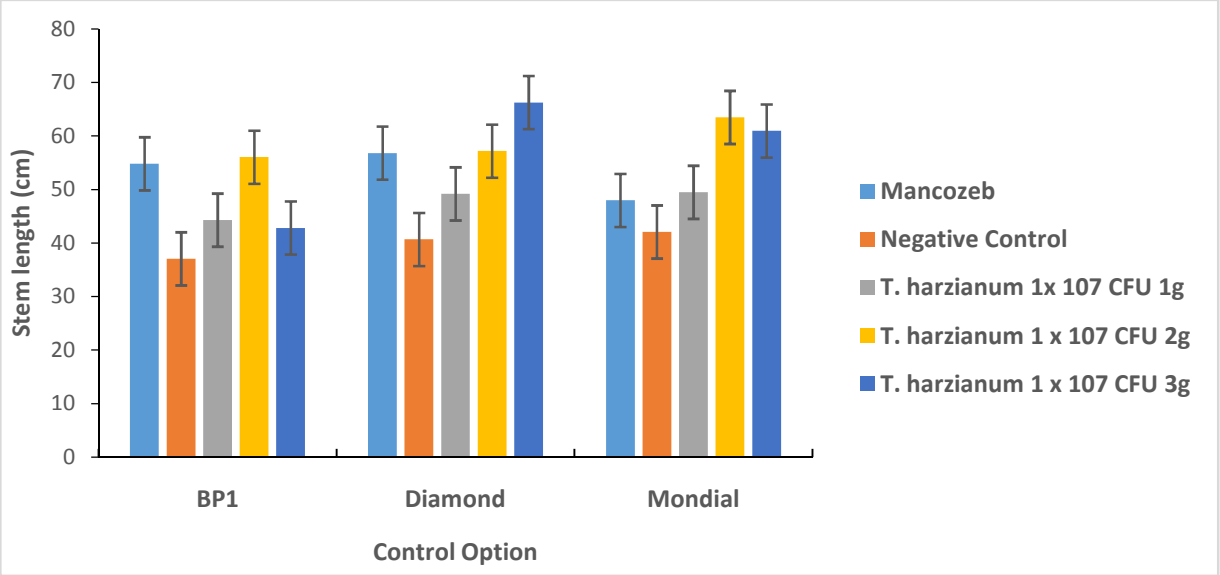


Figure 19: Interaction effects of *T harzianum* and variety on stem length at 84 DAP

4.5.3.1 Effect of *T harzianum* on potato yield (kg/plant)

There was no interaction ($p>0.05$) between *T harzianum* and variety on potato yield. However, significant differences ($p<0.05$) were noted on effect of *T harzianum* on potato yield. *T harzianum* 2g resulted in higher yield by 59% compared to the positive control, Mancozeb (Table 12).

Table 12: Effect of *T harzianum* on potato yield (kg/plant)

Treatment	112 DAP
T harzianum 1x 10^7 CFU 1g	0.49 [b]
T harzianum 1 x 10^7 CFU 2g	1.11 [d]
T harzianum 1 x 10^7 CFU 3g	1.13 [d]
Mancozeb	0.70 [c]
No control amendment	0.23 [a]
P value	<0.001
sed	1.843
CV%	17.5%

*Means with the same letter in the column are not significantly different at P<0.05

4.5.3.2 Effect of variety on potato yield (kg/plant)

There was no interaction between *T. harzianum* and variety on potato yield. Variety had a significant effect ($p<0.05$) on potato yield. Mondial had the highest yield of tubers by 26% compared to BP1 and Diamond whose yield was not significantly different from each other (Figure 20).

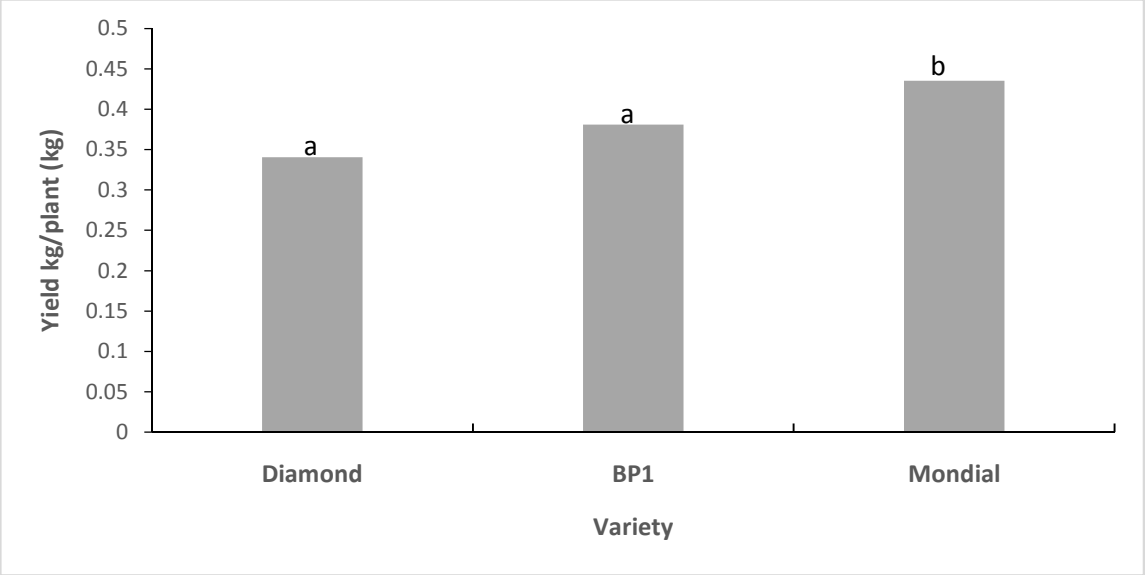


Figure 20: The effect of variety on potato yield (kg/plant)

4.5.4 Effect of *T harzianum* and variety on extra-large grade of tubers

There was a significant interaction ($p<0.05$) effect of *T harzianum* and variety on extra-large tuber grade. Mondial under *T harzianum* 2g had the highest number of extra-large tubers by 343% which was not significantly different from *T harzianum* 3g compared to Mondial under the positive control, Mancozeb (Figure 21).

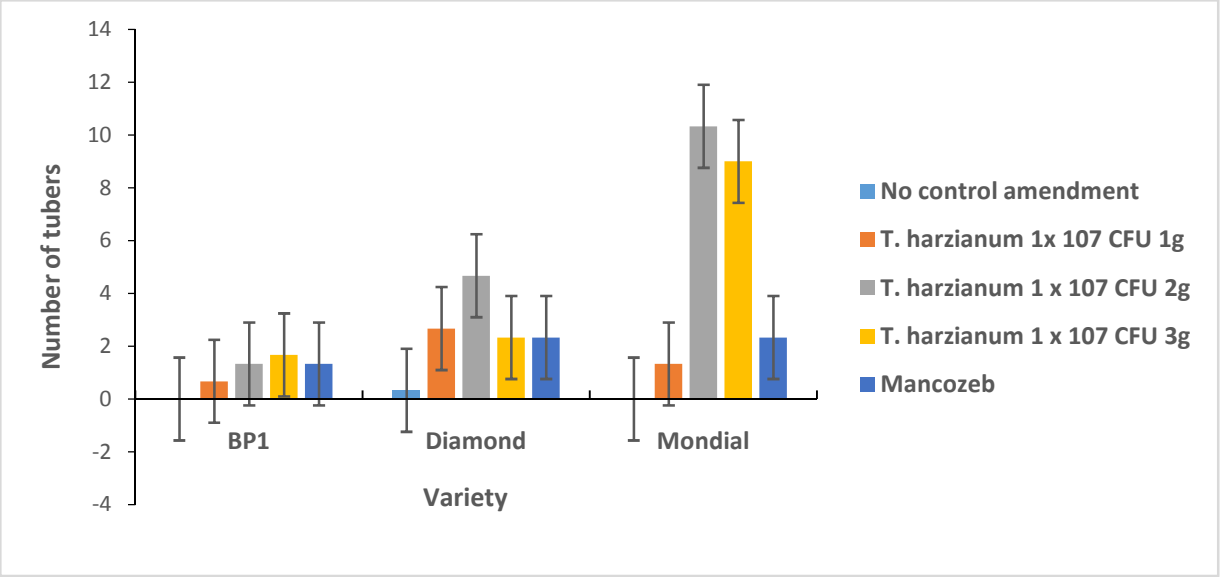


Figure 13: Interaction effects of *T harzianum* and variety on number of extra-large potato tuber size

4.5.6.2 Effect of *T harzianum* and variety on tuber grades large, medium and small

Effect of *T harzianum* and variety on tuber grades large, medium and small was not significant ($p>0.05$).

4.5.7.1 Effect of *T harzianum* on disease incidence

There was no interaction ($p>0.05$) effect between *T harzianum* and variety on disease incidence. However, *T harzianum* had a significant effect ($p<0.05$) on disease incidence. *T harzianum* 2g reduced disease incidence by 35% which was not significantly different from *T harzianum* 3g compared with the positive control, Mancozeb (Figure 22).

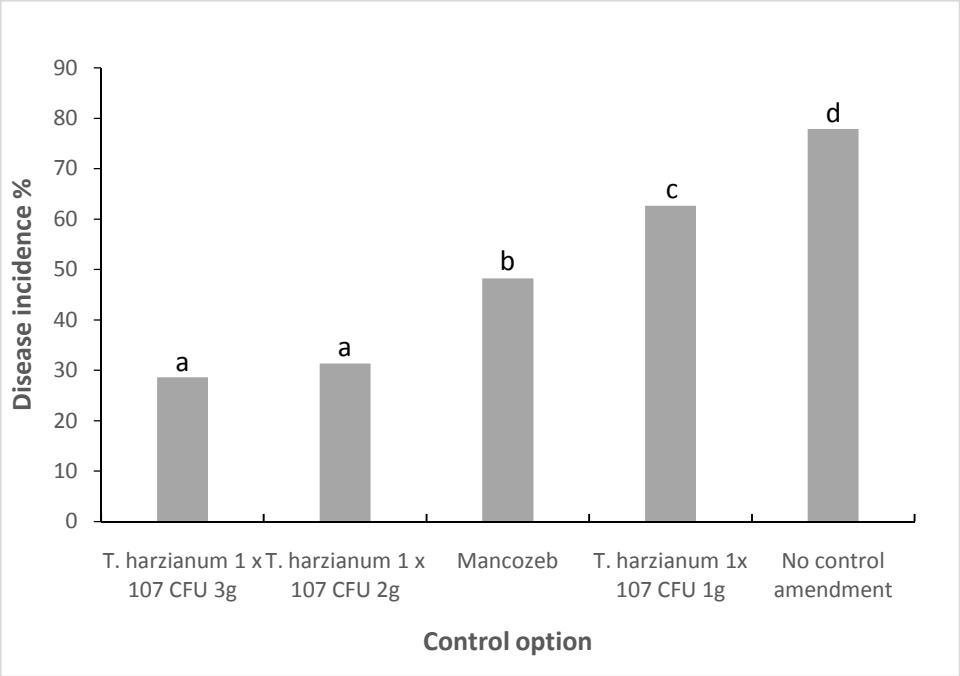


Figure 14: Effect of *T harzianum* on disease incidence on potato tubers

4.5.7.2 Effect of variety on disease incidence

There was no interaction ($p>0.05$) effect between *T harzianum* and variety on disease incidence. Mondial had the least severity by 42% compared to BP1and Diamond which were not significantly different from each other. (Figure 23).

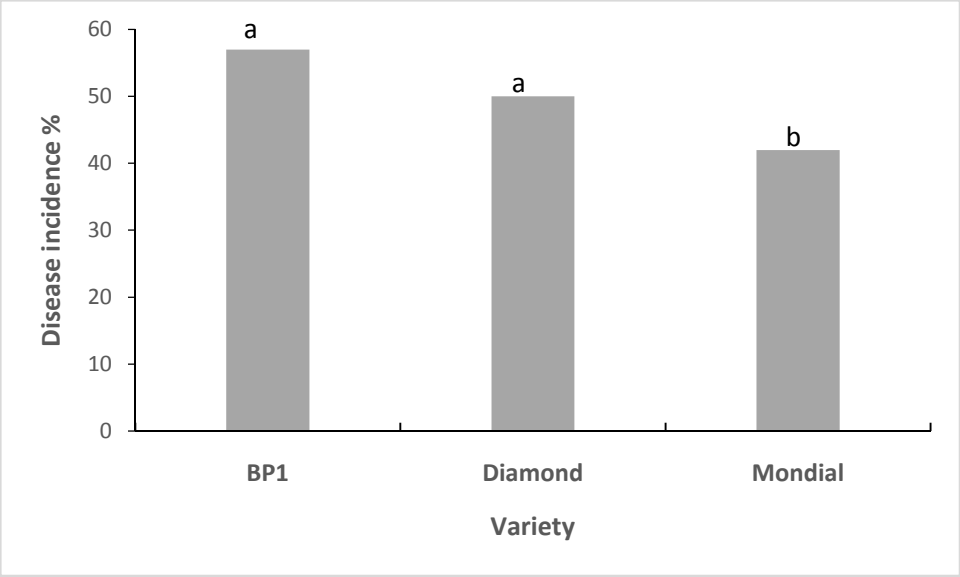


Figure 23: Effect of variety on disease incidence on potato tubers

4.5.8. Effect of *T harzianum* severity of root galls on potato roots

There was no interaction ($p>0.05$) between *T harzianum* and variety on severity of root galls on potato roots. However significant differences ($p<0.05$) were observed on *T harzianum*. *T harzianum* 2g resulted in the lowest severity of root galls on potato roots which was not significantly different from *T harzianum* 3g and the positive control, Mancozeb. *T harzianum* 2g reduced severity of root galls by 38% compared to the negative control (Figure 24).

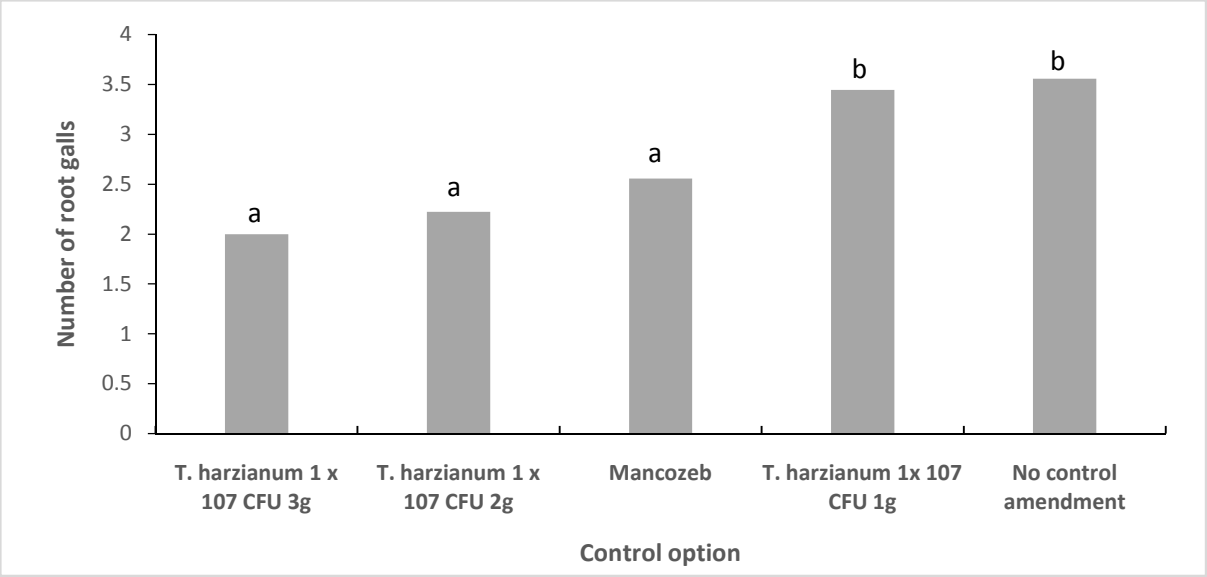


Figure 154: Effect of *T harzianum* on severity of root galls on potato roots

4.5.9 Effect of *T harzianum* on severity of powdery scab on potato tubers

There was no interaction ($p>0.05$) observed between *T harzianum* and variety on severity of powdery scab on potato tubers. However significant differences ($p<0.05$) were observed on *T harzianum*. *T harzianum* 2g had the lowest severity of powdery scab which was not significantly different from *T harzianum* 3g and the positive control, Mancozeb. *T harzianum* 2g reduced the severity of powdery scab on potato tubers by 59% as compared to the negative control where no control amendment was applied (Figure 25). Variety had no significant effect ($p>0.05$) on severity of powdery scab.

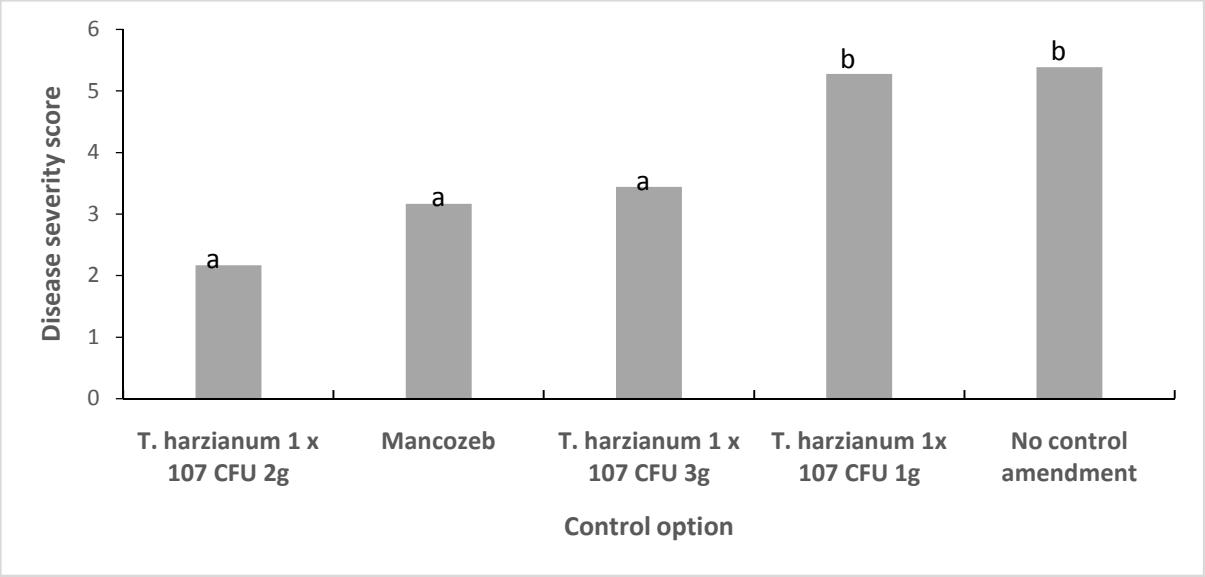


Figure 16: Effect of *T harzianum* on severity of powdery scab on potato tubers

4.6 DISCUSSION

4.6.1 Effect of *T harzianum* and variety on growth parameters (stem diameter, stem length, yield) and proportion of marketable tubers (grades) of potato

T harzianum and variety interacted on stem diameter (84DAP), stem length (84DAP), and the proportion of extra-large tubers at 112 DAP. *T harzianum* application on potato varieties resulted in increasead stem diameter, stem length starch content and low reducing sugar content. This could probably be due to the suppressive action through parasitism of *T harzianum* on cystori of *S. subterranea.* This concurs with the findings of D'ambra and Mutto, 1986 in Nielsen and Larsen 2004 who reported that cystori of *Polymyxa betae*, a member of the Plasmophorids to which *S. subterranea* belongs, were parasitized and degraded by *T harzianum*. Other possible modes of action of *T harzianum* on *S. subterranea* could possibly be due to the ability of *T harzianum* to effectively suppress the population of *S. subterranea* through competition with *S. subterranea*, stimulate plant growth which may allow plants to quickly outgrow any *S.*

subterranea effects, or damage *S. subterranea* by means of toxins produced (Cook, 2000; Gilreath, 2002).

The negative control where no control amendment was applied in all varieties resulted in the thinnest stems, shortest stems, lower starch content, low proportion of extra-large graded tubers and high reducing sugar content. This could be attributed to the infection of the roots with *S. subterranea* resulting in lowered root absorptive capacity, low net assimilation rate hence the reduction in these growth parameters (Falloon *et al*., 2005;Gilchrist*et al.,* 2009). This is in line with research carried out by Nielsen and Larsen, 2004, on controlling *S. subterranea* with *T harzianum*, they discovered that plant growth parameters examined were markedly lower in the infected controls compared with the uninfected plants.

T harzianum and variety did not interact on yield but *T harzianum* had a significant effect on yield of potatoes. Application of *T harzianum* resulted in increased yield as seen at *T harzianum* 3g which was not significantly different from *T harzianum* 2g compared to the negative control. This could be attributed to *Trichoderma* spp. causing increased nutrient uptake and fertilizer efficiency utilization (Harman, 2001; Yedidia *et al*., 2001) and leaf greenness that is probably related to increased photosynthetic rate (Harman and Shoresh, 2007) which increases net assimilation rate hence increase in yield. This concurs with the report that *Trichoderma* spp. can colonize plant roots leading to induce growth and nutrient adsorption (Harman *et. al*., 2004).

4.6.2 Effect of *T harzianum* and variety on incidence and severity of *S. subterranea*

T harzianum and variety did not interact on disease incidence. The reduced incidence of *S. subterranea* at *T harzianum* 3g could have be attributed to the success of *T harzianum* in suppressing *S. subterranea* through parasitism and degradation of cystori. This concurs well with

what Falloon *et al*., 1997 that *T harzianum* is known to parasitize cystori of *S. subterranea*. The same phenomenon could be used to explain the severity of both root galls and powdery scabs on roots and tubers of potatoes respectively. Research by Neilsen and Larsen 2004 has indicated that biological control agents, particularly *Trichoderma* spp., have potential for reducing activity of *S. subterranea*, presumably through effects on either resting spore viability or zoospore activity and infectivity. Nielsen and Larsen, 2003 found that *T harzianum* significantly reduced the root infection level of tomato by zoosporangia of *S. subterranea*.

T harzianum could have probably induced systemic resistance in potato to the pathogen, *S. subterranea*. Furthermore, *T harzianum* could have produced protease enzymes that inactivated *S. subterranea*. This concurs with the report by Sharon *et al*., 2001 were protease production by *T. hazianum* was associated with biocontrol of the root-note nematode Meloidgyne javanica on tomato plants. *T. harzianum* could have coiled its mycelia around *S. subterranea* or attached onto the pathogen by forming hook like structures (Siddiqui *et al.*, 2008). A cocktail of cell wall degrading enzymes able to hydrolyse the wall of *S. subterranean* might have been produced (Monte and Llodell, 2003). These enzymes include chitinases and glucanases as well as fungal proteases. Degradation of*S. subterranea* wall leads to dissolution of the pathogen's cytoplasm hence reduction in incidence and severity of the disease.

4.7 Conclusion

- ery scab by 59% compared to the negative control were no control amendment was applied
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ on BP1 resulted in higher stem diameter by 77% compared to the positive control, Mancozeb

- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ on Mondial resulted in higher stem length by 32% compared to the positive control, Mancozeb
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ resulted in higher yield by 59% compared to the positive control, Mancozeb
- Mondial had higher yield by 26% compared to BP1 and Diamond
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ on Mondial resulted in higher extra-large graded potato tubers compared to the positive control, Mancozeb, on Mondial
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ reduces disease incidence by 35% compared to the positive control, Mancozeb
- Mondial tubers were less sever by 57% compared to BP1 and Diamond
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ reduced severity of root galls by 38% compared to the negative control were no control amendment was applied
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-1}$ reduced powd

CHAPTER 5

5.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General Discussion

Demand for potatoes in sub-Saharan Africa is projected to have a 250% increase between 1993 and 2020, with an annual growth in demand of 3.1% and the growth in area under production is estimated at 1.25% a year (Scott *et al*., 2000). However, this growth is under threat from soil borne diseases like *S. subterranea* which can reduce the growth, yield and quality of potatoes. Control with chemicals like Mancozeb is possible though not solely effective. There is therefore need to adopt other environmentally friendly methods to control the disease like biofumigation with *B. napus* and biocontrol with *T harzianum.*

B. napus and variety did not interact on most of the growth parameters measured. However, *B. napus* had a significant on the growth parameters. The increased stem diameter, stem length, yield and proportion of extra-large tubers could be attributed to the green manuring effect which was greatest at *B. napus* 30% which was not significantly different to *B. napus* 20%. Green manuring has been reported to have agronomic benefits on crops through positively affecting the soil physical and chemical properties hence providing optimum conditions for growth of the potato plants hence the increase in the growth parameters measured.

B napus and variety did not interact on incidence and severity of *S. subterranea* on roots and tubers of potato. However, *B. napus* and variety were shown to independently and significantly affect disease incidence and severity. B napus 20% had the least incidence, *B. napus* 20% had the least severity of root galls on roots which was not significantly different from *B. napus* 30% and B.napus 20%, *B. napus* 30% and the positive control Mancozeb had the least severity of

powdery scabs and were not significantly different from each other. This was probably because *B. napus* contains GSL which upon hydrolysis produce isothiocyanates which have biocidal effect on *S. subterranea*. This biocidal effect is rate dependent as seen by the difference in degree of incidence and severity between *B. napus* at 20 and 30% compared to *B. napus* at 10%. Mondial and Diamond had the least visual expression of powdery scab on tubers compared to Diamond. This shows that as reported before that no variety is immune to *S. subterranea* but varieties respond differently due to differences in susceptibility conferred by differences in genetic make-up of varieties.

T. harzianum and variety interacted on most growth parameters measured. The increase in stem diameter, stem length, starch content, proportion of extra-large tubers and low reducing sugar content, low disease incidence and severity on application of *T. harzianum* could be attributed to the suppressive effect of *T. harzianum* on *S. subterranea*. This could have been achieved through parasitism, production of proteous enzymes that degraded the cystori of the pathogen, induction of systemis resistance on the plant, competition with *S. subterranea*, stimulate plant growth which may allow plants to quickly outgrow any *S. subterranea* effects, or damage *S. subterranea* by means of toxins produced and mycoparasitism. This allowed for maximum root absorptive capacity of nutrients to occur resulting in increased growth and reduction of incidence and severity of *S. subterranea* on potato.

T. harzianum and variety did not interact on yield but *T. harzianum* had a significant effect on yield. This could be attributed to *Trichoderma* spp. causing increased nutrient uptake and fertilizer efficiency utilization and leaf greenness that is probably related to increased photosynthetic rate which increases net assimilation rate hence increase in yield. This concurs

with the report that *Trichoderma* spp. can colonize plant roots leading to induce growth and nutrient adsorption.

Generally, the effect of *T. harzianum* 2g was not significantly different from T harzianum 3g and the positive control, Mancozeb. *T harzianum* 1g showed that its effect was not enough to cause optimum suppressive effect on *S. subterranea*.

5.3. The conclusions derived from this study were that:

Experiment 1

- *B. napus* 20% reduces disease incidence by 31% compared to the positive control, Mancozeb.
- *B. napus* 20% reduces severity of root galls by 37% compared to the positive control, Mancozeb
- *B. napus* 20% reduces severity of powdery scab by 67% compared to the negative control were no control amendment was applied and both Mondial and Diamond had 20% reduction in severity of powdery scab compared to BP1
- *B. napus* 20% had higher stem diameter by 13% compared to the positive control, Mancozeb and both Mondial and Diamond had higher stem diameter by 14% compared to BP1
- B napus 20% had higher stem lengths by 29% compared to the negative control
- *B. napus* 20% increased yield of potato by 55%
- *B. napus* 20% on Mondial resulted in 100% increase in extra-large graded tubers compared to Mondial under the positive control
- *B. napus* 20% on Mondial resulted in 175% increase in large graded tubers compared to

the positive control, Mancozeb

- BP1 had the highest number by 124% of medium graded tubers compared to Diamond and Mondial
- Mondial had the least number of small graded tubers by 57%

Experiment 2

- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ reduces disease incidence by 35% compared to the positive control, Mancozeb
- Mondial tubers were less sever by 57% compared to BP1 and Diamond
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ reduced severity of root galls by 38% compared to the negative control were no control amendment was applied
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ reduced powdery scab by 59% compared to the negative control were no control amendment was applied
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ on BP1 resulted in higher stem diameter by 77% compared to the positive control, Mancozeb
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ on Mondial resulted in higher stem length by 32% compared to the positive control, Mancozeb
- *T. harzianum* 1 x 107 $CFUg^{-1}$ $2g^{-l}$ resulted in higher yield by 59% compared to the positive control, Mancozeb
- Mondial had higher yield by 26% compared to BP1 and Diamond

- *T. harzianum* 1 x 107 $CFUg^{-1}2g\ l^{-1}$on Mondial resulted in higher extra-large graded potato tubers compared to the positive control, Mancozeb, on Mondial

5.4 Recommendations are that:

Experiment 1

- *B. napus*20% can be used for biofumigation as a substitute for Mancozeb in soils infected with *S. subterranea.*
- *B. napus* 20% on Mondial can be used as a substitute for Mancozeb as it results in higher proportion of extra-large graded potato tubers.
- Mondial variety has some resistance towards *S. subterranea* can be used in soils with known infection with *S. subterranea*.

Experiment 2

- *T harzianum* 1 x 10^7 $CFUg^{-1}2g\ l^{-1}$ can be grown in soils infested with *S. subterranea* as a substitute for Mancozeb.
- Mondial variety has some resistance towards *S. subterranea* can be used in soils with known infection with *S. subterranea*.

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APPENDICES

A 1: ANOVA of effect of *B. napus* and variety on disease incidence in potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	22256.6	5564.2	50.40	<.001
VARIETY	2	592.6	296.3	2.68	0.085
CO.VARIETY	8	1355.4	169.4	1.53	0.187
Residual	30	3311.7	110.4		
Total	44	27516.3			

A 2: ANOVA of effect of *B. napus* and variety on the severity of root galls in potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	27.1111	6.7778	21.79	<.001
VARIETY	2	1.9111	0.9556	3.07	0.061
CO.VARIETY	8	2.7556	0.3444	1.11	0.386
Residual	30	9.3333	0.3111		
Total	44	41.1111			

A 3: ANOVA of effect of *B. napus* and variety on the severity of powdery scabs in potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	185.2222	46.3056	75.77	<.001
VARIETY	2	8.0111	4.0056	6.55	0.004
CO.VARIETY	8	5.7111	0.7139	1.17	0.350
Residual	30	18.3333	0.6111		
Total	44	217.2778			

A 4: ANOVA of effect of *B. napus* and variety on the stem diameter of potato at 28DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	0.61613	0.15403	15.27	<.001
VARIETY	2	0.07170	0.03585	3.55	0.041
CO.VARIETY	8	0.08595	0.01074	1.06	0.413
Residual	30	0.30267	0.01009		
Total	44	1.07644			

A 5: ANOVA of effect of *B. napus* and variety on the stem diameter of potato at 42DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	1.69630	0.42408	25.95	<.001
VARIETY	2	0.07929	0.03965	2.43	0.106
CO.VARIETY	8	0.08635	0.01079	0.66	0.721
Residual	30	0.49033	0.01634		
Total	44	2.35228			

A 6: ANOVA of effect of *B. napus* and variety on the stem diameter of potato at 56DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	2.44970	0.61243	48.08	<.001
VARIETY	2	0.14828	0.07414	5.82	0.007
CO.VARIETY	8	0.14747	0.01843	1.45	0.218
Residual	30	0.38213	0.01274		
Total	44	3.12759			

A 7: ANOVA of effect of *B. napus* and variety on the stem diameter of potato at 70DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	3.72621	0.93155	54.08	<.001
VARIETY	2	0.04448	0.02224	1.29	0.290
CO.VARIETY	8	0.09820	0.01228	0.71	0.678
Residual	30	0.51673	0.01722		
Total	44	4.38563			

A8: ANOVA of effect of *B. napus* and variety on the stem diameter of potato at 84DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	3.40370	0.85093	40.93	<.001
VARIETY	2	0.04885	0.02443	1.17	0.323
CO.VARIETY	8	0.09171	0.01146	0.55	0.808
Residual	30	0.62372	0.02079		
Total	44	4.16799			

A 9: ANOVA of effect of *B. napus* and variety on the stem length of potato at 28DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	250.434	62.609	18.27	<.001
VARIETY	2	44.764	22.382	6.53	0.004
CO.VARIETY	8	64.260	8.033	2.34	0.043
Residual	30	102.833	3.428		
Total	44	462.292			

A 10: ANOVA of effect of *B. napus* and variety on the stem length of potato at 42DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	777.39	194.35	12.72	<.001
VARIETY	2	111.90	55.95	3.66	0.038
CO.VARIETY	8	185.45	23.18	1.52	0.193
Residual	30	458.47	15.28		
Total	44	1533.22			

A11: ANOVA of effect of *B. napus* and variety on the stem length of potato at 56DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	1268.89	317.22	6.20	<.001
VARIETY	2	288.95	144.47	2.82	0.075
CO.VARIETY	8	331.87	41.48	0.81	0.599
Residual	30	1535.64	51.19		
Total	44	3425.35			

A 12: ANOVA of effect of *B. napus* and variety on the stem length of potato at 70DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	2110.71	527.68	9.22	<.001
VARIETY	2	346.12	173.06	3.02	0.064
CO.VARIETY	8	430.44	53.80	0.94	0.499
Residual	30	1717.02	57.23		
Total	44	4604.29			

A 13: ANOVA of effect of *B. napus* and variety on the stem length of potato at 84DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	2429.76	607.44	8.93	<.001
VARIETY	2	513.57	256.78	3.77	0.034
CO.VARIETY	8	476.74	59.59	0.88	0.547
Residual	30	2040.84	68.03		
Total	44	5460.91			

A 14: ANOVA of effect of *B. napus* and variety on the yield of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	0.74756	0.18689	17.59	<.001
VARIETY	2	0.02483	0.01241	1.17	0.325
CO.VARIETY	8	0.05222	0.00653	0.61	0.759
Residual	30	0.31876	0.01063		
Total	44	1.14336			

A 15: ANOVA of effect of *B. napus* and variety on extra-large grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	76.000	19.000	12.57	<.001
VARIETY	2	38.711	19.356	12.81	<.001
CO.VARIETY	8	47.733	5.967	3.95	0.003
Residual	30	45.333	1.511		
Total	44	207.778			

A 16: ANOVA of effect of *B. napus* and variety on large grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	44.444	11.111	3.47	0.019
VARIETY	2	13.733	6.867	2.15	0.135
CO.VARIETY	8	73.822	9.228	2.88	0.017
Residual	30	96.000	3.200		
Total	44	228.000			

A 17: ANOVA of effect of *B. napus* and variety on medium grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	57.022	14.256	4.03	0.010
VARIETY	2	120.178	60.089	17.01	<.001
CO.VARIETY	8	16.711	2.089	0.59	0.777
Residual	30	106.000	3.533		
Total	44	299.911			

A18: ANOVA of effect of *B. napus* and variety on small grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	117.42	29.36	1.83	0.150
VARIETY	2	228.40	114.20	7.11	0.003
CO.VARIETY	8	69.38	8.67	0.54	0.817
Residual	30	482.00	16.07		
Total	44	897.20			

A 19: ANOVA of effect of *T harzianum* and variety on disease incidence in potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	15702.0	3925.5	33.52	<.001
VARIETY	2	1657.3	828.6	7.08	0.003
CO.VARIETY	8	1196.2	149.5	1.28	0.292
Residual	30	3513.5	117.1		
Total	44	22069.0			

A 20: ANOVA of effect of *T harzianum* and variety on the severity of root galls of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	32.7556	8.1889	40.94	<.001
VARIETY	2	1.7333	0.8667	4.33	0.022
CO.VARIETY	8	2.7111	0.3389	1.69	0.141
Residual	30	6.0000	0.2000		
Total	44	43.2000			

A 21: ANOVA of effect of *T harzianum* and variety on the severity of scabs of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	70.778	17.694	6.07	0.001
VARIETY	2	6.811	3.406	1.17	0.325
CO.VARIETY	8	11.856	1.482	0.51	0.841
Residual	30	87.500	2.917		
Total	44	176.944			

A 22: ANOVA of effect of *T harzianum* and variety on the stem diameter of potato at 28DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	0.575213	0.143803	18.83	<.001
VARIETY	2	0.032013	0.016007	2.10	0.141
CO.VARIETY	8	0.176720	0.022090	2.89	0.016
Residual	30	0.229133	0.007638		
Total	44	1.013080			

A 23: ANOVA of effect of *T harzianum* and variety on the stem diameter of potato at 42DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	1.15848	0.28962	10.09	<.001
VARIETY	2	0.01046	0.00523	0.18	0.834
CO.VARIETY	8	0.27165	0.03396	1.18	0.341
Residual	30	0.86080	0.02869		
Total	44	2.30139			

A 24: ANOVA of effect of *T harzianum* and variety on the stem diameter of potato at 56DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	2.76749	0.69187	19.47	<.001
VARIETY	2	0.00289	0.00145	0.04	0.960
CO.VARIETY	8	0.26228	0.03279	0.92	0.512
Residual	30	1.06613	0.03554		
Total	44	4.09880			

A 25: ANOVA of effect of *T harzianum* and variety on the stem diameter of potato at 70DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	5.21637	1.30409	47.14	<.001
VARIETY	2	0.00192	0.00096	0.03	0.966
CO.VARIETY	8	1.00056	0.12507	4.52	0.001
Residual	30	0.82987	0.02766		
Total	44	7.04872			

A 26: ANOVA of effect of *T harzianum* and variety on the stem diameter of potato at 84DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	5.90744	1.47686	56.85	<.001
VARIETY	2	0.06947	0.03473	1.34	0.278
CO.VARIETY	8	0.70988	0.08873	3.42	0.007
Residual	30	0.77941	0.02598		
Total	44	7.46620			

A 27: ANOVA of effect of *T harzianum* and variety on the stem length of potato at 28DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	192.150	48.037	12.33	<.001
VARIETY	2	32.507	16.254	4.17	0.025
CO.VARIETY	8	35.820	4.477	1.15	0.361
Residual	30	116.833	3.894		
Total 44 377.310					

A 28: ANOVA of effect of *T harzianum* and variety on the stem length of potato at 42DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	256.090	64.023	10.34	<.001
VARIETY	2	37.314	18.657	3.01	0.064
CO.VARIETY	8	138.462	17.308	2.79	0.019
Residual	30	185.833	6.194		
Total 44 617.699					

A 29: ANOVA of effect of *T harzianum* and variety on the stem length of potato at 56DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	1003.88	250.97	21.16	<.001
VARIETY	2	72.16	36.08	3.04	0.063
CO.VARIETY	8	282.51	35.31	2.98	0.014
Residual	30	355.78	11.86		
Total	44	1714.33			

A 30: ANOVA of effect of *T harzianum* and variety on the stem length of potato at 70DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	1799.92	449.98	23.02	<.001
VARIETY	2	307.12	153.56	7.86	0.002
CO.VARIETY	8	589.06	73.63	3.77	0.004
Residual	30	586.39	19.55		
Total	44	3282.50			

A 31: ANOVA of effect of *T harzianum* and variety on the stem length of potato at 84DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	2094.57	523.64	21.28	<.001
VARIETY	2	421.74	210.87	8.57	0.001
CO.VARIETY	8	800.88	100.11	4.07	0.002
Residual	30	738.37	24.61		
Total	44	4055.56			

A 32: ANOVA of effect of *T harzianum* and variety on the yield of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	0.691966	0.172991	33.92	<.001
VARIETY	2	0.067831	0.033915	6.65	0.004
CO.VARIETY	8	0.093137	0.011642	2.28	0.049
Residual	30	0.153001	0.005100		
Total	44	1.005934			

A 33: ANOVA of effect of *T harzianum* and variety on extra-large grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	168.311	42.078	17.06	<.001
VARIETY	2	98.311	49.156	19.93	<.001
CO.VARIETY	8	133.022	16.628	6.74	<.001
Residual	30	74.000	2.467		
Total	44	473.644			

A 34: ANOVA of effect of *T harzianum* and variety on large grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	20.000	5.000	0.63	0.645
VARIETY	2	4.311	2.156	0.27	0.764
CO.VARIETY	8	28.800	3.600	0.45	0.878
Residual	30	238.000	7.933		
Total	44	291.111			

A 35: ANOVA of effect of *T harzianum* and variety on medium grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	33.422	8.356	0.96	0.445
VARIETY	2	9.644	4.822	0.55	0.581
CO.VARIETY	8	51.911	6.489	0.74	0.654
Residual	30	262.000	8.733		
Total	44	356.978			

A 36: ANOVA of effect of *T harzianum* and variety on small grade of potato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CO	4	40.09	10.02	0.41	0.803
VARIETY	2	122.18	61.09	2.48	0.101
CO.VARIETY	8	180.71	22.59	0.92	0.517
Residual	30	740.00	24.67		
Total	44	1082.98			