STUDIES OF INTEGRATED CONTROL OF SELECTED ROOT DISEASES OF SUNFLOWERS USING *TRICHODERMA HARZIANUM* (ECO-T®) AND SILICON

by

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in the

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DECLARATIONS

I, **Konis Elungi**, declare that

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(ii) This dissertation has not been submitted for any degree or examination at any other university.

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Dr Patricia May Caldwell

DISSERTATION ABSTRACT

The soil-borne fungi *Rhizoctonia solani* Kuhn and *Sclerotinia sclerotiorum* De Bary are ubiquitous plant pathogens with a wide host range. They are among the most widespread and destructive diseases of many crops, including sunflowers. Although in many cases, the use of chemicals appears to be the most economical and efficient means of controlling plant pathogens, their environmental concerns and the development of tolerance in pathogen populations have led to drastic reduction in their usage and increased the need to find alternative means of disease control.

The potential benefits of applying *Trichoderma harzianum* Rifai and silicon (Si) nutrition to plants have been extensively reviewed. In this study, the ability of *T. harzianum* (Eco-T®), soluble silicon, and their combination was evaluated on sunflower (*Helianthus annuus* L.), for their potential to suppress pathogenic strains of *R. solani* and *S. sclerotiorum*. The ability of this crop to take up and accumulate Si in different plant parts was also investigated.

In vitro assessment of fungal responses to Si in PDA showed that both *R. solani* and *S. sclerotiorum* were inhibited in the presence of Si. More inhibition was observed as the Si concentration increased with a relative increase in pH. Maximum growth inhibition was observed at 3000 mg ℓ^{-1} – 6000 mg ℓ^{-1} of PDA. No difference in inhibition between the two pathogens was observed, thus confirming the fungitoxic/suppressive ability of high Si concentrations to fungal growth. In addition, *in vivo* trials showed that the Si concentration of 200 mg ℓ^{-1} applied weekly significantly increased the dry weight of plants inoculated with *R. solani* and *S. sclerotiorum* and was therefore considered the optimum concentration.

Assessments on *in vitro* antifungal activities of Eco-T® on *R. solani* and *S. sclerotiorum*, showed that Eco-T® significantly inhibited mycelial growth, in both dual culture methods and volatile and non-volatile compounds produced by Eco-T®.

In addition, the combination of Eco-T® and Si was most effective in suppressing damping-off and increasing plant dry weight of sunflower seedlings in the greenhouse. The combination of Si and Eco-T® significantly increased percentage germination, number of leaves and head dry weight of the sunflower cultivars tested. Silicon alone increased growth but was unable to control *R. solani* and *S. sclerotiorum* effectively. Rhizotron studies showed that *S. sclerotiorum* infected the host through the roots and the stem, whereas *R. solani* only *infected* the host through the roots.

A study on Si uptake and distribution showed that sunflower accumulates Si in various plant tissues. Analysis of plant tissues revealed that more Si was accumulated in leaves > stems > roots, with the Si levels in leaves being significantly higher than in stems and roots.

In conclusion, Si alone could be used to increase growth but was unable to control *R. solani* and *S. sclerotiorum.* However, Si together with Eco-T® provides an environmentally friendly alternative for the control of *R. solani* and *S. sclerotiorum,* and enhanced plant growth and yield.

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FOREWORD

Soil-borne pathogens are often difficult to control. Despite the effectiveness of fungicides, their widespread use has not eliminated seedling damping-off caused by soil-borne pathogens, but have become a subject of public concern mainly due to their potential harmful effect on non-target organisms. The current research was a result of a collaborative investigation driven by the need to examine other non-chemical approaches as alternative means for the control of *Rhizoctonia solani* Kuhn and *Sclerotinia sclerotiorum* De Bary on sunflower (*Helianthus annuus* L.) seedlings under greenhouse conditions using *Trichoderma* spp. (Eco-T®) and silicon (Si).

The main focus and overall aim of this research was to investigate the efficacy of using Eco-T® and soluble Si, and their combination, to manage *R. solani* and *S. sclerotiorum* infection of sunflower seedlings.

The approaches taken to achieve these aims were:

1. A literature review on sunflower production in relation to *R. solani* and *S. sclerotiorum*, and the basis and approaches of controlling these diseases (Chapter One).
2. Evaluation of the toxicity of Si on *R. solani* and *S. sclerotiorum in vitro* (Chapter Two).
3. An *in vitro* investigation of integrating Eco-T® and Si for the control of *R. solani* and *S. sclerotiorum* in pot trials with regard to percentage germination and seedling dry weight (Chapter Three).
4. Evaluation of the effects of Eco-T®, Si and the integration of Si+Eco-T® on percentage germination and shoot growth of different sunflower cultivars, post inoculation with *R. solani* or *S. sclerotiorum* (Chapter Four).
5. Investigation of Si uptake and distribution in sunflower seedlings and the relationship between the level of Si fertilization and its uptake (Chapter Five).
6. An overview of the experimental work conducted (Dissertation Overview)

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CHAPTER ONE

LITERATURE REVIEW

STUDIES OF INTEGRATED CONTROL OF SELECTED ROOT DISEASES OF SUNFLOWERS USING *TRICHODERMA HARZIANUM* (ECO-T®) AND SILICON

1.1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) (Fig. 1.1) is a widely cultivated plant species in the genus *Helianthus* and is grown for oilseed. Its centre of origin has been identified as North America. Spanish, English and French explorers introduced the plant to Europe as early as the 16th Century (Semelczi-Kovacs, 1975). Presently, sunflower is grown worldwide for oilseed production and as a non-oilseed crop for bird food markets. The development of hybrids with high oil content has been linked to the expanded world production of the crop (Carter, 1978).

Figure 1.1. Sunflower plants at flowering (Elungi, 2008).

Soil-borne pathogens cause a range of root and stem diseases, e.g., early stage damping-off, root rot, stem cankers and foliar and fruit diseases that may cause severe economic losses and therefore require effective control measures (Sneh *et al.,* 1996).

Various disease control methods have been investigated including crop rotation, tillage practices (Yang *et al.,* 1995), biological control and chemical control. However, none of these methods controls these diseases sufficiently, so there is a need for integrated control. Multiple control methods such as a combination of host resistance, reduction of inoculum or infection by cultivation practices, a fungicidal sprays, soil treatments, use of pathogen-free seeds, and biological control may be required for effective control (Baker and Cook, 1974).

Silicon (Si), even though it is not included in the list of essential elements in plant nutrition, plays an important role in plant growth, structural development and reproduction (Epstein, 1999). In plants, its effects include enhancement of growth and increased resistance to biotic stresses, drought, salinity and toxic metals (Fauteux *et al.,* 2005). As a result, it may be beneficial to determine its efficacy when integrated with other disease control methods, in particular, the use of biocontrol agents such as *Trichoderma harzianum*.

Examples of the successful use of *Trichoderma* spp. as biocontrol agents to reduce or prevent damage to plant roots are numerous and can be found for almost all the major root pathogens that affect all major crops. Some isolates of *Trichoderma* spp. do effectively control a wide range of soil-borne pathogens (Uphoff, 2006). Therefore, given all the known benefits that are associated with both Si and *T. harzianum* in plant disease control, their integration can be an alternative to other methods of soil-borne pathogens such as *Rhizoctonia solani* Kuhn and *Sclerotinia sclerotiorum* De Bary.

1.2 CROP HISTORY

Beard (1981) cites the earliest published description of the sunflower in 1568 by Rembert Dodoens. Although he cites Peru or Central America as the plant's place of origin, it is now believed that wild sunflowers are native to North America (Heiser, 1955). This has been confirmed by archaeological explorations at several sites in North America, where traces of both wild and cultivated sunflower have been found (Semelczi-Kovacs, 1975). Selection for better yield was practiced in the old Union of Soviet Socialist Republic (USSR) and by 1880, a large number of cultivars, all open-pollinated, were available. In 1910, breeding and selection commenced at official experimental stations in the USSR. Since then sunflower research has expanded dramatically in most of developed and developing countries where the crop is grown commercially (Leeuvner, 2005).

In the 1960s, the introduction of USSR cultivars that had a high oil content made sunflower seed economically attractive for processing and marketing by the United States, (US) oilseed processing industry, which promoted sunflower breeding and cultivation. Sunflower oil is also of great commercial importance in Australia, China, India, South Africa and Turkey (Semelczi-Kovacs, 1975).

1.3 CROP USAGE

Although man has used every part of the sunflower, its oil is the most valuable (Heiser, 1976). The oil is used for frying, or to produce salad dressing, shortening and margarine (Robbelen *et al.*, 1989). Sunflower oil is not commonly used for industrial purposes because of a generally higher price relative to soybean oil and other alternative fats and oils. However, it can be used to some extent in certain paints, varnishes and plastics because of good semidrying properties without the yellowing problems associated with oils high in linolenic acid (Heiser, 1976). Along with other vegetable oils, it has potential value for use in the production of agri-chemicals, surfactants, plastics and plastic additives, synthetic lubricants, coatings and other products (Robbelen *et*

al., 1989). Sunflower oil has been researched as a potential diesel fuel substitute (Heiser, 1976).

After the oil is extracted from the sunflower seed, the remaining seed material (meal) is used as a protein supplement in livestock feed and usually contains about 35% protein (Robbelen *et al*., 1989). Use of sunflower seed by the birdseed industry is growing. In some countries, it is considered as the premium component in most birdseed mixes, and is priced higher than other typical birdseed components such as sorghum, millet, or cracked corn (Robbelen *et al*., 1989).

1.4 SUNFLOWER INDUSTRY IN SOUTH AFRICA

In South Africa (SA), sunflower is the most important oilseed crop. Its oil market has shown a steady increase of approximately 3% per year in the past few years, with a current demand of 600 000 tons of seed for oil extraction (Pakendorf, 1998). In the past, sunflower in SA was considered an alternative crop to maize, i.e., if a maize crop could not be successfully produced due to drought or any other constraints (Schoeman, 2003), or as a crop rotation.

During the 2008/09 cropping season, about 843 530 tons of sunflower was produced on 635 800 ha within SA. The areas planted during the 2008/09 season were Free State 280 000 ha, Gauteng 7 000 ha, Limpopo 90 000 ha, Mpumalanga 27 000 ha and North West 230 000 ha and the rest (12 000) ha in the Western and Northern Cape (Dredge, 2009). It is evident that the largest concentration of sunflower is in the Free State and North West provinces. This is generally the drier or western part of SA, with sandy soils (Schoeman, 2003).

1.4.1 Typical sunflower farming practices

As sunflowers have highly efficient root systems, they can be grown in areas which are too dry for many other crops, but they grow best in soils ranging from sand to clay (Duke, 1983). However, sunflower cultivation has been limited to soils where the clay percentage varies between 15 and 55 %, especially sandy

loam to clay soil types (Carter, 1978). In SA the crop often performs well even during a dry season, especially in deeper soils (Anonymous, 2009).

In SA sunflower is usually planted from the beginning of November to the end of December. Compared to other crops, it performs well under drought conditions. However, sunflower is intolerant of acid or waterlogged conditions (Duke, 1983) and to high soil temperatures during seedling emergence. This is an issue especially in the sandy soils of the Western Free State and the North West Province, where this problem often leads to poor or erratic plant populations.

As a crop, sunflower yields are reduced, but rarely eliminated, by weeds that compete for moisture and nutrients and occasionally for light. Sunflower is a strong competitor with weeds, especially for light, but does not form a canopy early enough to prevent weed establishment (Duke, 1983). Therefore, successful weed control that includes mechanical and chemical practices are always a prerequisite for high sunflower yields. Sunflowers are generally mature long before they are dry enough for harvesting. Therefore, harvesting normally commences as soon as 80% of the sunflower heads are brown, in order to minimise losses caused by birds, lodging and shattering (Duke, 1983).

1.4.2 Production constraints (diseases)

Some of the most important pathogens of sunflower in SA are *Sclerotinia* wilt (*S. sclerotiorum*), damping-off (*R. solani*), rust (*Puccinia graminis* Pers) and downy mildew (*Plasmopara viticola* Berk) (Berlin and Arthur, 2000). Sunflowers are particularly sensitive to soil-borne pathogens during emergence, which may cause poor or patchy plant populations. In SA, *R. solani* and *S. sclerotiorum*, are major the soil-borne pathogens on sunflowers.

1.5 *RHIZOCTONIA SOLANI* KUHN

The soil-borne fungus *R. solani* is the causal agent of seed decay, pre- and post-emergence damping-off, stem canker, root rot, fruit decay and foliage diseases (Agrios, 1997). This pathogen occurs worldwide and causes disease in a broad range of host plants (Anderson, 1982; Ogoshi, 1987). Individual isolates of *R. solani* vary greatly in growth characteristics and virulence. However, they can be arranged in groups based on anastomosis, i.e., the process of hyphal fusion between isolates (Sneh *et al.,* 1991).

1.5.1 Taxonomy and morphology

Rhizoctonia solani is a member of Deuteromycetes (teleomorph: *Thanatephorus cucumeris*) and most strains do not produce spores; hence, it is indentified only from mycelial characteristics. The vegetative mycelium of *R. solani* and other *Rhizoctonia* fungi are colourless when young but become brown as they grow and mature (Sneh *et al.,* 1991). The mycelium consists of multinucleate hyphal cells (Adams, 1988). The hyphae are 4-15 µm wide, often branch at right angles and usually possess more than three nuclei per hyphal cell (Anderson, 1982). A septum near each hyphal branch and a slight constriction at the base of the branch are diagnostic (Parmeter *et al.,* 1969; Ogoshi, 1987). Since *R. solani* and other *Rhizoctonia* spp. do not produce conidia and only rarely produce basidiospores, the classification of these fungi has often been difficult, until 1969, when J. R. Parmeter reintroduced the concept of "hyphal anastomosis" to characterize and indentify *Rhizoctonia (*Parmeter *et al.,* 1969). The concept implies that isolates of *Rhizoctonia* that have the ability to recognize and fuse with each other are genetically related (Parmeter *et al.,* 1969; Ogoshi, 1987).

1.5.2 Isolation

The pathogen can be isolated from infected plant tissues, by cutting small samples from infected tissues and transferring them onto an isolation medium,

such as alkaline water agar. Alkaline water agar provides a faster way of isolating the fungus than other general media (Guttierez *et al.,* 1997).

1.5.3 Host plants and host specialization

There are many different strains of *R. solani* (Fig 1.2). Some grow more rapidly than others in the soil (Anderson, 1982; Adams, 1988). Strains also differ in host range, pathogenicity, pH response, and temperature requirements, but there is a continuous variation in character and much overlapping occurs (Adams, 1988). One principle used in fungal grouping is based on the ability to anastomose between members of the same group. Four main anastomosis groups (AG) have been recognized, i.e., AG 1 contains isolates causing seed and hypocotyl rot, aerial blight and web blight; AG 2 isolates cause canker of root crops and root rots of conifers; AG 3 isolates are pathogens of potato and barley and can also cause seed rot; AG 4 isolates cause seed and hypocotyl rots of many angiosperm species (Agrios, 1988).

1.5.4 Distribution

This pathogen is a very common soil-borne plant pathogenic fungus with a wide host range and worldwide distribution and is probably found in most arable soils (Ogoshi, 1987; Smith, 1988).

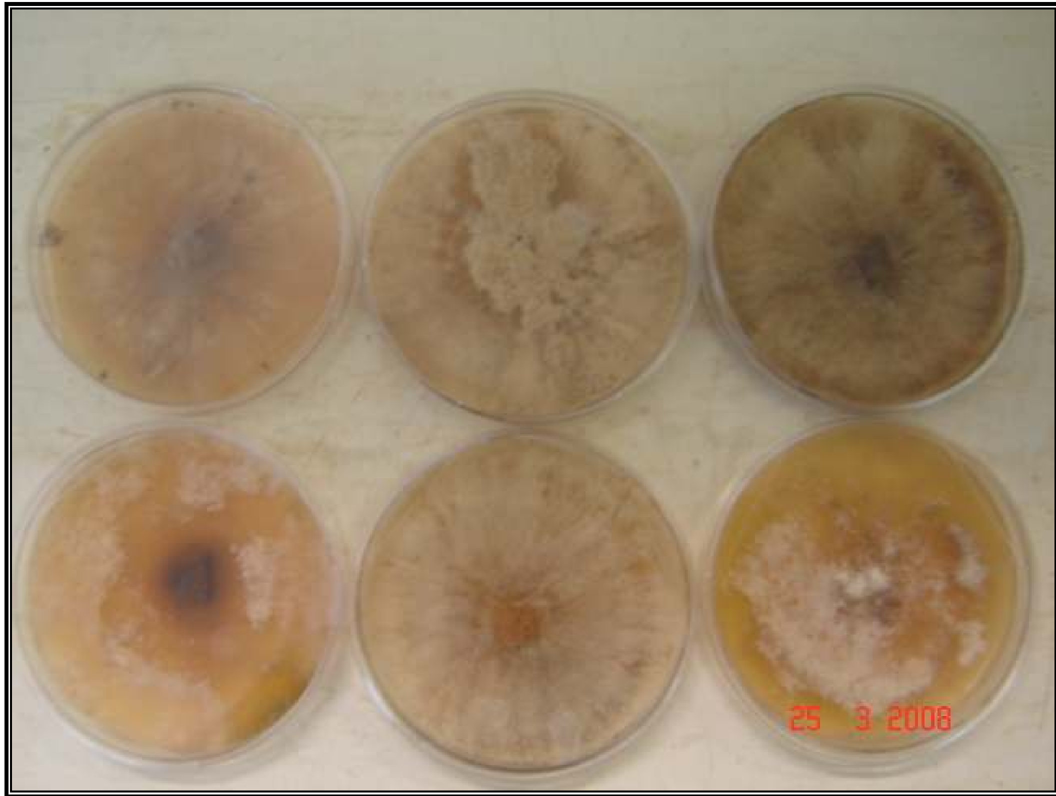

Figure 1.2. Isolates of *Rhizoctonia solani* growing on agar in Petri dishes. The isolates vary in colour, abundance of aerial mycelia, sclerotial formation, and their pathogenicity (Elungi, 2008).

1.5.5 Economic importance

Rhizoctonia solani has been recognized as one of the most widespread and economically important plant pathogenic fungi responsible for serious damage to many economically important agricultural and horticultural crops and forest trees, worldwide (Adams, 1988). Strains of this species cause a variety of diseases such as seed decay, damping-off, stem cankers, root rots, seed decay and foliar diseases (Banniza and Rutherford, 2001). In temperate climates, for example, the fungus is a well-known pathogen of potatoes and cereals, causing black scurf and bare patch disease respectively (Banniza and Rutherford, 2001). High yield losses have been reported, i.e., up to 50% for sugar beet (Kiewnick *et al*., 2001), up to 70% for field-grown lettuce (Davis *et al*., 1997), and about 20 % for potato (Grosch *et al*., 2005).

1.5.6 Disease symptoms

In sunflowers, *R. solani* invades and kills seeds either before or soon after germination. Often the rotted seed provides food for the fungus, which then grows through the soil and infects adjacent seeds (Davis *et al*., 1997). In other instances the seed germinates, but the fungus attacks and destroys the seedling before it emerges from the ground (Smith, 1988). Young seedlings are also attacked after they emerge from the soil. Initial root rot symptoms appear as elongated, water-soaked areas on roots 1-3 weeks after planting (Adams, 1988; Agrios, 1997). The pathogen destroys much of the main root system, thus reducing overall plant growth. The water-soaked regions may extend several centimeters above the soil line, with little, if any, visible evidence of the fungus. The water-soaked area eventually dries out, becomes somewhat sunken, and tan to brown in colour (Agrios, 1988; Smith, 1988).

Infection during the juvenile seedling phase after emergence causes typical damping-off, as a result of polygalacturonase enzymes produced by the fungus, that cause tissue decay and collapse of the stems (Smith, 1988). Damage is most common when seed quality is poor and/or seed has been damaged by freezing, herbicides or the presence of other pathogens (Smith, 1988).

1.5.7 Epidemiology

Rhizoctonia solani survives in soil within diseased host material or as sclerotia (Agrios, 1988). It can persist in soil for many years and after initial infection, the disease spreads. Environmental conditions greatly influence all phases of the disease. It is favoured by high air humidity, extreme soil temperatures and over-moist or saline soil (Agrios, 1988). Such conditions delay germination of the host, while favouring the spread of the pathogen to adjacent and surrounding plants (Smith, 1988). In the field, inoculum from soil is transported in infested soil or through movement of diseased plants (Davis *et al*., 1997).

1.5.8 Disease life cycle

The fungus survives indefinitely in the soil, passing through unfavorable conditions primarily as sclerotia (Agrios, 1997; Davis *et al*., 1997). Sclerotia are extremely resistant to cold, heat, drought, and most chemicals (Guttierez *et al.,* 1997). They germinate in damp weather by forming a mycelium, that spreads through the soil for several centimetres and penetrate roots and leaves of susceptible plants with which they come in contact (Agrios, 1988; Smith, 1988). Moisture on the surface of the host is required for penetration through natural openings, wounds, or intact tissues (Smith, 1988). After the fungus has depleted nutrients in the host's tissue, or as other environmental conditions become unfavourable, the mycelium produces sclerotia to complete the life cycle (Agrios, 1997). The fungus is also capable of infecting the seed without exhibiting symptoms (Smith, 1988; Guttierez *et al.,* 1997). It remains in the seed in a quiescent state until the seed begins to germinate before the fungus attacks the seed (Fig.1.3).

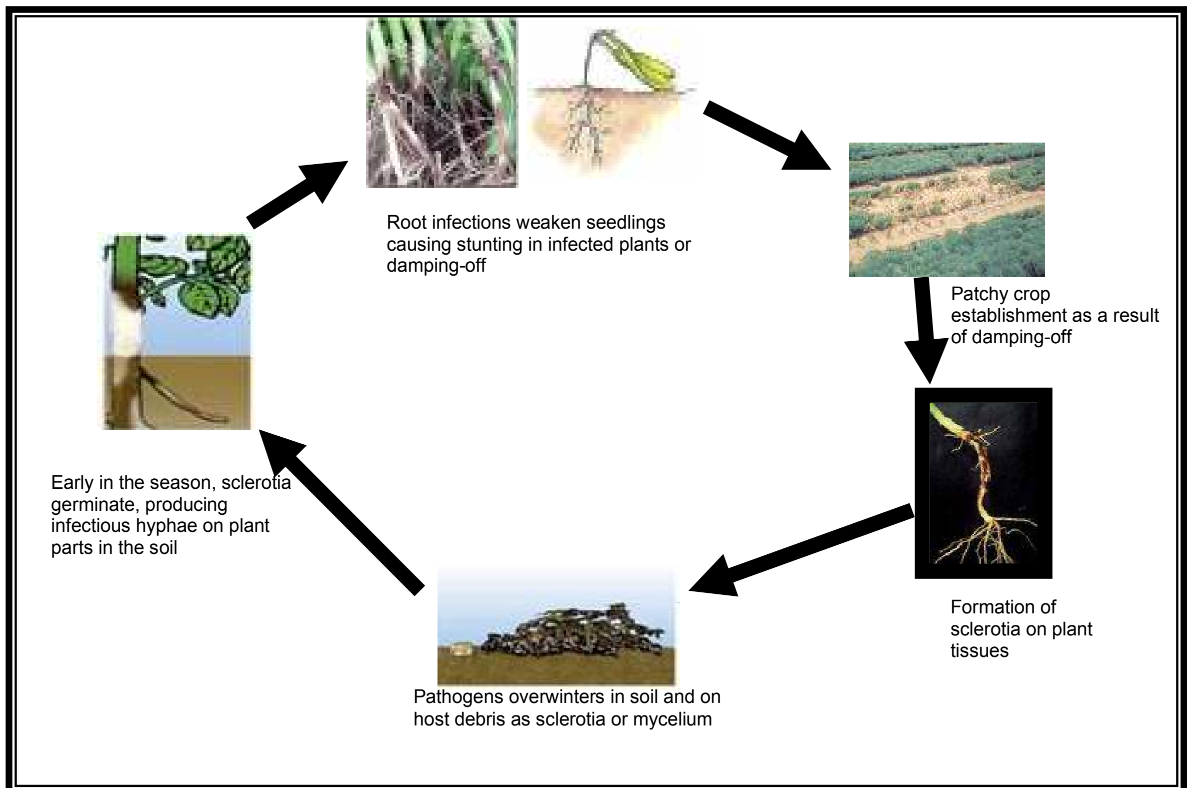

Figure 1.3. Life cycle of *Rhizoctonia solani* (Elungi, 2008).

1.5.9 Disease management

Diseases caused by *Rhizoctonia* spp. are usually difficult to control, as is common with most soil-borne pathogens because of its ecological behavior, its extremely broad host range and the high survival rate of sclerotia under various environmental conditions. In most cases, it is difficult to attack the pathogen, especially when it is embedded in debris of infected tissues. In addition, drastic methods of control, although effective, may also adversely affect non-target beneficial organisms (Sneh *et al.,* 1996).

Many methods have been developed for the control of diseases caused by *Rhizoctonia* spp. These include fungicide applications, breeding for resistant cultivars, sanitation, crop rotation, biological control and soil disinfection (Sneh *et al.,* 1996). The need for many different control methods rises from the fact that usually none of them is perfect, nor can one chemical be used in all circumstances.

The best cultural method to reduce infection by *Rhizoctonia* spp. is ploughing, which buries the sclerotia (Smith, 1988). Other cultural practices include soil compaction, weed control (alternative hosts) and irrigation management. Excessive irrigation leads to favourable conditions for the pathogen and increases root rot (Staple and Toenniessen, 1981), while soil compaction reduces aeration and water drainage resulting in death or poor growth of the pathogen. In SA, benomyl (Benlate) and tolclofos methyl (Rhizolex) are registred fungicides for the control of this pathogen (Anonymous, 2009).

1.6 *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

Sclerotinia sclerotiorum De Bary causes one of the most important diseases of sunflower. The most recognized symptoms in the field are *Sclerotinia* wilt caused by sclerotia (Figure 1.4), and middle stalk rot and head rot caused by airborne asco spores (Berlin and Arthur, 2000). This is the same fungus that causes white mould of soybean, dry bean, canola and other susceptible crops (Smith, 1988).

1.6.1 Taxonomy and morphology

Sclerotinia sclerotiorum De Bary belongs to: kingdom Fungi, phylum Ascomycota, class Discomycetes, order Helotiales, family Sclerotiniaceae, genus Sclerotinia is a member of Deuteromycetes. In culture, the fungus grows rapidly, producing abundant white, cottony mycelia on which black sclerotia develop, (Fig 1.4), up to 10 mm diameter with a slightly pitted surface, often exuding droplets of liquid usually under saturated atmosphere (Smith, 1988; Berlin and Arthur, 2000). Sclerotia exhibit myceliogenic or carpogenic germination that give rise to the vegetative hyphae, and apothecia. Apothecia are cup-shaped, yellowish-brown, up to 10 mm in diameter on a smooth cylindrical stalk, which emanates from the sclerotium. According to Smith (1988), Deuteromycetes asci are cylindric – clavate, up to 130 X 10 µm, and 8-spored; the ascospores are uniseriate and elliptical, 9 -13 X 4-6.5 µm.

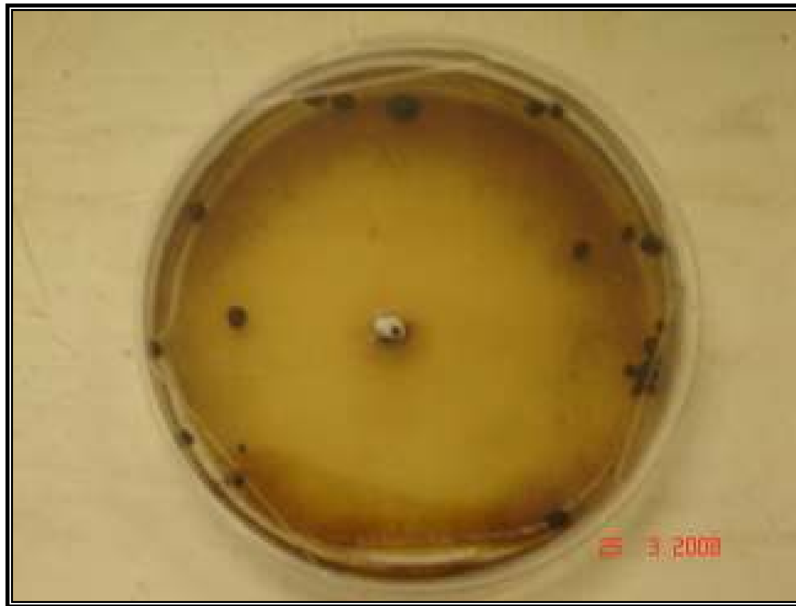

Figure 1.4. Sclerotia of *Sclerotinia sclerotiorum* formed on potato dextrose agar (Elungi, 2008).

1.6.2 Host plants

Sclerotinia sclerotiorum causes destructive diseases of numerous plants, particularly vegetables, flowers, and some shrubs (Williams and Western, 1965). *Sclerotinia sclerotiorum* has one of the widest host ranges of any pathogen. Purdy (1979) lists 225 genera from 64 families of higher plants as hosts, mainly angiosperms. Woody plants, grasses and cereals are rarely attacked (Smith, 1988).

1.6.3 Distribution

Sclerotinia diseases are worldwide in distribution and affect plants in all stages of growth, including seedlings, mature plants and harvested products in transit and storage, usually under in cool and moist conditions (Agrios, 1988; Smith, 1988).

1.6.4 Economic importance

The damage caused by *S. sclerotiorum* tends to be sporadic and it is usually regarded as a lesser pathogen. However, due to the nature of the disease, where outbreaks do occur, losses can be severe. Most reports refer to sunflower, rape, dwarf bean and lettuce as major hosts (Smith, 1988).

Out of the three diseases caused by *S. sclerotiorum*, *Sclerotinia* wilt is the most important, and can destroy entire fields (Berlin and Arthur, 2000). Crop damage is often determined by the number of plants infected and their growing stage during infection, This results in the greater damage if a greater number of plants are infected at the early growing stages.. On average, an infected plant yields 50% less than a healthy plant (Bruehl, 1975). Equally important is the fact that infection leads to increased levels of Sclerotia in the soil (Agrios, 1988). This results in reduced sunflower production from certain fields for many years, reducing yield, but it affects future production and economic gain from sunflowers (Berlin and Arthur, 2000).

Head rot and middle stem rot occur sporadically and only following periods of wet weather (Agrios, 1988). Head rot reduces seed weight and seed number and lowers oil content of sunflower. The presence of sclerotia in seed can reduce the grade and market value of the crop. Infected heads often disintegrate in the field, resulting in all the seed falling to the ground (Berlin and Arthur, 2000). Fortunately, no toxins are produced by *Sclerotinia* in sunflower seed, but heavy contamination with sclerotia is considered unacceptable for human or animal consumption (Berlin and Arthur, 2000). In addition, sunflower seed for export may be rejected at foreign ports if it contains sclerotia.

1.6.5 Disease symptoms

The most obvious and typical early symptom of *Sclerotinia* disease is the appearance on the infected plant of a white fluffy mycelial growth which soon develops large, compact resting bodies called sclerotia.

The most characteristic symptoms of *Sclerotinia* wilt are sudden wilting of leaves, root rot and water-soaked lesions that develop at the base of the plant. The lesions become a greyish green to brown canker (Fig. 1.5). As decay progresses the stalk becomes bleached and has a shredded appearance. The decayed portion may extend up the stem (Agrios, 1988; Berlin and Arthur, 2000). The pith decays at the base of the stem and form sclerotia. Sclerotia provide a positive identification of the disease (Agrios, 1988). During wet weather, a white mycelium (mould) often develops at the base of the stem, hence the name "white mould" (Williams and Western, 1965; Agrios, 1988; Berlin and Arthur, 2000).

Figure 1.5. Early symptoms of *Sclerotinia* infection on sunflower with water-soaked lesions at the base of the plant (Elungi, 2008).

Infected plants may or may not produce seed, depending on when infection occurs. Heads on wilted plants are generally smaller than those on healthy plants (Berlin and Arthur, 2000). Wilt can significantly reduce seed yield primarily through reduced seed weights. Wilted plants in advanced stages of root decay are often blown over during high winds (Berlin and Arthur, 2000).

Middle stalk rot is usually observed around or past flowering in the middle to mid-upper portion of the stalk. It begins as a brownish to grey water-soaked lesion, most commonly at, or near, the leaf node (Williams and Western, 1965; Agrios, 1988). Stalk decay may proceed downward to the stem base or upward to the head (Smith, 1988).

The fungus grows profusely within the receptacle, decays it, and produces a white mycelium and many large black sclerotia (Agrios, 1988; Smith, 1988; Berlin and Arthur, 2000). The receptacle is usually bleached and can be easily distinguished from an uninfected head (Berlin and Arthur, 2000). Seeds do not usually rot but may not fill (Smith, 1988; Berlin and Arthur, 2000).

1.6.6 Epidemiology

Sclerotia are the most important means of propagation. Their survival time in the soil is very variable but can be as long as 6 to 8 years (Bruehl, 1975; Willets

and Wong, 1980; Agrios, 1988; Berlin and Arthur, 2000). Survival of mycelium in seeds may also occur but epidemiologically it is of little consequence (Smith, 1988). Where sclerotia and susceptible plants are in close proximity, devastating stem base infections may result. A prerequisite for carpogenic germination is a period of chilling to break dormancy, followed by rising temperatures and high humidity (Smith, 1988).

The apothecial stipes elongate in response to light and ascospores are dispersed by wind. Spores landing on potential hosts need water to germinate for about 16 to 24 h at 0 to 25°C (Smith, 1988). Wounded, dead, or senescent tissues are readily colonized and serve as a food base from which infection of healthy tissues can take place. Spores usually enter the host by direct penetration through the cuticle, assisted by extensive pectolytic and cellulolytic dissolution of the host cell structure (Agrios, 1988). When infected plants die, sclerotia are returned to the soil, increasing inoculum levels for infection of the next crop. Sclerotia spread from field to field by natural or human-assisted soil movement (Smith, 1988).

1.6.7 Disease life cycle

Sclerotinia overwinters as sclerotia in the soil or in plant debris. In summer when growing sunflower roots encounter sclerotia, the sclerotia germinate and infect the roots. The fungus grows up the infected root into the taproot, then into the stem, and the plant dies (Agrios, 1988; Berlin and Arthur, 2000). Because there is contact between roots of adjacent plants within rows, the fungus can grow from one root system to another within the row, resulting in a series of wilted plants (Fig 1.6). The rapid development of wilt is one reason why an apparently healthy field appears to become infected in just a few weeks (Berlin and Arthur, 2000). Apothecia develop from sclerotia, produce ascospores which also infect plants. This airborne phase is crucial to the infection of sunflower heads.

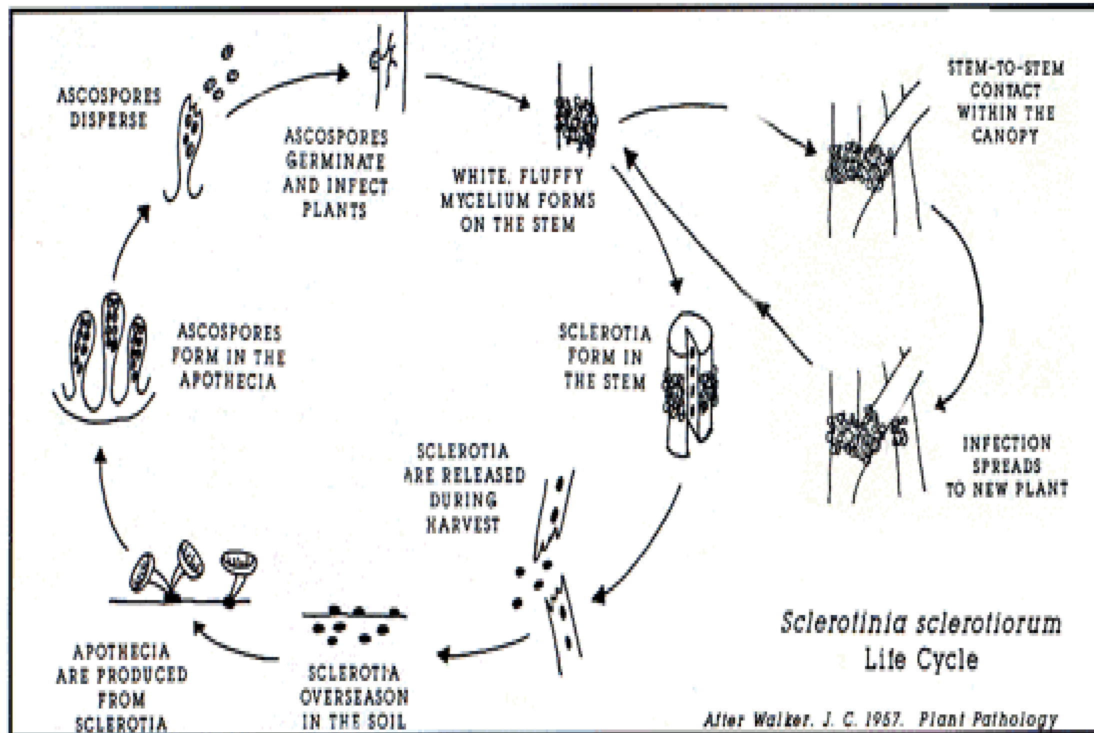

Figure 1.6. Life cycle of *Sclerotinia sclerotiorum* (Anonymous, 2009).

1.6.8 Disease management

Control of *Sclerotinia* diseases depends on a number of cultural practices, with the prevention of inoculum build-up being the most important control practice. Because the occurrence of middle stalk rot and head rot is sporadic, disease control is usually aimed at the control of *Sclerotinia* wilt (Bruehl, 1975).

Cultural practices have proven ineffective but have aided in the reduction of disease incidence (Bardin and Huang, 2001). These practices include crop rotation, fungicide treated seeds, increasing spacing between rows and plants in the field, high level of irrigation to induce rotting of sclerotia, and burying sclerotia (Gulya *et al*., 1997; Bourdot *et al*., 2000; Bardin and Huang, 2001).

Encouraging results of biological control of *Sclerotinia* diseases in some crops have been obtained by incorporating mycopararastic fungi in *Sclerotinia*-infested soils. (Bruehl, 1975; Agrios, 1988; Berlin and Arthur, 2000). The mycoparasites destroy existing sclerotia or inhibit the formation of new sclerotia by the fungus and can markedly reduce the fungus population in the soil (Agrios, 1988).

Benomyl, a (benzimidazole) compound or dichloran have generally been used as fingicide sprays to inhibit sclerotial development and to protect plants from infection (Smith, 1988). On the other hand, certain dinitroaniline and triazine herbicides have been shown to stimulate apothecial production while certain phenolic herbicides inhibit production, but their effect on disease in the field is not understood. Some herbicides are also reported to inhibit mycelial growth of the pathogen (Berlin and Arthur, 2000).

1.7 BIOLOGICAL CONTROL OF ROOT (SOIL-BORNE) DISEASES

Burge (1988) defines biological control as the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through, one or more organisms other than man.

Cook and Baker (1983) listed 41 biocontrol agents with potential for immediate application as biocontrol agents. The main problem in application is in managing the host, the prevailing environmental conditions and the agent itself so that the activities of the biological agents are enhanced. Organisms that have been found to successfully control *Rhizoctonia* and *Sclerotinia* spp. are *Trichoderma* spp. and *Bacillus* spp., which are produced commercially (Benhamou and Chet, 1997). Biocontrol with beneficial microorganisms seems to be a promising approach to managing crop diseases (Howell, 1982).

1.8 *TRICHODERMA* AS A BIOCONTROL AGENT (BCA)

The ability of a fungus to exist in a particular habit such as the soil or on the surface of a plant organ is partly determined by its ecological relationships with other microorganisms. These inter-relationships are often antagonistic in nature. One or more of the organisms may be harmed or have their activities curtailed. This provides another approach to the manipulation of fungi as biocontrol agents (Burge, 1988). *Trichoderma, Penicillium* and *Gliocladium* spp. are some useful fungi that antagonize plant pathogens on or near the surface of hosts such as seeds and seedlings and near wounds (Blakeman and Fokkema, 1982).

The potential of *Trichoderma* spp. as BCAs was noticed in the early 1930s. Subsequently they were found to effectively control pathogens such as *Botrytis cinerea* Pers, *Pythium ultimum* Trow, *R. solani* and *Sclerotium rolfsii* Sac. (Elad *et al*., 1980; Howell, 2003). Many researches have suggested the different mechanisms responsible for their biocontrol activity, which includes competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (Haram *et al*., 1996; Zimad *et al*., 1996).

1.8.1 Mode of action

The choice of a microbial antagonist for use as a biocontrol agent against a fungal pathogen will depend on both the nature of its antagonistic properties and the type of inhibitory mechanism to which the pathogen is responsive (Marjorie and Donald, 1985). Antagonistic microorganisms such as *Trichoderma* adversely affect another microorganism by utilizing different modes of action, i.e., competition, mycoparasitism and antibiosis. All these mechanisms may operate independently or together and their activities can result in suppression of plant pathogens (Fravel, 1988).

1.8.2 Antibiosis

Antibiosis refers to the synthesis and release of metabolic product(s) by one organism that directly inhibits or destroys another organism. Species of

Trichoderma are well-known biocontrol agents that produce a range of antibiotics that are active against pathogens *in vitro* and consequently, antibiotic production has been suggested as one mode of action for these fungi (Howell, 2003). The production of volatile and non-volatile compounds by biocontrol agents has a direct impact on pathogenic microorganisms, resulting in denaturation of cell contents before coming in contact with mycelia of antagonists (Uphoff *et al*., 2006). The production of volatile and non-volatile compounds by *Trichoderma* spp. inhibits the growth of a broad range of fungi e.g., species of *Fusarium, Rhizoctonia, Phytophthora and Sclerotinia* (Fravel, 1988).

Benhamou and Chet (1997) proposed the following scheme for the interaction of mycoparasitism, enzyme production, and antibiosis in the biocontrol of *Pythium* by *T. harzianum*:

1. Recognition of *pythium* by *T*. *harzianum* and attachment to the hyphae of *Pythium*.
2. Production of β-1,3-glucanases to weaken the host cell wall, accompanied by the production of cellulases to facilitate penetration by *Trichoderma* hyphae.
3. Production of antibiotic substances to deregulate host cell metabolism.
4. Host cell invasion and increased production of cellulases, resulting in the breakdown of the pathogen cells.

1.8.3 Competition

The basic principle involved in competition is that the pathogen must be deprived of a nutrient essential for pathogenesis. Thus biological control agents are used to create deficiencies of these essential elements (Marjorie and Donald, 1985; Burge, 1988). Competition involves an injurious effect of one organism to another because of the removal of some resources of the environment, thereby determining the growth of soil plant pathogens in competition with other microorganisms (Howell, 2003). A correlation between rhizosphere competence and its ability to utilize cellulose substrates associated with the root has been observed (Ahmad and Baker, 1987).

1.8.4 Mycoparasitisism/ hyperparasitism

Hyperparasitism has been implicated as a biological mechanism in a number of systems. It occurs when one fungus exists in intimate association with another from which it derives some or all of its nutrients while conferring no benefits in return. These mechanisms involve different kinds of interactions, e.g, coiling of hyphae, where hyphae of *T. harzianum* have been found to grow towards hyphae of susceptible fungi before contact is made (Chet *et al.*, 1981), presumably due to chemical signals originating from the host. Subsequently, excessive hyphal coiling and short branching on the host occurs, which in some cases may be lectin mediated (Barak *et al.,* 1985). Cytoplasmic degradation may occur before contact or penetration (De Oliveira and Bellei, 1984). Recent investigations have revealed that mycoparasites like *Trichoderma* spp. produce cell wall degrading enzymes such as chitinases, β-1,3-glucanases and proteases which enable the penetration of the host hyphae (Howell, 2003).

1.8.5 Siderophore production

Siderophores are compounds produced by root-inhabiting fluorescent pseudomonads and other microorganisms under low-iron conditions. These iron transport molecules not only enhance plant growth, but also cause reductions in root-zone populations of fungi and bacteria, which may include pathogens (Marjorie and Donald, 1985).

Most of fungal and bacterial biocontrol agents release siderophores, such as pseudobactines and pyoverdins (Horny, 1990). *Trichoderma* spp. have been shown to be prolific producers of siderophores (Casale, 1995). These compounds compete very efficiently for iron in the soil, to the extent that plant pathogens such as *Fusarium* are successfully suppressed.

With such a wide array of functional modes, *Trichoderma*-based biocontrol products are unlikely to suffer resistance build-up by pathogens, making them a sustainable alternative to traditional chemical fungicides.

1.9. APPLICATION TECHNIQUES

Application of biocontrol agents mainly depends on the nature of the pathogen. For control of soil-borne diseases, the biocontrol agent needs to be applied to the rhizosphere of the plant. Since both *R. solani* and *S. sclerotiorum* are both soil-borne pathogens, soil application of *Trichoderma* in the rhizosphere zone is therefore an appropriate mode of application.

1.9.1 Drawbacks

The biocontrol activity of *T. harzianum* can be influenced by environmental cues. The enzymes and antibiotics produced by *Trichoderma* spp. that appear to be involved in biocontrol are strongly influenced by the substrate on which the fungus is grown, and conditions in the laboratory probably occur only rarely in nature or not at all (Howell, 2003). Temperature also has a profound effect on the production and activities of enzymes and antibiotics associated with biocontrol by *Trichoderma* spp. What occurs at 25°C in a Petri dish may not occur at all in the soil around germinating seed at 35°C. The presence of members of the soil microflora may also influence biocontrol activity by inhibiting growth and development of the biocontrol agent or by metabolizing its enzymatic and/or antibiotic products. This may not entirely negate the biocontrol activities of *Trichoderma* spp. but may limit its efficacy in terms of the length of time that they are effective and the distance from the biocontrol focal point where they exhibit influence (Howell, 2003).

Therefore, although potential biocontrol agents with suitable antagonistic characteristics may be found, they must be carefully screened to take into consideration conditions of temperature, moisture and nutrient availability that approximate those found in nature (Chamola *et al.,* 2001).

1.10 SILICON IN CONTROL OF PLANT DISEASES

Silicon (Si) is the second most abundant element in the earth's crust and is common in most soils (Epstein, 1994; Marschner, 1995; Datnoff *et al.,* 1997).

Most recent reviews and experimentation reveal a multitude of Si functions in the life of plants. These include the reduction of disease susceptibility to fungal pathogens (and insects) and tolerance of toxic metals such as aluminum (Datnoff *et al.,* 1997). In addition, application of Si often minimizes lodging of cereals and often causes leaves to assume orientation more favourable for light interception (Fauteux *et al.,* 2005).

Various workers have suggested that the effects of Si in providing disease control are because of the creation of a mechanical barrier to penetration (Kim *et al.,* 2002). This seems logical given the reports of Si accumulation at sites of attempted pathogen penetration (Blaich and Grundhofer, 1998). However, as pointed out by Fauteux *et al.* (2005), this probably reflects the higher transpiration rates at infection sites, rather than active transport of Si as part of a plant defense response.

In fact, a number of studies have disputed the role of Si as a mechanical barrier to penetration by fungal pathogens. For example, when Si application to cucumber plants was interrupted, prophylactic effects against powdery mildew were lost, despite accumulation of Si in plant tissue (Samuels *et al.,* 1991). Heine *et al.* (2007) found that accumulation of Si in root cell walls did not represent a physical barrier to the spread of *Pythium aphanidermatum* (Edson) in roots of bitter gourd and tomato, although maintenance of elevated symplastic Si content was a prerequisite for Si-enhanced resistance to the pathogen.

A number of studies since the 1990s, mainly on dicotyledons like cucumber, have suggested that Si activates plant defense mechanisms (Cherif *et al.,* 1992; Cherif *et al.,* 1994). More recent work on monocotyledons supports an active role for Si in stimulating natural defense reactions in plants. Thus, in the wheat-*Blumeria graminis* f. sp. *tritici* pathosystem, epidermal cells of Si-treated plants were shown to react to attempted infection with specific defense reactions including papilla formation and callose production (Belanger *et al.,* 2003). Similarly, Si-mediated resistance in rice to *Magnaporthe grisea* (T.T. Hebert) was shown to be associated with accumulation of antimicrobial

compounds at infection sites, including diterpenoid phytoalexins (Fauteux *et al.,* 2005). Interestingly, Si-mediated resistance to pathogens in both dicotyledons and monocotyledons has been associated with phytoalexin accumulation. As phytoalexins are highly specific in each plant species, Fauteux *et al.* (2005) suggests that Si might be acting on mechanisms shared by all plant species, e.g., those leading to the expression of plant stress genes. The exact mechanism by which Si modulates plant signaling remains unclear, although it could act as a potentiator of defense responses or as an activator of signaling proteins (Fauteux *et al.,* 2005).

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CHAPTER TWO

EFFECT OF POTASSIUM SILICATE ON DAMPING-OFF CAUSED BY *RHIZOCTONIA SOLANI* AND *SCLEROTINIA SCLEROTIORUM* ON SUNFLOWER SEEDLINGS

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ABSTRACT

The potential of silicon (Si) to control *Rhizoctonia solani* and *Sclerotinia sclerotiorum* diseases in sunflower (*Helianthus annuus*) was investigated. *In vitro* assessments of fungal responses in the presence of Si were carried out and the levels of in mycelial growth inhibition were tested. More inhibition was observed as the Si concentration increased from 0 to 1000, 2000, 3000, 4000 and 6000 mg ℓ^{-1} of PDA with relative increases in pH from 5.3 to 9.5, 10.2, 10.5, 10.7 and 11.1, respectively. The effect of pH on the pathogens in the absence of Si was also examined. As the pH increased from 5.3 to 9.5 and subsequently to 11.1 colony diameter decreased. At a pH 9.5 there was no growth inhibition of *R. solani*, but growth of *S. sclerotiorum* was reduced by 17.7%. However, at pH 11.1 the colony diameters of both *R. solani* and *S. sclerotiorum* were inhibited by 44 and 43%, respectively. Greenhouse trials showed that Si caused a increase in dry weight, of inoculated plants. Optimal Si concentration and application frequency was 200 mg ℓ^{-1}, applied weekly, with an increase in dry weight of plants inoculated with *R. solani* and *S. sclerotiorum* of 66.1 and 63.8% respectively. These results suggest that integrating Si with other control methods such as *Trichoderma* spp. could increase the germination percentage of sunflower by protecting seed from pathogen invasion during germination.

2.1 INTRODUCTION

Root diseases are one of a number of factors that prevent the full expression of the inherited potential of a crop. They are among the most widespread and destructive diseases of many crops around the world, including sunflowers. Several unspecialized soil-borne pathogens such as different species of *Fusarium*, *Phytophthora, Pythium*, *Rhizoctonia* and *Sclerotinia* coexist in most soils and are responsible for severe damage of crop seedlings such as seed rot, pre-emergence and post-emergence damping-off and can lead to significant stand losses during plant establishment (Altier and Thies, 1995).

Multiple control measures are required to obtain optimum stand density in the presence of these pathogen complexes. Despite the effectiveness of fungicides, their widespread use has not eliminated seedling damping-off caused by *R. solani* Kuhn and other seedling pathogens (Bell, 1984). Moreover, the widespread use of chemicals has become a subject of public concern and scrutiny mainly due to their potential harmful effect on non-target organisms, development of resistant races of pathogens, and possible carcinogenicity of some chemicals (Misaghi *et al.,* 1998). There is, therefore, a need to examine the potential for non-chemical approaches to disease management.

Use of silicon (Si) nutrition is potentially an environmentally friendly method for the control of numerous diseases. Many researchers report successful disease control using Si for various combinations of plant pathosystems under field conditions. More recent work on monocotyledons supports the theory of an active role for Si in stimulating natural defense reactions in plants. In the dicotyledons, most results relating to Si and disease control are reported from cucumber trials where Si amendments to nutrient solutions have increased resistance to *Sphaerotheca fuliginea* (Schlecht.) Pollacci (Adatia and Besford, 1986; Belanger *et al*., 1995) and reduced *Fusarium* wilts (Miyake and Takahashi, 1983).

This study was undertaken to evaluate the potential of Si to control damping-off caused by *Rhizoctonia solani* Kuhn and *Sclerotinia sclerotiorum* de Bary in sunflower. Research objectives were to determine (i) the *in vitro* inhibitory effects of Si on *R. solani* and *S. sclerotiorum* growth; (ii) the *in vitro* effects of pH on Si activity; (iii) the efficacy of different concentrations and frequencies of application of Si for the control of seedling root diseases caused by *R. solani* and *S. sclerotiorum* on sunflower grown under greenhouse conditions.

2.2 MATERIALS AND METHODS

2.2.1 Sources of materials

A virulent isolate of *R. solani* was donated by K.S. Yobo[1]. *Sclerotinia sclerotiorum* isolate was obtained from sunflowers (*Helianthus annuus* Heiser) in Delmas, Mpumalanga, South Africa (SA) in February, 2005 (McLaren[2]) in the form of sclerotia. The *Sclerotinia* isolate was confirmed to be *S. sclerotiorum* (PPRI Accession number 8374) by the Plant Protection Research Institute in 2005. Silicon (Si) in the form of liquid potassium silicate containing 20.5-20.9% SiO_2 was provided by PQ Corporation (Pty) Ltd[3].

2.2.2 Laboratory bioassays

2.2.2.1 Antifungal activity of silicon

The effects of Si on the growth of *R. solani* and *S. sclerotiorum* were studied in 90 mm diameter Petri dishes containing Potato Dextrose Agar (PDA) (Merck[4]). Silicon was autoclaved in 0, 1000, 2000, 3000, 4000 and 6000 mg ℓ^{-1} and poured into a 1000 ml Erlenmeyer flask filled with PDA (1000 ml) prior to

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[4]Merck, Biolab Diagnostics (Pty.) Ltd., 289, Davidson Rd, Wadeville,1428, Gauteng, South Africa.

cooling and solidification. The pH of the Si supplemented PDA medium was determined before it was poured into sterile Petri dishes. Petri dishes were incubated for two days at room temperature to verify that no contamination occurred. After incubation, plates were inoculated with either *R. solani* or *S. sclerotiorum* by placing a 10 mm diameter disc of an actively growing culture of the fungus at the centre of each plate and then further incubated at room temperature. Each fungus was also grown on unsupplemented PDA plates to serve as controls. After the control plates were fully colonised by the fungus, agar plugs from treatments showing no fungal growth were aseptically transferred to fresh PDA plates to determine whether Si has fungicidal/fungistatic effects on the tested fungi. The trial was arranged as a randomized complete blocks design (RCBD) with 4 PDA plates per treatment as replications. The trial was run twice and the data pooled.

2.2.2.2 Effect of pH on pathogens

The effect of pH in the absence of Si was investigated. The pH of unsupplemented PDA was determined to be 5.3. However, after addition of Si at 1000, 2000, 3000, 4000 and 6000 mg ℓ^{-1}, the pH of the PDA increased from 5.3 to 9.5, 10.2, 10.5, 10.7 and 11.1, respectively. The effects of pH on fungal mycelial growth was examined on PDA by adjusting the pH level to (similar to pH level observed when minimum and maximum amount of Si was used) 9.5, and 11.1 using potassium hydroxide (KOH) and sodium chloride (NaCl). For both pathogens, mycelial plugs, (10 mm diameter), were removed from actively growing margins of the culture and transferred into the centre of each adjusted pH plate. Four plates per treatment were used for each pathogen. Plates were incubated at room temperature to evaluate mycelial growth. The trial was run twice and the data pooled.

2.2.2.3 Measurement of mycelial growth

After the control plates were fully colonized by the fungi, fungal growth diameters were recorded for the Si amended treatments. Colony diameter of both pathogens was measured horizontally and vertically for each individual

plate and averaged. The degree of antagonism was calculated according to Kucuk and Kõvanc (2003). Percentage reduction in growth was determined as: $[(D_c - D_t)/D_c] \times 100$, where D_c is the average diameter increase of the fungal colony in the control, and D_t is the average diameter increase of the fungal colony in each treatment.

2.2.3 Greenhouse bioassays

2.2.3.1 Preparation of pathogen inoculum

Fungal inoculum for *in vivo* experiments was prepared by soaking barley seeds in 500 ml Erlenmeyer flasks overnight with 40 ml of tap water per 100 g of seed. Soaked seeds were then drained and autoclaved at 121°C for 15 min on two consecutive days. Autoclaved barley seeds were then incubated for 4 days at room temperature to ensure that no contamination had taken place. After incubation at room temperature, flasks were inoculated with agar discs (4 x 4 mm) of either *R. solani* and *S. sclerotiorum* and then incubated for a further three weeks to allow the fungus to completely colonize the barley seeds. The colonized barley seeds were then air dried, and stored in paper bags. The inoculum for *S. sclerotiorum* was stored at 4°C while *R. solani* was kept at room temperature until needed.

2.2.3.2 Greenhouse trials

Greenhouse trials were conducted in Speedling 24® trays filled with composted pine bark (Growmor[5]). One sunflower seed was planted into each cell of the Speedling 24® trays, and inoculated with two fungal colonized barley grains placed equidistant from the seed at planting. During the trial, six treatments (T) were evaluated on biweekly and weekly basis; (T_1) application of 100 mg ℓ^{-1} of Si, (T_2) application of 200 mg ℓ^{-1} of Si, (T_3) a drench with 3000 mg ℓ^{-1} of Si at planting, (T_4) a combination of T_1 and T_3, (T_5) a combination of T_2 and T_3 and

[5] Growmor, PO Box 89, Cato Ridge, 3680, KwaZulu-Natal, South Africa.

(T_6) Control. Trays were left in the planting room overnight and only watered the following day to avoid immediate leaching of Si from Speedling trays. At the first sign of germination, trays were transferred to the greenhouse maintained at 26 to 28°C with a 75 to 85% relative humidity (RH).

The number of seedlings with damping-off symptoms were recorded 30 days after planting to determine the effect of *R. solani* and *S. sclerotiorum* on percentage germination of sunflower post Si application using the following equation:

$$\text{germination (\%)} = \frac{\text{no. of healthy seedlings / tray X 100}}{\text{no. of sown seeds / tray}}$$

While still at vegetative stage, seedlings were harvested at soil level and dry weight was taken after the biomass was dried in an oven for 72 h at 70°C. The trial was run twice and the data pooled.

2.3 STATISTICAL ANALYSIS

Data from *in vitro* trials were square root transformed before being subjected to an analysis of variance (ANOVA) using Genstat Release 9.1 Statistical Analysis Software (GenStat, 2006) to determine the differences between treatments. Least significant differences between treatment means were determined at $P \leq 0.05$.

2.4 RESULTS

2.4.1 Laboratory bioassays

2.4.1.2 Pathogen inhibition

In vitro growth of *R. solani* and *S. sclerotiorum* were inhibited in the presence of Si, with increased inhibition as the Si concentration increased (Table 2.1).

Inhibition of *R. solani* with the use of Si at ≥ 3000 mg ℓ^{-1} was significantly higher than 1000 mg ℓ^{-1}. Similar results were observed for *S. sclerotiorum.* Compared to *R. solani, S. sclerotiorum* was mostly inhibited between 1000 mg ℓ^{-1} and 2000 mg ℓ^{-1}. In addition, maximum growth inhibition was observed at ≥3000 mg ℓ^{-1} – 6000 mg ℓ^{-1} where no differences in inhibition between the two pathogens were observed. Plugs of both *R. solani* and *S. sclerotiorum* that failed to grow on Si amended PDA plates did, however, grow after being transferred to unamended PDA plates but their growth was still inhibited by 9.22% and 7.24%, respectively (Table 2.1).

2.4.1.2 pH effects

Mycelial growth of both *R. solani* and *S. sclerotiorum* varied with pH. As the pH increased, colony diameters decreased (Table 2.2). However, for *R. solani* no significant difference was seen in colony diameter between pH 5.3 (control) and pH 9.5 while growth *S. sclerotiorum* was significantly reduced at pH 9.5 and colony diameter was reduced from 9.11 to 6.77 mm with 17.7 %. At pH 11.1 the colony diameters of both *R. solani* and *S. sclerotiorum* were significantly inhibited, as their colony diameters were inhibited by 44.3 and 43.6%, respectively. Generally, increased inhibition was observed with an increase in pH (Table 2.2).

Table 2.1. *In vitro* inhibition (%) of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* mycelial growth at different silicon concentrations on potato dextrose agar (PDA) relative to a control grown on unamended PDA. Treatments exhibiting no colony formation were transferred to unsupplemented potato dextrose agar (PDA) and its subsequent growth measured.

	Silicon Inhibition (%)					
Pathogen	**1000 mg ℓ$^{-1}$ (pH 9.5)**	**2000 mg ℓ$^{-1}$ (pH 10.2)**	**3000 mg ℓ$^{-1}$ (pH 10.5)**	**4000 mg ℓ$^{-1}$ (pH 10.7)**	**6000 mg ℓ$^{-1}$ (pH 11.1)**	**0 mg ℓ$^{-1}$ Transferred plugs (pH 5.3)**
	Inhibition (%)	**Inhibition (%)**	**Inhibition (%)**	**Inhibition (%)**	**Inhibition (%)**	**Inhibition (%)**
R. solani	4.5 (2.1 a)	75.1 (8.7 d)	97.0 (9.9 f)	97.1 (9.8 f)	100 (10.0 f)	84.2 (9.2 e)
S. sclerotiorum	37.0 (6.1 b)	86.0 (9.3 e)	94.4 (9.8 ef)	100 (10.0 f)	100 (10.0 f)	52.2 (7.2 c)
F pr	**<.001**					
LSD	**0.51**					
CV%	**4.1**					

Data in brackets are presented as inhibition percentage square root transformed means of two trials each comprising four replicates.
Means within columns followed by the same letters are not significantly different at $P \leq 0.05$.

Table 2.2. Colony diameter of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* mycelia growth on potato dextrose agar (PDA) adjusted to pH 9.5 and 11.1 as compared to pH 5.3 of unammended PDA (control).

	pH 5.3 (control)	**pH 9.5**		**pH 11.1**	
Pathogen	Colony diameter (mm)	Colony diameter (mm)	Inhibition (%)	Colony diameter (mm)	Inhibition (%)
R. solani	86.2 (9.3 c)	83.1 (9.1 c)	1.5	26.8 (5.16 a)	44.3
S. sclerotiorum	83.2 (9.1 c)	53.4 (7.5 b	17.7	27.0 (5.16 a)	43.6
F pr	**0.18**				
LSD	**0.4**				
CV%	**12.3**				

Data in brackets are presented as the mycelial diameter square root transformed means of two trials each comprising four replicates.
Means within columns followed by the same letters are not significantly different at $P \leq 0.05$.

2.4.2 Greenhouse bioassays

For all Si concentrations and its application frequencies used, there was no significant effect on percentage germination of sunflower seedlings in the uninoculated trial (Table 2.3).

There was an increase dry weight associated with Si applications in comparison with untreated plants (Table 2.3). However, different application frequencies of the same treatment had no significant effect on plant dry weight. With no Si treatments germination (%) was reduced from 93.1% to 66.7% by *R. solani* (Table 2.4) and 70.8% by *S. sclerotiorum* (Table 2.5).
Additionally, Tables 2.4 and 2.5 shows that Si applications (100, 200 mg ℓ^{-1} and and drenching with 3000 mg ℓ^{-1}+ 200 mg ℓ^{-1} both weekly and biweekly) on inoculated plants showed a significant increase of plant dry weight compared to the untreated controls ($P \leq 0.05$). Drenching (3000 mg ℓ^{-1}) at planting has no significant effect on percentage seedling emergence and dry weight of both inoculated and un-inoculated plants.

The highest dry weight for plants inoculated with *R. solani* was 18.1 g, attained by applying 200 mg ℓ^{-1} of Si weekly, with 17 %, increase in dry weight (Table 2.4). The highest dry weight for plants inoculated with *S. sclerotiorum* was 17.9 g, attained by applying a drench of 3000 mg ℓ^{-1} +200 mg ℓ^{-1} weekly, which also resulted in 17%, increase in dry weight (Table 2.5).

Table 2.3. Effects of silicon concentrations and application frequencies on germination percentage and dry weight of sunflower (*Helianthus annuus*) seedlings, measured 30 days after planting, without inoculation.

Treatment	Frequencies	Emergence (%)	Dry Weight (g)	Increase in Dry Weight (%)
Control (No Si.)	None	93.1 a	15.4 a	0
100 mgℓ$^{-1}$ Si.	Weekly	88.9 a	17.2 abc	12.0
	Biweekly	86.1 a	18.0 c	17.0
200 mgℓ$^{-1}$ Si.	Weekly	90.3 a	20.2 d	31.0
	Biweekly	91.7 a	20.1 cd	31.0
3000 mg ℓ$^{-1}$ Si.	Drench @ planting	95.8 a	15.6 ab	0.01
3000 mg ℓ$^{-1}$ +100 mgℓ$^{-1}$ Si.	Weekly	91.7 a	17.3 bc	12.0
	Biweekly	90.3 a	18.3 c	19.0
3000 mg ℓ$^{-1}$ +200 mgℓ$^{-1}$ Si.	Weekly	94.4 a	20.4 d	32.0
	Biweekly	91.7 a	20.1 cd	31.0
F pr		**0.750**	**<.001**	
Lsd		**NS**	**1.85**	
CV %		**6.5**	**5.9**	

Data are presented as the means of two trials each comprising 3 replicates.
Percentage germination and dry weight were determined 30 days after planting.
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
3000 mg ℓ$^{-1}$ represents a drench with Si at planting only or followed by weekly or biweekly applications of 100 or 200 mg ℓ$^{-1}$ Si.
NS = not significant

Table 2.4. Effects of silicon concentrations and application frequencies on percentage germination and dry weight of sunflower (*Helianthus annuus*) seedlings, measured 30 days post inoculation with *Rhizoctonia solani.*

Treatment	Frequencies	Emergence (%)	Dry weight (g)	Increase in Dry weight (%)
Control (No Si.)	None	66.7 a	10.9 a	0
100 mgℓ$^{-1}$ Si.	Weekly	65.3 a	15.5 b	42.2
	Biweekly	63.9 a	15.4 b	41.3
200 mgℓ$^{-1}$ Si.	Weekly	66.7 a	18.1 c	66.1
	Biweekly	66.7 a	18.1 c	66.1
3000 mg ℓ$^{-1}$ Si.	@ planting	63.9 a	10.7 a	-1.8
3000 mg ℓ$^{-1}$ +100 mgℓ$^{-1}$ Si.	Weekly	68.1 a	15.5 b	42.2
	Biweekly	62.5 a	15.4 b	41.3
3000 mg ℓ$^{-1}$ +200 mgℓ$^{-1}$ Si.	Weekly	68.1 a	17.9 c	64.2
	Biweekly	66.7 a	18.0 c	65.1
F pr		**0.642**	**<.001**	
Lsd		**NS**	**0.9297**	
CV %		**5.6**	**3.5**	

Data are presented as the means of two trials each comprising 3 replicates.
Percentage germination and dry weight were determined 30 days after planting.
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
3000 mg ℓ$^{-1}$ represents a drench with Si at planting only or followed by weekly or biweekly applications of 100 or 200 mg ℓ$^{-1}$ Si.
NS = not significant

Table 2.5. Effects of silicon concentrations and application frequencies on percentage germination and dry weight of sunflower (*Helianthus annuus*) seedlings, measured 30 days post inoculation with *Sclerotinia sclerotiorum*.

Treatment	Frequencies	Emergence (%)	Dry weight (g)	Increase in Dry weight (%)
Control (No Si.)	None	70.8 ab	10.9 a	0.0
100 mgℓ$^{-1}$ Si.	Weekly	68.1 a	15.9 b	45.5
	Biweekly	69.4 ab	15.9 b	45.5
200 mgℓ$^{-1}$ Si.	Weekly	69.4 ab	17.8 c	63.8
	Biweekly	72.2 ab	17.9 c	63.8
3000 mg ℓ$^{-1}$ Si.	@ planting	73.6 ab	11.3 a	3.1
3000 mg ℓ$^{-1}$ +100 mgℓ$^{-1}$ Si.	Weekly	72.2 ab	16.0 b	46.4
	Biweekly	73.6 ab	16.1 b	47.0
3000 mg ℓ$^{-1}$ +200 mgℓ$^{-1}$ Si.	Weekly	70.8 ab	18.1 c	65.3
	Biweekly	76.4 b	17.9 c	63.8
F pr		**0.612**	**<.001**	
Lsd		**8.138**	**1.011**	
CV %		**6.6**	**3.7**	

Data are presented as the means of two trials each comprising 3 replicates.
Percentage germination and dry weight were determined 30 days after planting.
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
3000 mg ℓ$^{-1}$ represents a drench with Si at planting only or followed by weekly or biweekly applications of 100 or 200 mg ℓ$^{-1}$ Si.

2.5 DISCUSSION AND CONCLUSION

The results presented in Table 2.1 show the potential of Si to inhibit both *R. solani* and *S. sclerotiorum* colony growth *in vitro*. However, these fungi have differential sensitivities to Si concentrations, as demonstrated by the different levels of inhibition of colony growth. According to Palmer *et al*. (1997) different growth rates among fungi may be related to their ability to compensate for elevated concentrations of most cations. Silicon inhibits several other pathogens such as *Phytophthora cinnamoni* Rands both *in vitro* and *in vivo* (Kaiser *et al*., 2005). However, the ability of Si to produce inhibition of radial colony growth *in vitro* does not necessarily ensure its effectiveness in the field because its mechanisms may be influenced by soil type, temperature, pH and moisture of the plant and soil environment.

Many compounds with high pH values (basic pKa) inhibit fungal colony growth (Palmer *et al*., 1997), indicating that pH may be a reason for their control by Si. Most fungi grow better in acidic to neutral conditions than in alkaline environments. For example, *S. rolfsii* sclerotial germination is inhibited above pH 7.0 (Palmer *et al*., 1997). Data show that *R. solani* and *S. sclerotiorum* are inhibited on media with high pH values. Kaiser *et al*. (2005) conducted similar studies with Si and observed that the mycelia growth of *Alternaria solani* Sorauer*, Fusarium solani* Sacc*, Stemphylium herbarum* Rank and *Verticillium fungicola* Hassebr was reduced at increased pH but not to the same extent as with Si. The present findings confirm these results suggesting that there may be other mechanisms involved. Kaiser *et al*. (2005) proposed that Si by itself inhibits pathogens.

Greenhouse studies showed that no relationship between Si application and the incidence of damping-off could be discerned, indicating that Si alone cannot control damping-off of *R. solani* and *S. sclerotiorum*. Several studies describe the ability of Si to enhance the resistance of *Cucurbitaceae* against root rot caused by *Pythium* spp (Cherif *et al.,* 1994). However, this did not occur in sunflowers with *R. solani* and *S. sclerotiorum*.

Two different mechanisms have been proposed regarding the role of Si nutrition in disease control. The first approach is the barrier theory by which polymerized Si mechanically impedes the growth of fungal hyphae in the plant tissue. The other theory is emphasizing a metabolic role of Si on stimulating plant defence mechanisms (Fawe *et al*., 2001). In the light of these results, the mechanical barrier theory with regard to Si in roots was ruled out as a defense mechanism of sunflower against *R. solani* and *S. sclerotiorum,* since these pathogens could be re-isolated from the hypocotyls of diseased and symptomic plant tissues of inoculated sunflowers, irrespective of the Si treatment.

On the other hand, some Si treatments resulted in a better shoot dry weight of sunflower compared to plants not treated with Si (Tables 2.3, 2.4 and 2.5). Similar results were recorded on monocotyledons, e.g., maize *(Zea mays)* in which addition of Si in the early experiments of Williams and Vlamis (1957) enhanced growth; after 6 weeks, the roots of the Si-treated plants had nearly twice the weight of the Si deficient ones; the weight of the Si-treated shoots were greater than of the Si-deficient plants. In other instances where Si also enhanced growth the effect was due to alleviation by Si of nutrient imbalances (Epstein, 1994). However, not all instances in which Si promoted growth can be attributed to its effect in moderating or alleviating nutrient imbalances (Emadian and Newton, 1989).

The ability of Si to inhibit colony growth of *R. solani* and *S. sclerotiorum in vitro* may not only be attributed to its high pH. It also has a positive influence in enhancing dry weight of diseased plants. Therefore, these is a possibility to integrate Si with other control methods that will boost the germination and emergence of sunflower seed by protecting it from pathogen invasion during the seedling stages.

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CHAPTER THREE

INTEGRATION OF *TRICHODERMA HARZIANUM* (ECO-T®) AND POTASSIUM SILICATE FOR CONTROL OF *RHIZOCTONIA SOLANI* AND *SCLEROTINIA SCLEROTIORUM* ON SUNFLOWER

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ABSTRACT

The aim of this study was to evaluate the efficacy of a combining *Trichoderma harzianum* (Eco-T®) and potassium silicate (Si) for the control of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* on sunflower. *In vitro* antifungal activities of Eco-T® on *R. solani* and *S. sclerotiorum* were assayed and resulted in a significant inhibition of their mycelial growth in a dual culture assay, as well as the effects ofvolatile and non-volatile compounds produced by Eco-T®. In addition, the effect of combining Eco-T® with Si was assayed on sunflower seedlings artificially inoculated with *R. solani* or *S. sclerotiorum* under greenhouse conditions with regard to germination and increased plant dry weight. Additionally, the effects of potassium and pH in potassium chloride (KCl) and potassium hydroxide (KOH) as controls were tested, to compare the effect of potassium and pH in the absence of silicon. Germination of sunflower plants infected with *R. solani* and *S. sclerotiorum* was significantly improved by the application of Eco-T®+Si from 65.3% to 84.7% and 68.1% to 80.6%, respectively. Treatments with Eco-T®+Si, Eco-T®+KCl or Eco-T®+KOH in combination also resulted in increased dry weight of plants inoculated with pathogens. However, the combination of Eco-T®+Si produced the largest increase in dry weight.

3.1 INTRODUCTION

Chemical control methods have been widely used to control soil-borne plant pathogens. Although in many cases these chemicals appear to be the most economical and efficient means of controlling plant pathogens, their effect on the environment and the development of tolerance in pathogen populations have led to a drastic reduction in their use and increased the need for farmers to find alternative means of disease control. One of the alternative and effective methods to control these pathogens is the use of biological control agents (Harman *et al.*, 2004).

Trichoderma spp. are common fungi, present in substantial quantities in nearly all agricultural soils, and their use as biocontrol agents is now being recognized as an alternative in plant disease control (Harman *et al*., 2004). Examples of the successful use of *Trichoderma* spp. as biocontrol agents to reduce or prevent damage to plant roots are numerous and can be found for almost all the major root pathogens that affect all major crops. *Trichoderma* can inhibit the pathogen by utilizing different modes of action, i.e., competition, mycoparasitism and antibiosis (Ridout *et al.,* 1986; Cotes *et al.,* 1994; Inbar and Chet, 1995).

Additionally, silicon (Si) has been reported to have a suppressive effect on both soilborne and foliar fungal diseases of plant species (Datnoff *et al.*, 2001). However, the exact mode of action of this element is unknown (Ma and Yamaji, 2006). The two most common theories that have been presented are that Si accelerates the defense system resulting in the release of phytoalexins and induces biochemical defense mechanisms in plants or by forming a layer or sheath immediately below the cuticle which acts as a physical barrier to hinder hyphal penetration of fungi and prevent infections (Fawe *et al.,* 1998; Epstein, 1999; Datnoff *et al.,* 2001).

There have been considerable efforts to find and develop a sustainable natural means to control soil-borne pathogens (Stephens *et al.,* 1983). Therefore, given all the benefits associated with biological agents and

nutritional amendments as alternative means to avoid chemical control of soilborne diseases, the current study was aimed to investigate the compatibility and efficacy of integrating *Trichoderma* spp. and Si to control soil-borne pathogens.

The current study focuses on two of the most important and economically damaging soil-borne sclerotial pathogens of sunflower, i.e., *Rhizoctonia solani* Kuhn and *Sclerotinia sclerotiorum* de Bary (Wheeler and Rush, 2001).

3.2 MATERIALS AND METHODS

3.2.1 Sources of materials

A virulent isolate of *R. solani* was donated by K.S. Yobo[6]. The *Sclerotinia sclerotiorum* isolate was obtained from infected sunflowers (*Helianthus annuus* L.) in Delmas, Mpumalanga, South Africa (SA) in February, 2005 (McLaren[7]) in the form of sclerotia. The *Sclerotinia* isolate was confirmed to be *S. sclerotiorum* (PPRI Accession number 8374) by the Plant Protection Research Institute in 2005. *Trichoderma harzianum* Rifai (Eco-T®) was supplied by Plant Health Products[8]. Silicon (Si) in the form of liquid potassium silicate containing 20.5-20.9% SiO_2 was provided by PQ Corporation (Pty) Ltd[9].

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[8] Plant Health Products (Pty) Ltd, PO Box 207, Nottingham Road, 3280, KwaZulu-Natal, South Africa
[9] PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

3.2.2. Laboratory bioassays

3.2.2.1 Dual culture tests

Interactions between Eco-T® and the pathogenic fungi (*R. solani* and *S. sclerotiorum*) were evaluated by the dual culture method as described by Kucuk and Kovanc (2003). Mycelial disks (10 mm diameter) of a 7 day old culture of *R. solani* and *S. sclerotiorum* grown on potato dextrose agar (PDA) was aseptically transferred to one edge of a Petri dish containing PDA (Merck[10]), while mycelial disks of Eco-T® grown in the same manner were placed on the opposite side of the plate. The experimental design used was a randomized complete blocks design (RCBD) using 3 Petri dishes for each isolate. In control plates (without *Trichoderma*), a sterile agar disc was placed on the opposite side of the pathogenic fungus on PDA in Petri dishes. All samples were incubated at room temperature. After the control plates were fully colonized by the fungi, the radial growth of the pathogens in experimental plates was recorded by measuring the diameter of the fungal colonies to determine their inhibition (%). The degree of antagonism was calculated according to Kucuk and Kovanc (2003), i.e., percentage reduction in growth was determined as $[(D_c - D_t)/D_c] \times 100$, where D_c is the average diameter increase of the fungal colony in the control, and D_t is the average diameter increase of the fungal colony with treatment. The trial was run twice and the data pooled.

3.2.2.2 Non-volatile antagonistic properties bioassay

To determine the antifungal effects of Eco-T® on mycelial growth of the pathogenic fungi, 100 ml sterilized potato dextrose broth (PDB) (Anatech Instruments[11]) was inoculated with 0.5 g of Eco-T® in 250 ml conical flasks. Flasks were incubated at 25 ± 1°C on a rotary shaker set at 50 rpm for 14 days (Abd-El-Moity and Shatla, 1981). The culture was filtered through a Millipore filter to remove spores and mycelial mats. The filtrate extract was

[10]Merck Biolab Diagonstics (Pty) Ltd., 289, Davidson Rd, Wadeville, 1428, Gauteng, South Africa
[11] Anatech Instruments, PO Box 98485, Sloane Park, 2152, South Africa

tested for inhibitory activity against the growth of *R. solani* and *S. sclerotiorum.*

A volume of 2 ml of this filtrate was placed in sterile Petri dishes and 15 ml of PDA at 45ºC was added. After the agar cooled and solidified, mycelial discs (10 mm diameter) of the pathogens were aseptically placed in the centre of the agar plates. Control plates were not amended with the filtrate extract. Radial growth of the pathogens was recorded by measuring the diameter of the fungal colonies after control plates were fully overgrown with mycelia. The degree of antagonisim was calculated according to Kucuk and Kõvanc (2003) as described in section3.2.2.1. There were 3 replicates for each treatment. The trial was run twice and the data pooled.

3.2.2.3 Volatile antagonistic properties bioassay

Determination of any antagonistic activity of volatile compounds generated by Eco-T® was carried out using the technique of Dennis and Webster (1971). Eco-T® plugs (10 mm diameter) taken from 3 day old cultures were placed centrally on PDA plates, which were then incubated at 25±1°C for 7 days. After 7 days, the lid of each Petri dish was replaced with the bottom of another Petri dish containing PDA inoculated with a plug from a young culture of either *R. solani* or *S. sclerotiorum*. Each double Petri dish was sealed with parafilm (Industricord[12]). The pathogens were therefore exposed to any volatile metabolites emitted by Eco-T®. Petri dishes containing PDA without Eco-T® but inoculated with the pathogen, were used as controls. Radial growth of the pathogens was measured in the same manner as described in section 3.2.2.1 and compared with mycelial growth on control plates. Each treatment was replicated 3 times. The trial was run twice and the data pooled

[12] Industricord, PO Box 243, Pavilion, 3611, KwaZulu-Natal, South Africa.

3.2.3 Greenhouse bioassays

3.2.3.1 Seed treatments

Sunflower seeds (150 g) were treated by soaking them in a conidial suspension (10 ml) of Eco-T® (2 x 10^8 spores ℓ^{-1}) (Mao *et al.,* 1998) using a 2% solution of carboxymethylcellulose (CMC) (Sigma[13]) as a sticking agent, stirred for 10 min to fully coat the seeds with Eco-T®, before drying on a laminar bench for 24 h.

3.2.3.2 Preparation of pathogen inoculum

Fungal inoculum for *in vivo* trials was prepared by soaking barley seeds in 500 ml Erlenmeyer flasks overnight with 40 ml of tap water per 100 g of seed. Soaked seeds were then drained and autoclaved at 121°C for 15 min on two consecutive days. Autoclaved barley seeds were then incubated for 4 days at room temperature to verify that no contamination had taken place. After incubation at room temperature (20 ± 2°C), flasks were inoculated with agar discs (10 mm diameter) of either *R. solani* or *S. sclerotiorum* and then incubated for a further three weeks to allow the fungus to completely colonize the barley seeds. The colonized barley seeds were then air dried, and stored in paper bags until needed.

3.2.3.3 Trial design and disease assessment

A RCBD trial was carried out under greenhouse conditions, in Speedling 24® trays, with three replicates. The trial was run twice and the data pooled.

The trial was conducted in Speedling 24® trays filled with composted pine bark (Growmor[14]) for both *R. solani* and *S. sclerotiorum* inoculated plants. One sunflower seed was planted into each cell of the Speedling 24® tray, and inoculated with two fungal colonized barley grains, placed equidistant

[13] Sigma Capital Enterprises, PO Box 62, New Germany, KwaZulu-Natal, 3620, South Africa.
[14] Growmor, PO Box 89, Cato Ridge, 3680, KwaZulu-Natal, South Africa.

from the seed during planting. Eight treatments were evaluated on sunflowers, i.e., (T_1) control; (T_2) application of Eco-T® (seed treatment); (T_3) application of 130 mg ℓ^{-1} of KCl (Merck Chemicals[15]) once every 7 days, as a control treatment to determine the effects of K in potassium silicate activity; (T_4) a combination of T_2 and T_3; (T_5) application of 200 mg ℓ^{-1} of Si once every 7 days; (T_6) a combination of T_2 and T_5; (T_7) application of KOH as a control treatment to determine the effects of pH in the absence of Si by adjusting it at the same pH level as 200 mgℓ^{-1} of Si once every 7 days, and (T_8) a combination of T_2 and T_7.

Plants were grown in a greenhouse maintained at 26 to 28°C with a relative humidity (RH) of 75 to 85%. The trial was terminated 30 days after planting (dap) to determine the effects of treatments on germination (%) and dry weight of sunflower seedlings, post inoculation with *R. solani* and *S. sclerotiorum* using the following equation:

$$\text{Germination (\%)} = \frac{\text{no. of healthy seedlings / tray X 100}}{\text{no. of sown seeds / tray}}$$

Additionally, living seedlings (30 dap) were harvested at soil level and dried in an electric oven at 70°C for 96 h to determine the dry weight (g) of the seedlings. The trial was run twice and the data pooled.

3.3 STATISTICAL ANALYSIS

Data from *in vitro* trials were square root transformed before being subjected to an analysis of variance (ANOVA) using Genstat Release 9.1 Statistical Analysis Software (GenStat, 2006) to determine the differences between treatment means. Least significant differences of treatment means were determined at $P \leq 0.05$.

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[15] Merck Chemicals (Pty.) Ltd., 259, Davidson Rd, Wadeville, Gauteng , South Africa

3.4 RESULTS

3.4.1 Laboratory bioassays

Eco-T® had a marked significant inhibitory effect on the radial growth of the pathogens in the Eco-T® and pathogen interactions in dual culture. Eco-T® caused significant growth inhibition with similar performances for isolates of both *R. solani* and *S. sclerotiorum* in dual culture (Table 3.1). Pathogen growth inhibition of 8.62% occurred with the Eco-T® and *R. solani* interaction*,* while an inhibition of 8.26% was recorded with the Eco-T® and *S. sclerotiorum* interaction. After 7 days, Eco-T® grew towards both fungi, stopping all further growth and started to overgrow the pathogens. Volatile and non-volatile inhibition of pathogens was recorded when the unamended plates (controls) were colonized by the tested fungi. Non-volatile compounds inhibited *R. solani* and *S. sclerotiorum* by 6.89% and 7.18% while volatile compounds inhibited *R. solani* and *S. sclerotiorum* by 5.44% and 5.71%, respectively

Table 3.1. Inhibition of radial growth of *R. solani* and *S. sclerotiorum* in dual culture with Eco-T®, non-volatile and volatile antibiotics produced by *Trichoderma harzianum* (Eco-T®) on potato dextrose agar at 25°C after 7 days.

Pathogens	**Inhibition by Eco-T® (%)**			
	Control	**Dual culture**	**Non-volatile**	**Volatile**
R. solani	0 a	74.2 (8.6 d)	47.2 (6.9 c)	29.4 (5.4 b)
S. sclerotiorum	0 a	69.1 (8.3 d)	50.9 (7.2 c)	32.9 (5.7 b)
F pr	**0.79**			
LSD	**0.59**			
CV%	**6.5**			

Data in brackets are presented as the square root transformed means of two trials each with three replicates.
Numbers followed by the same letters are not significantly different at $p \leq 0.05$.

3.4.2 Greenhouse bioassays

Greenhouse trials showed no differences between treatments in percentage germination compared to the control on uninoculated plants (Table 3.2). In

addition, Eco-T® when applied alone had no effect on plant dry weight. However, a single application of KOH, KCl or Si resulted in a significant increase in plant dry weight in comparison to the control ($P \leq 0.05$). Furthermore, in comparison to the control, dry weight was significantly increased by Eco-T®+KOH (13%), Eco-T®+Si (26%) and Eco-T®+KCl (17%) (Table 3.2).

Germination (%) was generally lower in treatments inoculated with *Rhizoctonia* or *Sclerotinia* (Table 3.3 and 3.4) than the positive control (Table 3.2). In comparison to the control, when KCl and KOH were applied to *Rhizoctonia* or *Sclerotinia* inoculated plants had no influence on germination (Tables 3.3 and 3.4). However, KCl increased the dry weight of plants inoculated with *Rhizoctonia* and *Sclerotinia* by 22.1% and 22.4%, respectively (Tables 3.3 and 3.4). Application of KOH resulted in an increase of 16.3% on *Rhizoctonia* and 27.1% on *Sclerotinia* inoculated plants (Tables 3.3 and 3.4). On the other hand, single applications of Si caused a significant increase in germination for plants inoculated with *Rhizoctonia* (Tables 3.3). Furthermore, there was an increase in dry weight of 23.1% for *Rhizoctonia* (Table 3.3) and 6.5% for *Sclerotinia* inoculated plants (Table 3.4).

Application of Eco-T®+KCl enhanced the dry weight of *Rhizoctonia* and *Sclerotinia* inoculated plants by 42.3 and 59.8% respectively, while, application of Eco-T®+KOH resulted in increases of 33.7 and 57.9% in dry weight. Application of Eco-T®+Si resulted in the largest increases in dry weight for *Rhizoctonia* and *Sclerotinia* inoculated plants of 65.4 and 63.6%, respectively (Tables 3.3 and 3.4).

Table 3.2. Effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), potassium chloride (KCl), potassium hydroxide (KOH), Si+Eco-T®, KOH+ Eco-T® and KCl+Eco-T® on germination and dry weight of sunflower (*Helianthus annuus*) seedlings 30 days after planting, without inoculation.

Treatment	**Germination (%)**	**Dry Weight (g)**	**Increase in Dry Weight (%)**
Control	86.1	12.5 a	0
Eco-T®	88.9	13.3 ab	6
KCl (once every 7 days)	80.6	14.4 bc	13
KCl + Eco-T®	81.9	15.0 c	17
Si 200 mgℓ^{-1} (once every 7 days)	81.9	14.6 bc	14
Si 200 mgℓ^{-1}+ Eco-T®	84.7	16.8 d	26
KOH (once every 7 days)	80.6	14.2 bc	12
KOH+Eco-T®	82.6	14.3 bc	13
F pr	**0.706**	**0.003**	
Lsd	**NS**	**1.606**	
CV %	**7.5**	**6.4**	

Data are presented as the means of two trials each comprising 3 replicates
Percentage germination and dry weight were determined 30 days after planting
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
NS = not significant

Table 3.3. Effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), potassium chloride (KCl), potassium hydroxide (KOH), Si+Eco-T®, KOH+ Eco-T® and KCl+Eco-T® on germination and dry weight of sunflower (*Helianthus annuus*) seedlings, inoculated with *Rhizoctonia solani*, 30 days after planting .

Treatment	Germination (%)	Dry Weight (g)	Increase in Dry Weight (%)
Control	65.3 a	10.4 a	0
Eco-T®	81.9 e	12.5 b	20.2
KCl (once every 7 days)	69.4 abc	12.7 bc	22.1
KCl + Eco-T®	80.6 de	14.8 d	42.3
Si 200 mgℓ^{-1} (once every 7 days)	73.6 bcd	12.8 bc	23.1
Si 200 mgℓ^{-1}+ Eco-T®	84.7 e	17.2 e	65.4
KOH (once every 7 days)	67.8 ab	12.1 b	16.3
KOH+Eco-T®	76.9 cde	13.9 cd	33.7
F pr	**<.001**	**<.001**	
Lsd	**7.532**	**1.246**	
CV %	**5.7**	**5.4**	

Data are presented as the means of two trials each comprising 3 replicates
Percentage germination and dry weight were determined 30 days after planting
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.

Table 3.4. Effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), potassium chloride (KCl), potassium hydroxide (KOH), Si+Eco-T®, KOH+ Eco-T® and KCl+Eco-T® on percentage germination and dry weight of sunflower (*Helianthus annuus*) seedlings, inoculated with *Sclerotinia sclerotiorum*, 30 days after planting

Treatment	Germination (%)	Dry Weight (g)	Increase in Dry Weight (%)
Control	68.1 ab	10.7 a	0
Eco-T®	80.6 c	14.2 b	32.7
KCl (once every 7 days)	76.4 bc	13.1 b	22.4
KCl + Eco-T®	83.3 c	17.1 c	59.8
Si 200 mgℓ^{-1} (once every 7 days)	66.7 a	11.4 a	6.5
Si 200 mgℓ^{-1}+ Eco-T®	80.6 c	17.5 c	63.6
KOH (once every 7 days)	69.4 ab	13.6 b	27.1
KOH+Eco-T®	80.6 c	16.9 c	57.9
F pr	**0.002**	**<.001**	
Lsd	**8.139**	**1.219**	
CV %	**6.1**	**4.9**	

Data are presented as the means of two trials each comprising 3 replicates
Percentage germination and dry weight were determined 30 days after planting
Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.

3.4 DISCUSSIONS AND CONCLUSION

Successful biocontrol of soil-borne plant pathogens has been reported in many cases (Schroth and Hancock, 1981). Damping-off diseases and root rots caused by *Pythium*, *Rhizoctonia* spp., *Sclerotinia* and other soilborne plant pathogens have been effectively reduced using tisolates of the antagonistic fungus *Trichoderma* (Sivan *et al.,* 1984; Papavizas, 1985).

In vitro results from the present study revealed that both volatile and non-volatile metabolites produced by Eco-T® had an inhibitory effect on the growth of the plant pathogenic fungi tested. Results in Table 3.1 show that the non-volatile metabolites of Eco-T® are more effective, when compared to nonvolatile metabolites. However, the dual culture evaluation produced the maximum inhibition. These results are in line with the findings of Kucuk and Kõvanc (2003).

Growth inhibition of the soilborne plant pathogens by *Trichoderma* metabolites have been reported in a number of studies and this phenomenon and related mechanisms have been explained by many authors (Kucuk and Kovanc, 2003). *Trichoderma* is able to produce volatile metabolite(s) having either a fungastatic effect e.g., acetaldehyde and/or a fungicide effect e.g., alkyl pyrones (Claydon *et al.,* 1987) and non-volatile metabolite(s). *Trichoderma* also exudes cellulase or chitinase enzymes that break down the cell walls of pathogens and subsequently inhibit their growth (Graeme-Cook and Faull, 1991).

The present study showed that Si and KCl, in association with Eco-T® ,has a suppressive effect on the pathogenic effects of both pathogens (*Rhizoctonia* or *Sclerotinia*) as measured by germination and dry weight of sunflowers. KOH+Si also increased germination and dry weight of sunflowers, but to a lesser extent.

Silicon content in plants is reported to strengthen plant cell walls to enhance disease and pest resistance (Datnoff and Rodrigues, 2005). In addition, Si improves light interception by keeping leaves erect, thereby stimulating canopy photosynthesis in rice (Ma and Takahashi, 2002). Silicon also has a role in enhancing root elongation and in protecting the stele by hardening the cell wall of the stele and endodermal tissues (Hattori *et al.*, 2003; Lux *et al.*, 2003). Additionally, according to Perrenoud (1990), potassium application improves plant health and vigour, making infection less likely to occur or enabling the plant to make a quick recovery as well as increase the production of disease inhibitory compounds, e.g., phenols, phytoalexins and auxins, around infection sites of resistant plants. Diseases reported to be reduced by potassium fertilization are; late blight (*Phytophthora infestan*s (Mont.) de Bary), dry rot (*Fusarium* spp.), powdery scab (*Spongospora subterranean* Wallr) and early blight (*Alternaria solani* Sorauer) (Perrenoud, 1993; Marschner, 1995).

In contrast, this study showed that the levels of disease control attained by a single application of either Si or KCl only, were limited (Tables 3.3 and 3.4). This demonstrated that except in quiescent infections, Si when used under environment conditions favourable for damping-off diseases and with high inoculum pressure might not prevent losses.

Treatment with the Eco-T® together with Si or KCl showed a synergistic effect against *Rhizoctonia* and *Sclerotinia*. However, the effectiveness of potassium silicate in combination with Eco-T® can be attributed to Si rather than potassium because this combination resulted in a higher level of germination and dry weight of sunflower than KCl in combination with Eco-T®.

This study showed that Si or K fertilizer can be important components in integrated pest management (IPM) used against *Rhizoctonia* and *Sclerotinia.*

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CHAPTER FOUR

USE OF *TRICHODERMA HARZIANUM* (ECO-T®) AND SILICON FOR THE CONTROL OF *RHIZOCTONIA SOLANI* AND *SCLEROTINIA SCLEROTIORUM* ON SELECTED SUNFLOWER CULTIVARS

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ABSTRACT

Seed treatment with biocontrol agents such as *Trichoderma harzianum*, provides an environmentally friendly method for the control of numerous plant diseases and may enhance plant growth. The current study was undertaken to evaluate the effects of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of the two treatments (Si+ Eco-T®) on germination, seedling dry weight, plant height, and head dry weight of four cultivars of sunflower (*Helianthus annuus*) inoculated with either *Rhizoctonia solani* or *Sclerotinia sclerotiorum* under greenhouse conditions. An interaction between cultivars, Si and Eco-T® had a significant effect on germination and seedling dry weight of plants inoculated with *R. solani*. The main effects of cultivars were significant for germination and head dry weight, while Si+Eco-T® had a significant effect on germination and seedling dry weight of plants inoculated with *S. sclerotiorum.* Among the sunflower cultivars tested cv. KKS 318 responded best to the Si and Eco-T® treatments. In addition, the sporulation behaviour of both pathogens and their interactions with the host were studied in rhizotrons. *S. sclerotiorum* infects the host through the stem and roots. On the other hand, *R. solani* infects the host only through the roots.

4.1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) is prone to attack by several infectious diseases, especially in the early stages of development (Carter, 1978). The loss incurred may vary, depending on the nature of the pathogen and the severity of the attack (Altier and Thies, 1995). A number of soil-borne diseases, e.g., *Sclerotinia* wilts and *Rhizoctonia* damping-off are not satisfactorily controlled by agrochemicals (Schoeman, 2003). The development of an integrated management approach, combining multiple control measures would be an advantage. Seed treatments, nutritional enhancement and use of resistant cultivars have been reported as environmentally friendly methods for the control of numerous diseases but also to enhance and stimulate plant growth (Merle and Alan, 1995).

Infection by The impact of *Sclerotinia sclerotiorum* De Bary at an early stage of crop growth may result in root rot symptoms or in the death of the whole sunflower plant (Smith, 1988; Agrios, 1997). If it appears at the time of harvesting, sclerotia of the fungus are mixed with seed, causing seed contamination, affecting the marketability of the crop (Kolte, 1985). *Rhizoctonia solani* Kuhn is responsible for severe damage of crop seedlings, causing seed rot, pre-emergence, and post-emergence damping-off, leading to significant stand losses during plant establishment (Altier and Thies, 1995).

The potential benefits of silicon (Si) nutrition in plants have been extensively reviewed (Belanger *et al.,* 1995). Although Si is not recognized as an essential plant nutrients, many plants accumulate Si from 0.1% to 10% of dry matter (Epstein, 1999). It has been reported in many recent reviews that Si is beneficial to some plant species, e.g., rice and sugarcane. These include growth enhancement and yield, effects on enzymes activity, improvement of soil penetration by roots, enhanced exposure of leaves to light, and improved resistance to lodging, drought stress, salinity and metal

toxicities (Epstein, 1994). It is also well documented that the Si content of rice and other crops is a contributing factor to the expression of resistance to several plant diseases (Epstein, 1999).

The ability of *Trichoderma harzianum* Rifai to reduce disease is also well known and related to its antagonistic properties, which involve parasitism, lysis of pathogens and/or competition for limiting factors in the rhizosphere and production of diffusible and/or volatile antibiotics and hydrolytic enzymes like chinase and β-13-glucanase (Ahmad and Baker, 1987; Kubicek *et al*., 2001). In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production/or control of plant hormones.

The aim of this study was to evaluate the effect of Si and Eco-T®, applied individually and in combination and their potential use in integrated control strategies against selected soil-borne diseases such, as *R. solani* and *S. sclerotiorum,* on different sunflower cultivars.

4.2 MATERIALS AND METHODS

4.2.1 Sources of materials and trial preparations

Four different cultivars of sunflowers (KKS 318, Hysun 333, NK FERT and DKF 68-22) were provided by Dr. A. Nell[16]. A virulent isolate of *R. solani* was donated by K.S. Yobo[17]. The *S. sclerotiorum* isolate was obtained from sunflowers (*Helianthus annuus* L.) in Delmas, Mpumalanga, South Africa (SA) in February 2005 (McLaren[18]) in the form of sclerotia. The *Sclerotinia*

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isolate was confirmed in 2005 to be *S. sclerotiorum* (PPRI Accession number 8374) at the Plant Protection Research Institute. *Trichoderma harzianum* (Eco-T®) was supplied by Plant Health Products[19] (Pty) Ltd. Silicon (Si) in the form of liquid potassium silicate containing 20.5-20.9% SiO_2 was provided by PQ Corporation (Pty) Ltd[20].

4.2.2 Preparation of pathogen inoculum

Fungal inoculum for *in vivo* experiments were prepared by soaking barley seeds in 500 ml Erlenmeyer flasks overnight with 40 ml of tap water per 100 g of seed. Soaked seeds were then drained and autoclaved at 121°C for 15 min on two consecutive days. Autoclaved barley seeds were then incubated for 4 days at room temperature to verify that no contamination had taken place. After incubation at room temperature (20 ± 2°C), flasks were inoculated with agar discs (10 mm diameter) of either *R. solani* or *S. sclerotiorum* and then incubated for a further three weeks to allow the fungus to completely colonize the barley seeds. The colonized barley seeds were then air dried, and stored in paper bags until needed.

4.2.3 Seed treatments

Sunflower seeds (150 g) were treated by soaking them in a conidial suspension of Eco-T® (2 x 10^8 conidia ℓ^{-1}) (Mao *et al.,* 1998), using a 2% solution of carboxymethylcellulose (CMC) (Sigma[21]) as a sticking agent, stirred for 10 min to fully coat the seeds with Eco-T®, before drying on a laminar flow for 24 h. Coated seeds were stored at 4°C and planted no longer than five days after treatment.

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[19] Plant Health Products (Pty) Ltd, PO Box 207, Nottingham Road, KwaZulu-Natal, 3280, South Africa

[20] PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

[21] Sigma Capital Enterprises, PO Box 62, New Germany, KwaZulu-Natal, 3620, South Africa.

4.2.4 Speedling 24® trial

The Trial was conducted in Speedling 24® trays filled with composted pine bark (Growmor[22]) for both *R. solani* and *S. sclerotiorum* inoculated plants. One sunflower seed was planted into each cell of the Speedling 24® trays, and inoculated with two fungal colonised barley grains placed equidistant from the seed during planting. Four treatments (T) with 4 replicates were evaluated on all four sunflower cultivars; (T_1) application of 200 mgℓ$^{-1}$ of Si once every 7 days, (T_2) application of Eco-T® as a seed treatment, (T_3) a combination of T_1 and $T_{2,}$ and (T_4) Control. The same treatments were applied on uninoculated plants to test their effect on growth enhancement. Plants were grown in a greenhouse maintained at 26 to 28°C with a relative humidity (RH) of 75 to 85%. The trial was terminated 30 days after planting (dap) to determine the effects of the treatments on germination and dry weight of sunflower seedlings, post inoculation with *R. solani* and *S. sclerotiorum* was detrminedusing the following equation:

$$\text{Germination (\%)} = \frac{\text{no. of healthy seedlings / tray X 100}}{\text{no. of sown seeds / tray}}$$

Additionally, surviving seedlings (30 dap) were harvested at soil level and dried in an electric oven at 70°C for 96 h to determine the dry weight (g) of the seedlings. The trial was run twice and the data pooled.

4.2.5 Pot trials

The trial was conducted in cylindrical plastic pots (300 mm diameter) filled with composted pine bark for both *R. solani* and *S. sclerotiorum* inoculated plants. Four replicates were used with two plants in each pot. Plants were inoculated with two fungal colonized barley grains placed equidistant from the seed during planting. Similar treatments applied in Speedling 24® trials were used in the Pot trials. After eighty days (80 dap) plant height (cm), and

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[22] Growmor, PO Box 89, Cato Ridge, 3680, KwaZulu-Natal, South Africa.

the head dry weight (g) were evaluated to determine the effect of treatments on sunflowers, in spite of inoculation with *R. solani* and *S. sclerotiorum*. The trial was run twice and the data pooled.

4.2.6 Rhizotron studies

Since *R. solani* and *S. sclerotiorum* have different sporulation behaviors' in the rhizosphere, it was assumed that it is effective to control *R. solani* by treating seeds with biocontrol agents such as *T. harzianum* in comparison with *S. sclerotiorum.* Based on that hypothesis rhizotron studies were carried out to prove the hypothesis. Plants were grown in rhizotrons (transparent containers, 20 x 100 x 100 mm) filled with steamed pine bark (Growmor). The units were infected with either *R. solani* or *S. sclerotiorum* on barley seed by placing two fungal colonized barley grains placed equidistant from the seed during planting. Moisture was maintained by compensating for water losses by adding 20 ml of water into each rhizotron daily. Seven days after planting, microscopic examination of the rhizotrons was carried out to reveal growth of the two pathogens, and their interaction with sunflower seedlings. A Leica dissecting light microscope was used for examination.

4.3 STATISTICAL ANALYSIS

Data were subjected to an analysis of variance (ANOVA) using Genstat Release 9.1 Statistical Analysis Software (GenStat, 2006) to determine the differences between treatment means. Least significant differences were determined at $P \leq 0.05$.

4.4 RESULTS

4.4.1 Uninoculated plants

The main effect of Cultivars were significant for germination and head dry weight, while Si+Eco-T® had a significant effect on germination and

seedling dry weight (Table 4.1 and 4.2). Application of Eco-T® as a seed treatment on KKS318, resulted in a significant increase in head dry weight (34.8%). Eco-T® also resulted in a significant increase of 16% on germination of Cultivar NK FERT (Table 4.2 and Figure 4.1). The combination of Si+Eco-T® on Cultivar KKS 318 increased, plant height by 27.6%, and head dry weight by 42.3% (Table 4.2 and Figure 4.1). In addition, the seedling dry weight of Cultivar DKF 68-22 was significantly increased with the combined application of Si+Eco-T® (26.3%).

4.4.2 Sunflower seeds inoculated with *Rhizoctonia solani*

When seeds were infected with *R. solani*, an interaction between Cultivars, Si and Eco-T® had a significant effect on germination and seedling dry weight (Table 4.3and 4.4). In response to Si application, among cultivars evaluated only head dry weight on Cultivar KKS318 was increased. Similarly, Si application significantly increased the germination of Cultivar Hysun 333 and NK FERT by 10.0% and 15%, respectively (Table 4.4 and Figure 4.2).

Furthermore, the effect of Eco-T® on Cultivar KKS 318 was significant in respect of germination and plant height with increases of 22.4 and 43.1, respectively. Effects of Eco-T® on Cultivar Hysun 333 only resulted in a significant increase on germination and plant height with increases of 12.9% and 21.2% respectively. When Eco-T® was evaluated on Cultivar NK FERT, there was an increase in germination, seedling dry weight and plant heights by 20.6%, 41.8% and 22.9%, respectively. However, Eco-T® only managed to significantly increase the germination of Cultivar DKF 68-22 by 17.6% among all assessed parameters (Table 4.4 and Figure 4.2).

Similar results were observed when Si+Eco-T® effects were evaluated on the same cultivars. On Cultivar KKS318, all other assessed parameters were increased significantly, with exception of head dry weight. Its germination, seedlings dry weight, and plant height, were increased by 24.5%, 15.1% and 37.9%. Furthermore, when Si+Eco-T® was tested on

Hysun 333 there was a increase in germination and seedling dry weight by 17.2% and 16.4%, respectively. Similarly, germination and seedlings dry weight of Cultivar NK FERT was also increased by 13.9% and 47.6%, respectively. However when tested on DKF68-22 it was only germination increased by 28.8%.

Among all tested cultivars, Cultivar KKS 318 responded positively to all treatments compared to the other cultivars, while Cultivar DKF 68-22 showed the least positive response to treatments (Table 4.4 and Figure 4.2).

Table 4.1. The effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting (dap), and on plant height (cm) and the head dry weight (DW), 80 days after planting, without inoculation.

		Speedling 24® Trial (30 dap)		**Pot Trial (80 dap)**	
Treatment	**Cultivar**	**Germination (%)**	**Seedlings' DW (g)**	**Height (cm)**	**Head DW (g)**
Control	**KKS 318**	78.1 e	18.5 bcd	110ab	19.2 abc
	Hysun 333	77.1 e	18.8 bcd	130 bcd	17.8 abc
	NK FERTI	65.1 ab	18.7 bcd	150 d	13.0 ab
	DKF 68-22	67.7 abcd	15.0 ab	100 a	14.4 abc
Si	**KKS 318**	71.3 bcde	16.7 bc	120 abcd	18.2 abc
	Hysun 333	74.9 de	17.3 bcd	130 bcd	22.9 cd
	NK FERTI	71.4 bcde	12.6 a	110 abc	14.9 abc
	DKF 68-22	62.9 a	17.4 bcd	110 ab	16.5 abc
EcoT	**KKS 318**	74.3 cde	17.1 bcd	130 abcd	29.5 de
	Hysun 333	72.2 bcde	15.4 ab	140 cd	22.7 cd
	NK FERTI	77.5 e	17.2 bcd	130 abcd	16.7 abc
	DKF 68-22	66.7 abc	16.5 abc	110 abc	21.5 bcd
Si + EcoT	**KKS 318**	78.1 e	20.9 d	150 d	33.3 e
	Hysun 333	74.9 de	17.3 bcd	110 ab	18.9 abc
	NK FERTI	71.8 bcde	19.5 cd	110 ab	11.9 a
	DKF 68-22	70.8 abcde	20.4 cd	130 abcd	15.2 abc
LSD		**8.**	**4.**	**30**	**9.**
CV%		**7.9**	**16.1**	**18.2**	**33**

Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
*dap = days after planting
*DW = dry weight

Table 4.2. ANOVA table of the effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting (dap), and on plant height (cm) and the head dry weight (DW), 80 days after planting.

		Trays trial			Pots trial		
		Germination (%)			Height (cm)		
	Stratum	D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	7.33	<.001 ***	3	2.26	0.094 NS
	Eco-T®		2.4	0.128 NS		0.68	0.413 NS
	Si		0.05	0.831 NS		0.63	0.432 NS
	Cultivars * Eco-T®		1.7	0.181 NS		3.04	0.038 *
Inter. effects	Cultivars *Si	3	0.09	0.966 NS	3	3.62	0.020 *
	Si+Eco-T®		1.19	0.281 NS		0.06	0.803 NS
	Cultivars * Si * Eco-T®		3.28	0.029 *		1.04	0.383 NS
	Residual	45			45		
		Seedling Dry Weight (g)			Head dry weight (g)		
		D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	0.66	0.580 NS	3	8.98	<.001 ***
	Eco-T®		2.61	0.113 NS		6.68	0.013 **
	Si		0.73	0.396 NS		0.06	0.809 NS
	Cultivars * Eco-T®		2	0.128 NS		3.42	0.025 *
Inter. effects	Cultivars *Si	3	2.22	0.098 NS	3	0.28	0.841 NS
	Si+Eco-T®		11.49	0.001 ***		2.32	0.135 NS
	Cultivars * Si * Eco-T®		1.14	0.343 NS		1.05	0.382 NS
	Residual	45			43		

NS = non-significant ($P > .05$); * = significant ($P \leq .05$); ** = highly significant ($P \leq .01$); *** = very highly significant ($P \leq .001$)

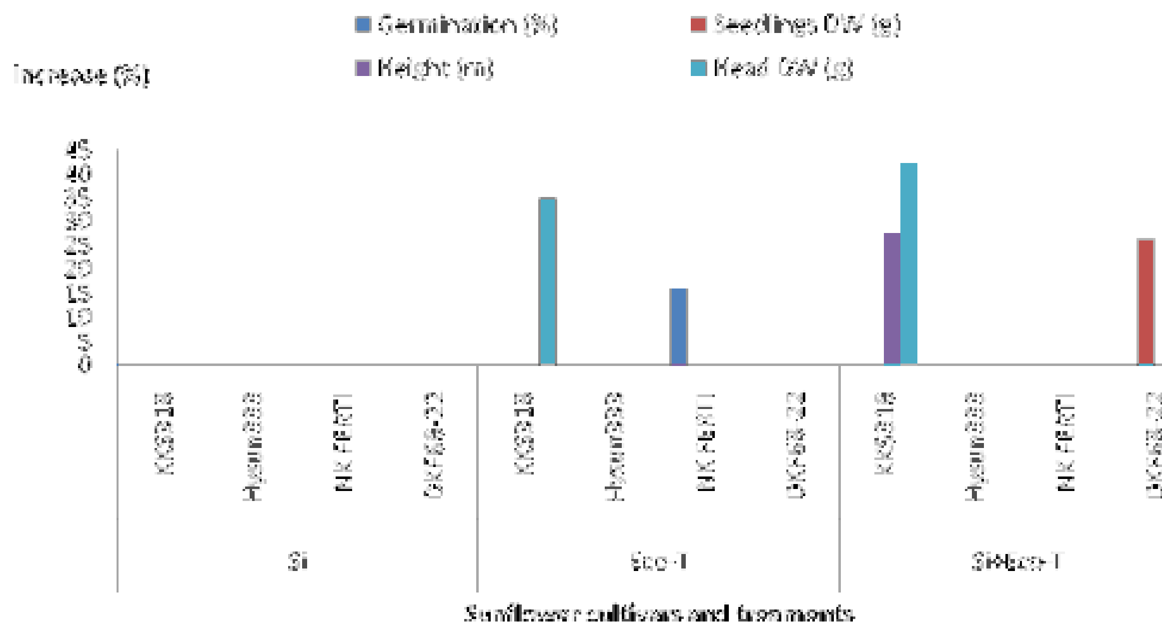

Figure 4.1. Percentage increases on assessed parameters of sunflower (*Helianthus annuus*) cultivar/s that responded positively to Si, Eco-T® and Si+Eco-T® treatments with regard to germination, seedling dry weight 30 days after planting (dap), plant height, and head dry weight (80 dap)

Table 4.3. The effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting, and on plant height (cm) and the head dry weight (DW) post inoculation with *Rhizoctonia solani*, 80 days after planting.

		Speedling 24® trial (30 dap)		**Pot trial (80 dap)**	
Treatment	**Cultivar**	**Germination (%)**	**Seedlings' DW (g)**	**Height (cm)**	**Head DW (g)**
Control	**KKS 318**	64.8 bc	18.7 def	100 a	12.5 a
	Hysun 333	69.3 c	18.1 def	160 bc	13.7 ab
	NK FERTI	67.6 c	10.9 a	150 b	13.1 a
	DKF 6822	65.2 c	15.1 bc	160 bcd	20.0 abc
Si	**KKS 318**	55.8 a	18.2 def	160 bc	29.3 c
	Hysun 333	77.1 d	20.1 efg	180 bcd	27.2 bc
	NK FERTI	79.1def	20.9 fg	180 bcd	20.6 abc
	DKF 68-22	57.9 ab	14.6 b	150 bc	18.8 abc
EcoT	**KKS 318**	82.6 def	18.7 def	180 bcd	16.8 abc
	Hysun 333	79.6 def	20.0 efg	200 d	21.9 abc
	NK FERTI	84.7 f	18.7 def	200 d	19.0 abc
	DKF 68-22	79.1def	17.7 cde	150 bc	19.5 abc
Si + EcoT	**KKS 318**	84.8 f	22.0 g	170 bcd	19.8 abc
	Hysun 333	83.8 ef	21.7 g	180 bcd	26.0 abc
	NK FERTI	78.1 de	20.7 fg	190 bcd	20.8 abc
	DKF 68-22	83.4 def	16.0 bcd	170 bcd	25.9 abc
LSD		**6.**	**2.**	**40**	**14.**
CV %		**5.9**	**10.8**	**15.1**	**47.8**

Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
*dap = days after planting, *DW = dry weight

Table 4.4. ANOVA table of the effect of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting, and on plant height (cm) and the head dry weight (DW) post inoculation with *Rhizoctonia solani*, 80 days after planting.

		Trays trial			**Pots trial**		
		Germination (%)			**Height (cm)**		
	Stratum	D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	9.03	<.001 ***	3	0.64	0.596 NS
	Eco-T®		184.33	<.001 ***		7.63	0.008 *
	Si		0.84	0.364 NS		0.08	0.775 NS
	Cultivars * Eco-T®		12.73	<.001 ***		2.26	0.094 NS
Inter. effects	Cultivars *Si	3	3.34	0.027 *	3	0.27	0.847 NS
	Si+Eco-T®		0	0.996 NS		1.15	0.289 NS
	Cultivars * Si * Eco-T®		10.16	<.001 ***		0.83	0.484 NS
	Residual	3	45		45		
		Seedling Dry Weight (g)			**Head dry weight (g)**		
		D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	14.21	<.001 ***	3	1.17	0.333 NS
	Eco-T®		23.14	<.001 ***		1.19	0.280 NS
	Si		17.58	<.001 ***		4.28	0.044 *
	Cultivars * Eco-T®		1	0.402 NS		0.08	0.972 NS
Inter. effects	Cultivars *Si	3	9.09	<.001 ***	3	0.15	0.927 NS
	Si+Eco-T®		1.95	0.169 NS		0.48	0.494 NS
	Cultivars * Si * Eco-T®		6.22	0.001 ***		0.8	0.503 NS
	Residual	45			45		

NS = non-significant (P > .05); * = significant (P ≤ .05); ** = highly significant (P ≤ .01); *** = very highly significant (P ≤ .001

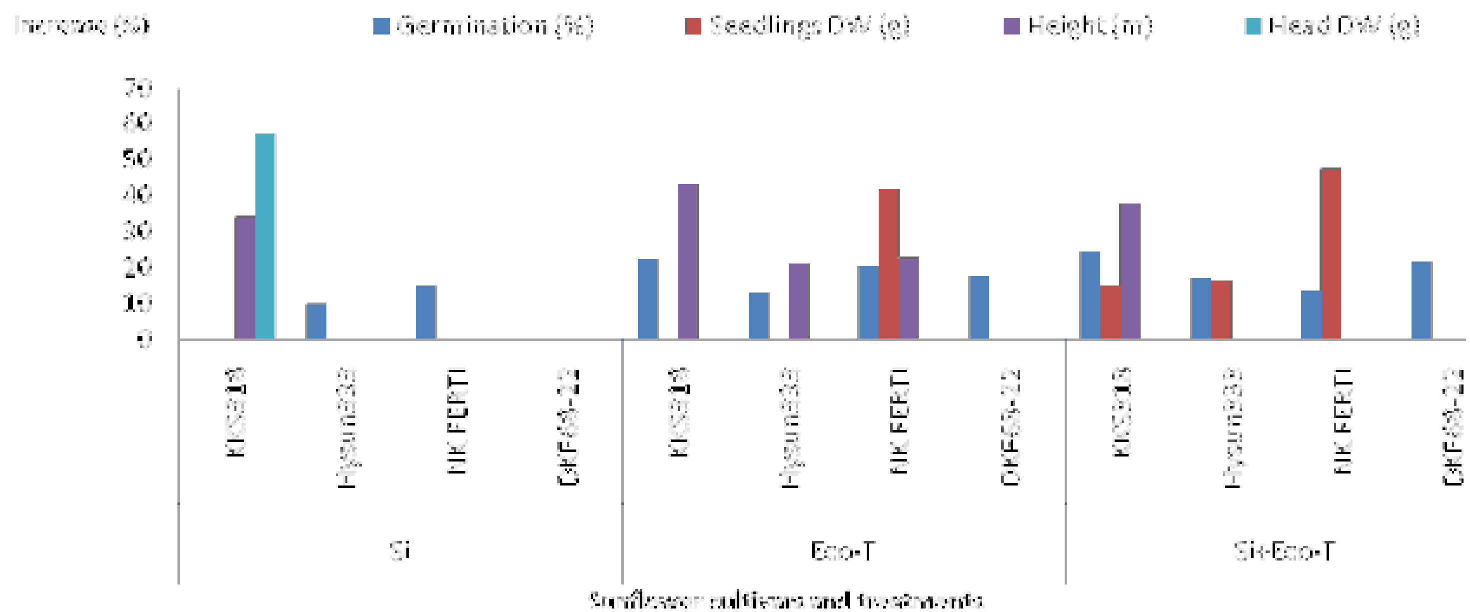

Figure 4.2. Percentage increases on assessed parameters of sunflower (*Helianthus annuus*) cultivar/s that responded positively to Si, Eco-T® and Si+Eco-T® treatments with regard to germination, seedling dry weight 30 days after planting (dap), plant height, and head dry weight (80 dap) post inoculation with *Rhizoctonia solani*.

4.4.3 Sunflower seeds inoculated with *S. sclerotiorum*

When plants were inoculated with *S. sclerotiorum, c*ultivars and Eco-T® had a significant effect on germination and seedling dry weight (Table 4.5 and 4.6). Table 4.6 and Figure 4.3, shows that when Si was applied alone it only manages to increase significantly the seedlings dry weight of cultivar DKF 68-22 by 10.1% among all assessed parameters of the evaluated cultivars. However, application of Eco-T resulted in a significant increase of germination, seedlings, dry weight and plant heights of 14.2%, 28.1% and 25.6% on cultivar KKS 318. Additionally, Eco-T® resulted in increased seedlings dry weight of Hysun 333 with 12.6% and germination of NK FERT with 11.7%.

Similarly, a combination of Si+Eco-T® yielded a significant results on cultivar KKS318 with respect to germination, seedlings dry weight, plant height and head dry weight of 13.7%, 5.5%, 24.2% and 41.8% respectively. Germination of cultivar NK FERT and seedlings dry weight of cultivar DKF 68-22 was also increased with 12.2% and 12.4% respectively.

With exception of all above mentioned effects of treatments, other assessed parameters on evaluated cultivars were either negatively affected or not significantly different to the control (Table 4.6 and Figure 4.3).

4.4.4 Rhizotron studies

Rhizotron studies showed that *R. solani* infections begin within the rhizosphere by entering the host though roots cells (Fig. 4.4 A). In contrast, *S. sclerotiorum* was able to enter the host through the stem and roots (Fig. 4.4 B). Fig. 4.4 C shows a stem of a healthy plant, not inoculated with both pathogen and Fig. 4.5 D shows a rotten root of the host plant resulting from *S. sclerotiorum* infection in comparison to the roots of an uninfected plant (Fig. 4.5 E).

Table 4.5. The effects of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting (dap), and on plant height (cm) and the head dry weight (DW) post inoculation with *Sclerotinia sclerotiorum*, 80 days after planting (dap).

		Speedling 24® trial (30 dap)		**Pot trial (80 dap)**	
Treatment	**Cultivar**	**Germination (%)**	**Seedlings' DW (g)**	**Height (cm)**	**Head DW (g)**
Control	**KKS 318**	65.2 a	15.5 a	130 a	17.8 (4.0 abc)
	Hysun 333	81.9 f	20.4 cd	160 bcde	20.4 (4.0 abc)
	NK FERTI	70.4 abc	19.0 bc	200 ef	13.5 (3.6 abc)
	DKF 68-22	73.5 bcde	17.2 ab	180 cdef	15.8 (3.9 abc)
Si	**KKS 318**	64.6 a	17.2 ab	160 abcd	18.4 (4.2 abc)
	Hysun 333	69.8 ab	19.9 cd	180 def	24.2 (4.9 abc)
	NK FERTI	69.8 ab	19.5 cd	200 f	24.8 (4.8 abc)
	DKF 68-22	72.4 bcd	19.6 cd	160 bcd	15.8 (3.9 abc)
Eco-T	**KKS 318**	76.0 bcdef	21.5 de	170 bcdef	22.2 (4.7 abc)
	Hysun 333	79.1 ef	23.4 e	180 cdef	30.4 (5.4 bc)
	NK FERTI	79.7 f	19.0 bc	180 cdef	20.6 (3.3 a)
	DKF 68-22	76.5 cdef	19.1 bc	150 abc	19.9 (3.4 ab)
Si + Eco-T	**KKS 318**	75.5 bcdef	20.8 cd	170 bcdef	30.6 (5.5 c)
	Hysun 333	80.2 f	19.9 cd	170 bcdef	19.5 (4.4 abc)
	NK FERTI	80.2 f	19.4 bcd	190 def	13.0 (3.5 ab)
	DKF 68-22	78.6 ef	19.7 cd	150 ab	14.1 (3.7 abc)
LSD		**6.**	**2.**	**30**	**2.**
CV%		**6.2**	**8.1**	**14.1**	**32**

Means within columns for a given parameter followed by the same letters are not significantly different at $P \leq 0.05$.
Means in the brackets are square root transformed means of head dry weight (g).
*dap = days after planting, *DW = dry weight

Table 4.6. ANOVA table of effects of silicon (Si), *Trichoderma harzianum* (Eco-T®), and integration of Si+Eco-T® on mean germination and dry weight on four cultivars of sunflower (*Helianthus annuus*) seedlings 30 days after planting (dap), and on plant height (cm) and the head dry weight (DW) post inoculation with *Sclerotinia sclerotiorum*, 80 days after planting (dap).

		Trays trial			**Pots trial**		
		Germination (%)			**Height (cm)**		
	Stratum	D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	7.08	<.001 ***	3	0.8	0.503 NS
	Eco-T®		39.31	<.001 ***		0.04	0.848 NS
	Si		1.44	0.237 NS		2.27	0.139 NS
	Cultivars * Eco-T®		2.37	0.083 NS		1.23	0.309 NS
Inter. effects	Cultivars *Si	3	1.44	0.244 NS	3	1.85	0.151 NS
	Si+Eco-T®		3.54	0.067 NS		0.35	0.556 NS
	Cultivars * Si * Eco-T®		1.66	0.189 NS		0.4	0.755 NS
	Residual	45			45		
		Seedling Dry Weight (g)			**Head dry weight (g)**		
		D.f	F val.	P val.	D.f	F val.	P val.
Main effects	Cultivars	3	6.32	0.001 ***	3	2.28	0.095 NS
	Eco-T®		21.55	<.001 ***		0.04	0.836 NS
	Si		0.1	0.758 NS		0.79	0.379 NS
	Cultivars * Eco-T®		7.06	<.001 ***		1.49	0.234 NS
Inter. effects	Cultivars *Si	3	3.47	0.024 *	3	0.24	0.865 NS
	Si+Eco-T®		5.61	0.022 *		0.46	0.503 NS
	Cultivars * Si * Eco-T®		0.56	0.643 NS		0.72	0.548 NS
	Residual	45			37		

NS = non-significant (P > .05); * = significant (P ≤ .05); ** = highly significant (P ≤ .01); *** = very highly significant (P ≤ .001

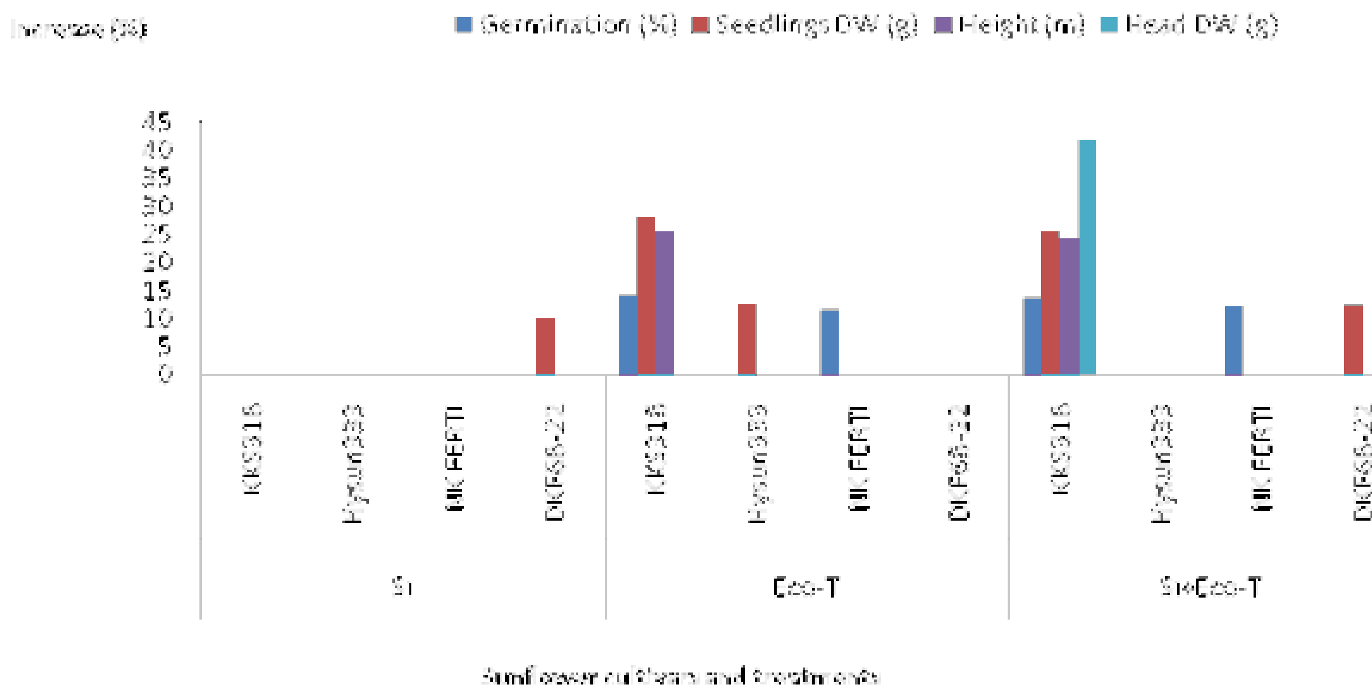

Figure 4.3. Percentage increases on assessed parameters of sunflower (*Helianthus annuus*) cultivar/s that responded positively to Si, Eco-T® and Si+Eco-T® treatments with regard to germination, seedling dry weight (30 days after planting), plant height, and head dry weight (80 days) post inoculation with *Sclerotinia sclerotiorum*.

Figure 4.4. Sunflower seedling stems inoculated with *Rhizoctonia solani* (A), and *Sclerotinia sclerotiorum* (B) showing early symptoms of damping-off compared to an uninoculated plant (C), seven days after germination in rhizotrons.

Figure 4.5. Sunflower seedling roots inoculated with *Sclerotinia sclerotiorum* (D) compared to roots of an uninoculated seedlings (E), seven days after germination in rhizotrons.

4.4. DISCUSSION

Trichoderma spp. have long been known to interact with other micro-organisms especially with fungi, through antibiosis, mycoparastism, competition of various types and other mechanisms (Haran *et al*., 1996; Radwan *et al*., 2006). Induced systemic resistance has been demonstrated by many strains on both monocotyledonous and dicotyledonous plants, resulting in control of bacterial, fungal and viral plant pathogens (Perello *et al*., 2003). For field crops, such as sunflower and wheat, seed treatments with biocontrol agents is one of the most suitable application methods for biocontrol of soil-borne pathogens in the rhizosphere (Harman, 1992). In addition, Si has also been reported to alleviate the negative effects of abiotic stress on sunflower (Gunes *et al*., 2008). Si-induced acceleration of dry-matter production has also been observed in wheat and sorghum. It ameriorates the reduction in dry weight caused by both abiotic and biotic stresses (Hattori *et al*., 2005).

The effect of Si and Eco-T® were measured on treatments on germination, seedling dry weight, plant height and head DW of the sunflower cultivars used. The significant effect observed on cv. KKS 318 was in line with earlier reports where fresh weight, shoot length, dry weight and leaf area of cucumber seedlings as well as seedling weight of cabbages were increased significantly by the application of *T. harzianum* and *T. viride* (Raviv *et al.,* 1998 and Yedidia *et al.,* 2001).

When plants were inoculated with pathogens, plants responded positively but although not always significantly, to application of Si, Eco-T®, and Si+Eco-T®, especially on the plants inoculated with *R. solani*. However, increases were more associated with the application of Eco-T® than Si. The use of Si alone had no effect on improving germination of both inoculated and uninoculated plants. However, it did improve the dry weight of inoculated plants. Eco-T® showed a tendency to improve both germination and dry weight of inoculated plants. All cultivars responded better the treatments when they were inoculated with *R. solani*

compared to *S. sclerotiorum*. These responses may be attributed to the differences of pathogen's growth behavior in the rhizosphere and their interaction with host plants. Cultivar KKS 318 showed the best responses to all used treatments compared to other cultivars tested.

The rhizotron study proves that *S. sclerotiorum* can enter the host through roots, stem and other tissues exposed to its mycelium and spores, making it difficult to control the pathogen with seed treatments.

Silicon alone could be used to increase growth but is unable to control *R. solani* and *S. sclerotiorum.* Nevertheless, combined with Eco-T® it provides an environmentally friendly alternative for the control of *R. solani* and *S. sclerotiorum*. Because Eco-T® protects seed from attack by *R. solani* and *S. sclerotiorum* during the period when seedlings are taking up Si from the soil resulted in increased germination.

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CHAPTER FIVE

SILICON ACCUMULATION AND DISTRIBUTION IN SUNFLOWER SEEDLINGS

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ABSTRACT

A number of recent studies have indicated that silicon (Si) nutrition does not only play an important role in growth and yield enhancement but also for pest and disease control of various crops. This study was conducted to investigate the Si accumulation capacity of sunflower seedlings by quantifying its uptake and distribution in different tissues. Plants were subjected to a range of Si fertilization levels, i.e., 0, 50, 100, 150, 200, 250, 300, 400 and 500 mg ℓ^{-1} of Si in hydroponic system. An increase in Si fertilization resulted in more Si uptake in leaves > stems and roots. There was no significant difference in the accumulation of Si in the leaves at 100 - 500 mg ℓ^{-1} Si. However, application of > 300 mg ℓ^{-1} of Si resulted in stunted plants. The Si concentration in the stems was not significantly different to the control at all Si application. However, in the roots, there was a significant increase in Si accumulation at > 300 mg ℓ^{-1} Si.

5.1 INTRODUCTION

The beneficial effects of silicon (Si) to plants have been reported in various situations, especially under biotic and abiotic stress conditions (Datnoff and Rodriques, 2005). Although Si is not universally considered as an essential plant nutrient, numerous plants species absorb it from soil in larger amounts than most of the elements that are considered essential macronutrients for plant function. Other plant species accumulate small amounts of Si. Accumulation of Si by plants is highly variable, and depends on genotype and environmental conditions (Epstein 1999). Silicon-fed plants will translocate Si as silicic acid (Ma and Takahashi, 2002), and accumulation may occur in leaves, seeds, fruits, roots and stem tissues (Piperno, 1988).

The most significant effect of Si on plants, besides improving their fitness and increasing agricultural productivity, is the restriction of parasitism (Yoshida *et al*., 1962). There are numerous examples of Si negatively affecting infection efficiency of pathogens on their hosts. Increased resistance through Si treatments has been associated with the density of silified buliform, long, and short cells in the leaf epidermis of rice, a model plant used in Si research. Based on the density, Si is believed to act as a physical barrier, a passive form of resistance, to impede penetration (Datnoff and Rodriques, 2005). Fawe *et al*. (1998) proposed that Si played an active role in reinforcing plant disease resistance by stimulating natural defense reactions.

This study was aimed to determine the ability of sunflower seedlings to accumulate Si. The trial examined firstly the accumulation of different Si concentrations and secondly whether the concentration of Si varied in different plant tissues (leaves, stems and roots), in order to indentify the plants tissues in sunflower that may utilize Si to enhance resistance against abiotic and biotic stresses.

5.2 MATERIALS AND METHODS

5.2.1 Experimental procedures and treatments

Plants were grown in a hydroponic culture system in a greenhouse maintained at 26 to 28°C with a relative humidity (RH) of 75 to 85%. Plants (1 plant/pot) were grown in 150 mm diameter pots containing composted pine bark. Pots were subjected to 8 treatments of aerated hydroponic solutions of Silicon (Si) (PQ Corporation (Pty) Ltd[23]). (0, 50, 100, 150, 200, 250, 300, 400 and 500 mg ℓ^{-1}) and arranged in a randomized complete blocks design (RCBD) with 4 replicates per treatment. Pots were fertilized weekly with 100 ml per pot of Hortichem 3:1:3 (28) (N:P:K) and $CaNO_3$ (19.5% Ca, 15.5% N) at a rate of 1 g ℓ^{-1} (Visser, 2007) to ensure that nutrients were not a limiting factor for growth. The trial was terminated 6 weeks after planting and roots, stems and leaves were harvested. The trial was run twice and the data pooled.

5.2.1.2 Inductively Coupled Plasma (ICP) analysis

For determination of Si concentrations in plant tissues, a Microwave-Assisted Digestion Method was used for digestion (Haysom *et al*., 2006). The elemental composition of the samples was determined by inductively coupled plasma (ICP) analysis (Cheng and Jan, 1987; Elliot and Snyder, 1991). Washed and dried leaves, stems and roots were milled and digested using Haysom *et al*. (2006) protocols with a few modifications.

Two hundred mg of plant tissue, together with 3 ml of 70% nitric acid and 2 ml of 30% H_2O_2 were added to the microwave digestion tube. Tubes were capped, microwaved and the acid digestion conducted. Upon completion of the digestion, 2 ml of 10% NaOH were added to the digestate and microwaved again. Silicon and other elements in the samples were then measured using an inductively coupled plasma optical emission spectrophotometer (ICP-OES), Varian 720-ES.

[23] PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

5.2.1.3 Scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis

To determine the localization and distribution of Si in plant tissues (roots, stems and leaves), harvested plant tissues were freeze dried. Freeze dried tissues were cross-sectioned with a blade into slices of < 2 mm. Cross-sectioned materials with the smoothest surface was chosen and mounted onto scanning electron microscope (SEM) stubs for examinations. The samples were examined under a Zeiss EVO MA15, equipped with INCA Microanalysis (Oxford Instruments).

5.3 STATISTICAL ANALYSIS

Data from ICP analyses were square-root transformed before being subjected to an analysis of variance (ANOVA) using Genstat Release 9.1 Statistical Analysis Software (GenStat, 2006) to determine the differences between treatment means. Least significant differences were determined at $P \leq 0.05$.

5.4 RESULTS

The EDX was mainly used to observe the composition of samples and the elemental distribution in specific plant tissues, while ICP was used to provide accurate results by taking representative samples. Hence, only the results of the ICP analyses were considered for analysis (Tesfagioris, 2008).

5.3.1 Inductively Coupled Plasma (ICP)

Application of Si resulted in marked increases in Si content in various plant tissues (Table 5.1 and Figure 5.1). Accumulation of Si in leaves was significantly higher than in stems and roots with the highest recorded level of 36.3 mg g^{-1} dry weight (DW) observed when plants were treated with 400 mg ℓ^{-1} Si. The lowest application level with optimal Si uptake was observed with 100 mg ℓ^{-1} which recorded

25.3 mg g^{-1} DW. Therefore, applications of >100 mg ℓ^{-1} had little impact and can be regarded as unnecessary and uneconomical. On the other hand, the Si content in stems were not significantly different for all application levels. However, in the roots there was significant difference in the Si concentration at > 300 mg ℓ^{-1} Si. As Si was also found in plant parts grown in silicon-free solution (controls), Si must be present in the pine bark used as a growing medium. It was observed that application of 200-300 mg ℓ^{-1} resulted in stronger and healthier sunflower seedlings than those treated with 400 and 500 mg ℓ^{-1} and the Control plants.

5.3.2 Scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis

Similarly, with ICP results from the EDX analysis and Si mapping showed that leaves accumulated more Si, with Si even in Si-deprived (Control) plants. The amount of Si in leaves was significantly higher compared to stems and roots (Fig. 5.1). The white dots (brightness) on the base of the trichomes in the EDX map shows the presence of Si in untreated plants (Fig. 5.2A). In plants treated with 100 mg ℓ^{-1} Si, Si appears to also be mainly concentrated in the trichomes (Fig. 5.2B), but at higher concentrations in relation to the untreated plant's leaf (Fig. 5.2A).

Silicon mapping of sunflower roots and stems showed that Si accumulated in the roots and stems in the epidermis but at relatively low levels (Figures 5.3 and 5.4).

Table 5. Soluble Si extracted from plant tissues of sunflower seedlings treated with Si

Plant tissue	Si applied (mg ℓ$^{-1}$)	Si in plant tissue (mg g^{-1} DW)
Leaves	0	5.6 (2.4 c)
	50	34.4 (4.8 d)
	100	25.3 (6.2 e)
	150	33.3 (5.8 de)
	200	30.7 (5.6 de)
	250	35.8 (6.0 e)
	300	30.9 (5.6 de)
	400	36.3 (6.4 e)
	500	24.4 (4.8 d)
Stems	0	2.5 (1.5 abc)
	50	3.7 (2.0 bc)
	100	4.8 (2.3 c)
	150	3.4 (1.8 bc)
	200	3.9 (2.0 bc)
	250	5.4 (2.3 c)
	300	5.8 (2.4 c)
	400	2.9 (1.7 abc)
	500	5.9 (2.5 c)
Roots	0	0.6 (0.7 a)
	50	2 .0 (1.4 abc)
	100	2.3 (1.2 ab)
	150	2.4 (1.5 abc)
	200	2.7 (1.6 abc)
	250	2.9 (1.7 abc)
	300	3.1 (1.9 bc)
	400	3.4 (1.9 bc)
	500	3.6 (1.9 bc)
F pr		**0.003**
LSD		**1.1**
CV%		**25.8**

Data in brackets are presented as the square root transformed means of two trials each comprising four replicates.
Values in the same column denoted by the same letter are not significantly different at $P \leq 0.05$.

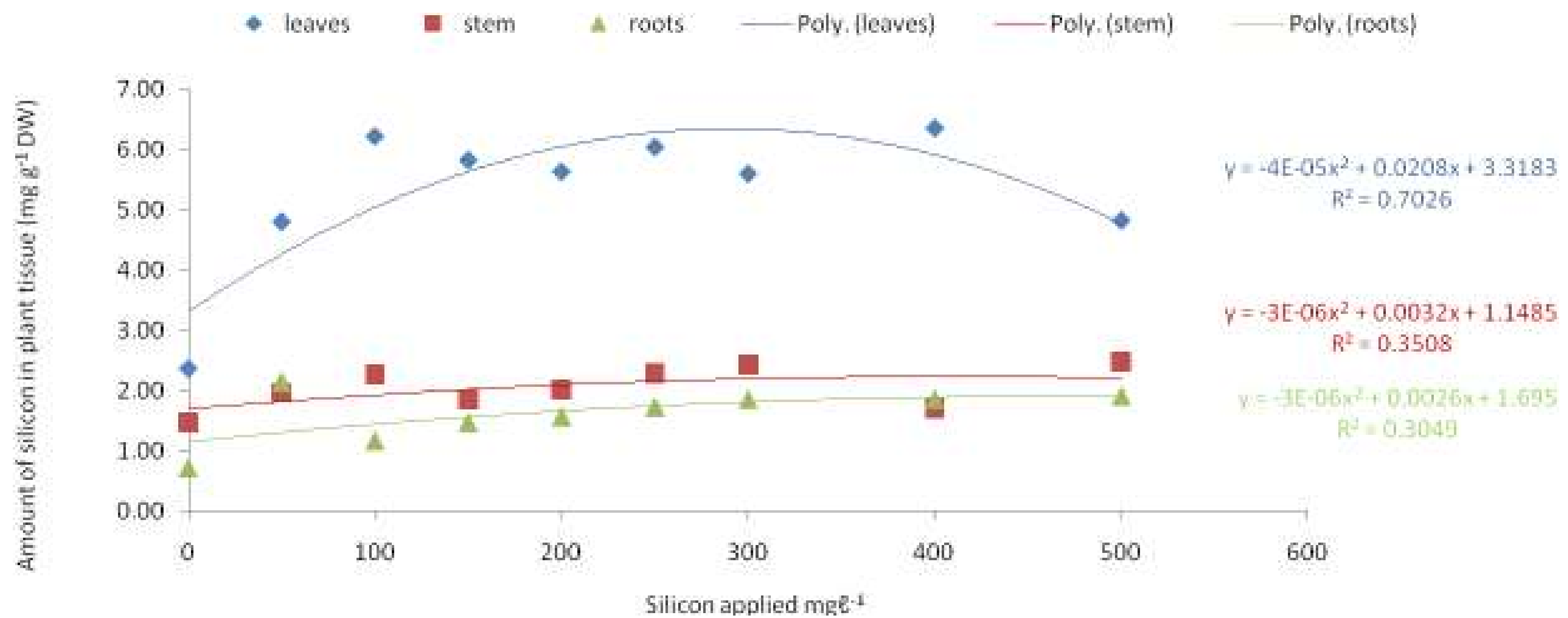

Figure 5.1. Silicon uptake and distribution in leaves, stems and roots of sunflower seedlings grown under a hydroponic system with different silicon applications (mg ℓ^{-1})

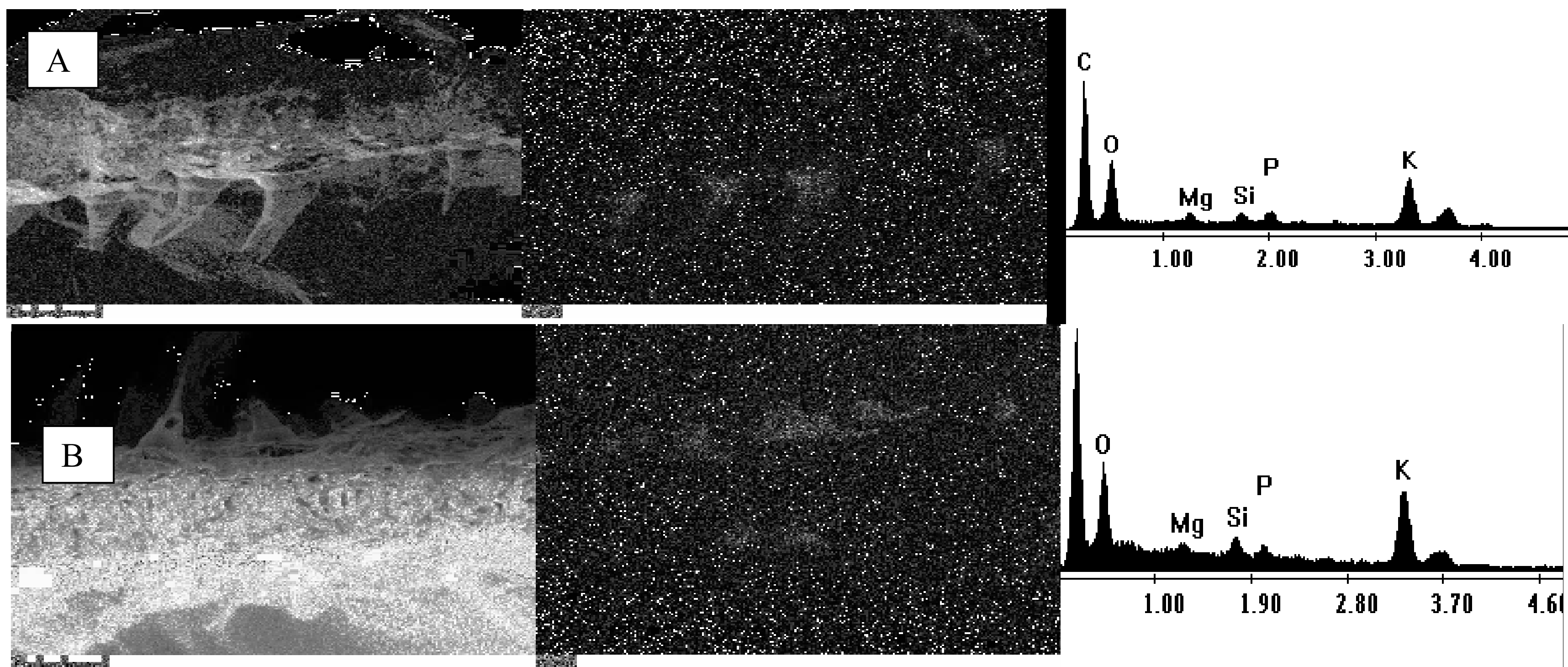

Figure 5.2. Cross-section micrograph and EDX maps of leaves from sunflower seedlings (*Helianthus annuus*) treated with (A) 0, and (B) 100 mg ℓ^{-1} of silicon (Si), in a hydroponic system. Concentrations of white dots show the concentration of Si in trichomes.

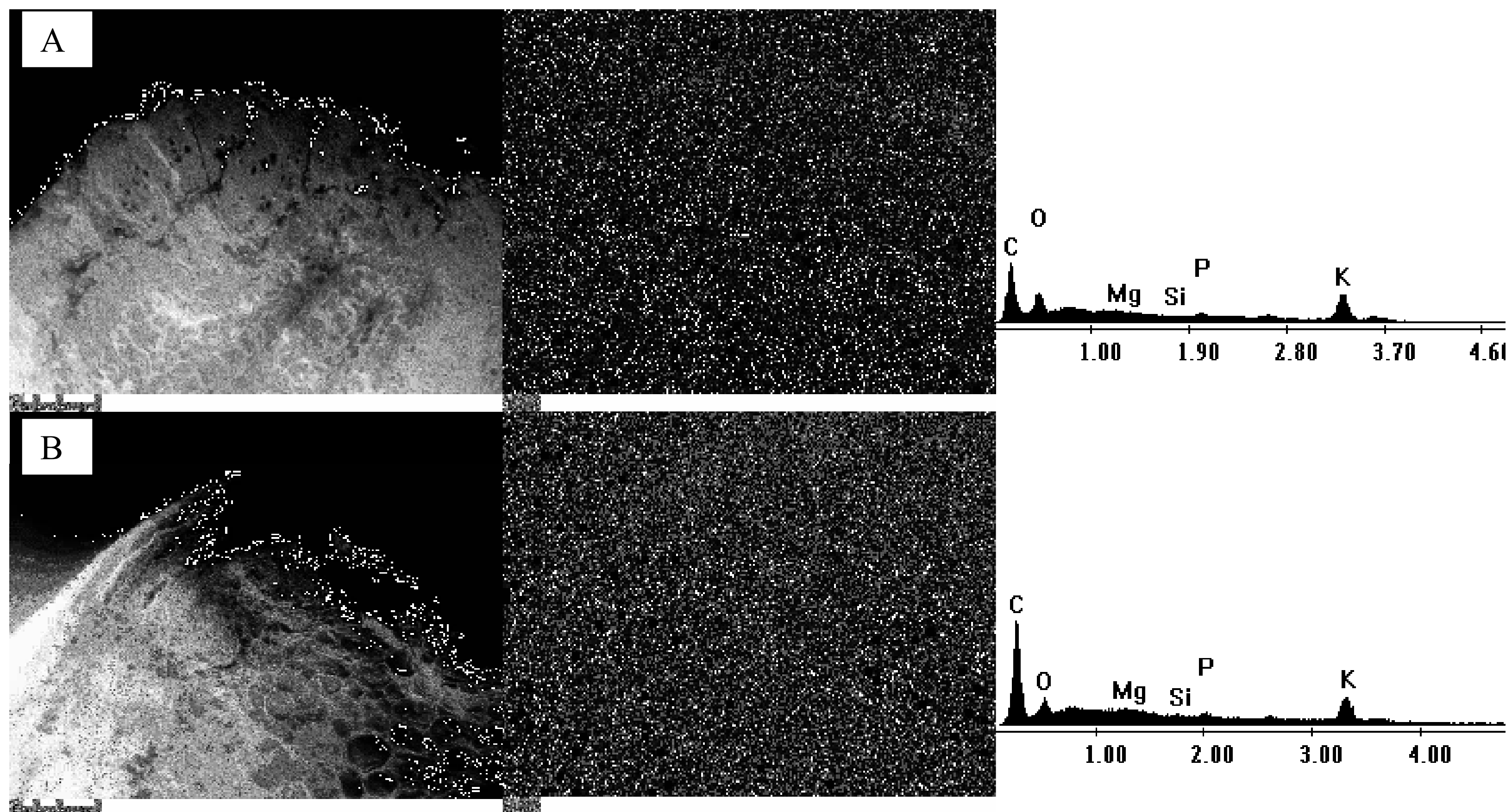

Figure 5.3. Cross-section micrograph and EDX dot maps of stems from sunflower seedlings (*Helianthus annuus*) treated with (A) 0, and (B) 100 mg ℓ^{-1} of silicon (Si), in a hydroponic system. Concentrations of white dots show the concentration of Si in stem epidermal cells.

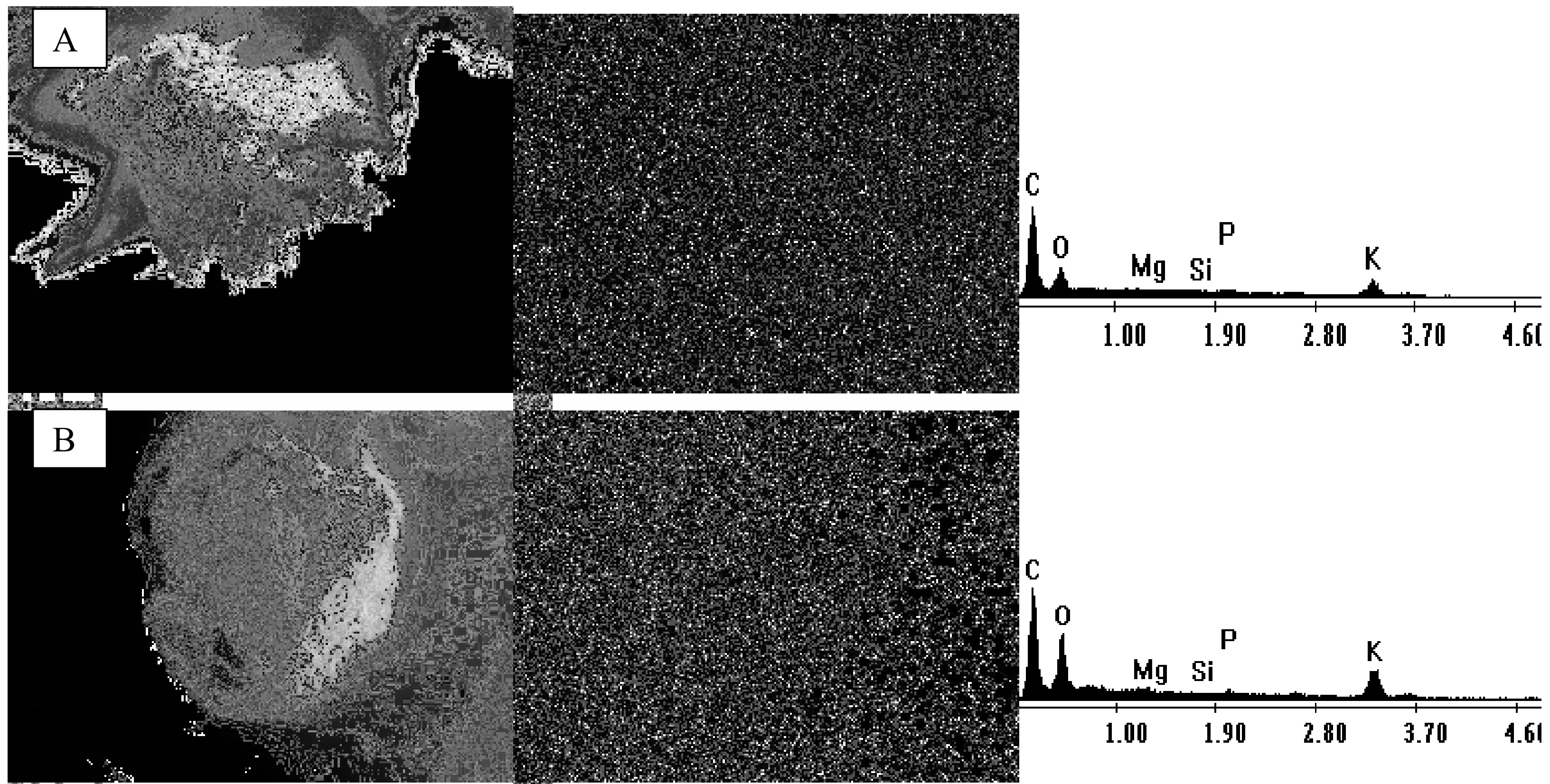

Figure 5.4. Cross-section micrograph and EDX dot maps of roots from sunflower seedlings (*Helianthus annuus*) treated with: (A) 0, and (B) 100 mg ℓ^{-1} of silicon (Si), in a hydroponic system. Concentrations of white dots show the concentration of Si in roots epidermal cells.

5.4 DISCUSSION

Silicon nutrition has several beneficial effects on plant growth largely due to its unique physiological role (Takahashi *et al*., 1990). Adatia and Besford (1986) reported a number of positive effects of Si on the growth of cucumber plants such as increased leaf thickness, increased dry matter per unit area of leaf, a small but significant added increment in root fresh and dry weight, and a reduced propensity of leaves to wilt (Tariq *et al.*, 2002).

Seedlings treated with Si showed different Si contents in their tissues (leaves > roots and stems). More Si is usually found in leaves than in both stems and root tissues in most plants (Tariq *et al.*, 2002). Jones *et al*. (1963), also reported similar patterns of Si distribution among vegetative parts of oats. This pattern is probably due to the greater transpiration activity of leaves compared to either stems or roots (Gali and Smith, 1992). Soni *et al.* (1971) also reported that Si accumulator plants, i.e., rice, wheat and barley continue to actively take up Si after the beginning of silicification and thus the shoot accumulates more Si than roots. However, Si concentrations in the leaves declined when Si application levels were raised above 400 mg ℓ^{-1}. This could be due to the reduced ability of the plant to respond to increases in soil Si levels (Kidane, 2008; Tesfagiorgis, 2008).

The accumulation of large quantities of Si in a cell appears to alter the accumulation of other elements (Soni *et al.,* 1971). Previous studies have suggested that Si reduces other nutrient uptake through reduction in transpirational bypass flow (Gong *et al*., 2006). In addition, Soni *et al.* (1971) reported that it is equally possible that Si acts as an ion absorbed and therefore keeps other elemental ions out of solution and hence excludes them from plants, resulting in stunted plants.

High deposition of Si in leaves results in reinforced cell walls and trichomes, thus, stimulate photosynthesis by improving light interception by keeping leaves erect,

and decrease susceptibility to disease and insect damage. In stem, Si reinforces the cell walls, thereby enhances its strength and rigidity and increases the resistance to lodging. Apoplastic bypass flow in roots can be reduced by presence of Si in roots, thus provides binding sites for metals, resulting in decreased uptake and translocation of toxic metals and salts from the roots to the shoots. However, since application of Si is associated with increase in pH in soils, at high soil pH levels, the uptake of nutrients such as nitrogen are limited, resulting in reduced plant growth and development (Tesfagiorgis, 2008). Therefore, in order to fully reap all the benefits associated with Si fertilization one needs a better understanding of its effects on the accumulation of other elements required for plant growth and development in order to avoid side effects and unwanted results.

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DISSERTATION OVERVIEW

Sunflower (*Helianthus annuus* L.) is grown worldwide for oilseed production and as food crop for the home and bird food markets. Its oil is also of great commercial importance in Australia, China, India, South Africa (SA) and Turkey (Semelczi-Kovacs, 1975). Along with other vegetable oils, it has potential value for use in the production of agrichemicals, surfactants, plastics and plastic additives, synthetic lubricants, coatings and other products (Robbelen *et al.*, 1989). Sunflower oil has also been researched as a potential diesel fuel substitute (Heiser, 1976). Unfortunately, the crop is particularly sensitive to some soil-borne pathogens during emergence and this problem often leads to poor or erratic plant density. Some of the most important diseases of sunflower in SA are *Sclerotinia* wilt (*Sclerotinia sclerotiorum* De Bary), damping-off (*Rhizoctonia solani* Kuhn), rust (*Puccinia graminis* Pers) and downy mildew (*Plasmopara viticola* Berk) (Sneh *et al.,* 1996; Berlin and Arthur, 2000).

The two soil-borne fungi, *R. solani* and *S. sclerotiorum* are ubiquitous plant pathogens with wide ranges of host plants. They are among the most widespread and destructive diseases of many crop including sunflowers.

The potential benefits of *Trichoderma harzianu*m Rifai and silicon (Si) nutrition in plants have been extensively reviewed. *Trichoderma* spp. are common fungi, present in substantial quantities in nearly all agricultural soils. *Trichoderma* can inhibit a pathogen by utilizing different modes of action, i.e., competition, mycoparasitism and antibiosis (Ridout *et al.,* 1986; Cotes *et al.,* 1994; Inbar and Chet, 1995). Silicon has been reported to have a suppressive effect on both soil-borne and foliar fungal diseases of plant species (Datnoff *et al.*, 2001). The two most common theories that have been presented are that Si accelerates the defense system resulting in the release of phytoalexins and induces biochemical defense mechanisms in plants or by forming a layer or sheath immediately below

the cuticle which acts as a physical barrier to hinder hyphal penetration of fungi and prevent infections (Fawe *et al.,* 1998; Epstein, 1999; Datnoff *et al.,* 2001). Other elements, i.e., Cu and Ma, have been reported to have the fungitoxic/suppressive ability to fungal growth (Kidane, 2008).

The research presented in this dissertation aimed to develop an integrated pest management (IPM) programme for the two major soil-borne pathogens of sunflower (*R. solani* and *S. sclerotiorum*) focusing on the use of *T. harzianum* (Eco-T®), Si, and their combination.

The assessments on fungal response to Si on PDA showed that *R. solani* and *S. sclerotiorum* were inhibited in the presence of Si *in vitro*. More inhibition was observed as the Si concentration increased with a relative increase in pH. Maximum growth inhibition was observed at 3000 mg ℓ^{-1} – 6000 mg ℓ^{-1} of PDA where no differences in inhibition between the two pathogens was observed, thus confirming the fungitoxic/suppressive ability of high Si concentrations to fungal growth as do elements such as Cu and Mn.

When the effect of pH on fungal growth was assessed, by raising the pH of PDA to similar pH levels which prevail due to the addition of Si in PDA media i.e. >3000 mg ℓ^{-1} of PDA, results showed that pH did contribute to the inhibition of fungal growth when compared to unameliorated PDA, but was not solely responsible in the presence of Si.

From *in vitro* antagonism bioassays it was found that Eco-T® exhibited antifungal activity against the tested pathogens resulting in significant inhibition of mycelial growth by dual culture tests. Volatile and non-volatile compounds produced by Eco-T® also inhibited both fungi. This suggests that mechanisms of both action and production of other types of antifungal compounds were involved in the antagonism status of Eco-T®. However, there is still a need for field-testing to

evaluate its efficacy under field conditions since biological agents are affected by biotic and abiotic factors.

Since both Eco-T® and Si exhibited antifungal activity against the tested fungi, *in vitro* bioassays were further tested under greenhouse conditions with percentage germination and shoot dry weight used as parameters to evaluate their efficacy. *In vivo* trials showed that Si applied at 200 mg ℓ^{-1} weekly, significantly increased the dry weight of sunflower plants inoculated with *R. solani* and *S. sclerotiorum*. Integration of Si and Eco-T® significantly increased percentage germination, number of leaves and head dry weight of some sunflower cultivars tested. Furthermore, rhizotron studies showed that *S. sclerotiorum* infected the host through the roots and the stem whereas *R. solani* only infects the host through the roots.

A study on Si uptake showed that sunflower accumulates Si in various plant tissues. Both techniques used (Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Environmental Scanning Electron Microscopy (ESEM-EDX)) in Si analysis of plant tissues showed that more Si was accumulated in leaves> stem and roots, with Si levels in leaves significantly higher than in stem and roots. In addition, Si was also found in plant parts grown in silicon-free media. This may be caused by the presence of Si in pine bark as Si is abundant in most soils (Epstein, 1999; Marschner, 1995; Datnoff *et al.,* 1997).

In conclusion, Si alone could be used to increase growth but was unable to control *R. solani* and *S. sclerotiorum.* However, together with Eco-T®, it provides an environmental friendly alternative for the control of *R. solani* and *S. sclerotiorum* with enhanced plant growth and yield. Eco-T® protects seed from attack by *R. solani* and *S. sclerotiorum* during the period when the roots are taking up Si from the soil. This study presents the basis of investigating the efficacy of Si and Eco-T against other soil-borne pathogens under field conditions.

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