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**Biological control of the soilborne pathogen *Rhizoctonia solani*  
in Asian cabbage**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Master of Horticultural Science (Plant Protection)

at  
Lincoln University  
by  
Helen Moe David

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Horticultural Science

**Biological control of the soilborne pathogen *Rhizoctonia solani* in Asian cabbage**

**By**

**Helen Moe David**

Brassicas are common vegetable crop in most parts of the world, including Papua New Guinea (PNG). However, pathogens can affect vegetable brassica production. *Rhizoctonia solani* is a serious fungal soil-borne pathogen that causes damping off and bottom rot of emerged seedlings. It attacks the hypocotyl or lower stem tissue of the plant and causes wire stem. In severe infections the stem separates from the root and dies. Asian cabbage (Pak Choi) is widely grown in PNG, but the effects of *R. solani* on its production, or methods for control to the pathogen are not known. This research programme investigated the efficacy of biological control methods to manage *R. solani* in Asian cabbage. Two New Zealand strains of *R. solani* (043-1 and 043-4) were evaluated for their virulence on Asian cabbage, with strain 043-4 being identified as more virulent and used in subsequent experiments. Four *Trichoderma* isolates (*T. atroviride* LU132, *T. hamatum* LU785, *T. harzianum* LU1347, and *T. polysporum* LU1358) from the Lincoln University culture collection were formulated into a seed coating and tested for their biocontrol potential in comparison with that of the seed treatment fungicide Apron XL using soil naturally infested with *R. solani*. *T. hamatum* LU785 significantly improved seedling emergence and survival, while *T. atroviride* LU132 enhanced plant growth parameters in a study. A further trial compared the effectiveness of *T. hamatum* LU785 and a ground mustard (*Brassica juncea*) biofumigation applied as seed treatments. Both treatments increased seedling emergence in the presence of the pathogen and increased early plant growth.

Additionally, the biofumigation properties of glucosinolates from Brassicaceae species were assessed in the glasshouse. The incorporation of Frozen cauliflower tissues into the growing medium provided better control of the pathogen and allowed greater plant growth than fresh or frozen tissues of other brassica species.

This study has identified the potential for using *Trichoderma* spp. and biofumigation to reduce the impact of *R. solani* on the production of Asian cabbage, but these results require confirmation in field studies.

**Keywords:** Pak Choi, *Trichoderma* species, biofumigation, *Rhizoctonia solani*, seed coating

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# Chapter 1: General Introduction

## 1.1 Introduction

Pak Choi (*Brassica campestris* subspecies *pekinensis*), also known as Chinese cabbage, is a vital leafy vegetable widely cultivated for its nutritional value and culinary versatility (Podsędek, 2007). The crop originated from China and became popular worldwide (Cartea et al., 2010), due to its health benefits (Jahangir et al., 2009). Pak Choi annual consumption accounts for 30% to 40% of the total annual vegetable consumption in China (Ding et al., 2018).

Pak Choi is well-adapted to the temperate climate of New Zealand, allowing for year-round cultivation, with optimal growth observed during the cooler months. It is a versatile crop for farmers (kingsseeds.co.nz, n.d.). The growing demand for Pak Choi in both domestic and international markets provide economic opportunities for New Zealand farmers. It is a profitable crop due to its relatively short growing cycle and high yield (GardenGrow, 2024).

However, the cultivation of Pak Choi faced challenges from soil-borne pathogens, particularly *Rhizoctonia solani*. *R. solani* is a necrotrophic fungus responsible for various plant diseases, including damping-off, root rot, and stem cankers, which can lead to substantial yield losses (Ajayi & Bradley, 2018). This pathogen affects a wide range of crops globally, making it a critical concern for sustainable agriculture (Senapati et al., 2022).

Growers face a continuous problem as a result of substantial yield losses, poor crop quality, and increased production costs caused by *R. solani* (Urdukhe & Mogle, 2024). Chemical fungicides are commonly used to manage *R. solani* by disrupting the cell metabolic processes and reduces the pathogens ability to infect plants (Babli & Tiwari, 2022). However, the excessive use of chemicals poses a threat to both the environment and human health (Punia et al., 2023). Overuse can also lead to fungicide resistance (Institute for Agriculture and Trade Policy, 2015), resulting in further environmental damage.

To combat the detrimental effects of *R. solani*, biocontrol methods have gained attention as environmentally friendly alternative methods such as the biological control methods.

Trichoderma spp. is a saprotrophic fungus (Abbas et al., 2022) known for their ability to suppress plant pathogens through mechanisms such as mycoparasitism, competition for nutrients, and the production of antifungal compounds (Alizadeh et al., 2024). These fungi

not only protect plants from diseases but also promote plant growth and enhance soil health (Yao et al., 2023a).

Biofumigation is another biocontrol method, which involves the incorporation of glucosinolate-containing Brassica plant materials into the soil as green manure or seed meals (Abdallah et al., 2020), which release bioactive compounds such as isothiocyanates (ITCs) upon decomposition (Santos et al., 2021). These compounds have been shown to possess strong antimicrobial properties, effectively reducing pathogen populations in the soil (Gimsing & Kirkegaard, 2009).

## 1.2 Objectives

(1.) To determine the effect of *Rhizoctonia solani* on Asian Cabbage (*Brassica. campestris* subspecies *pekinensis*) production. (2.) To identify the potential of four *Trichoderma* strains for biocontrol of *R. solani*. (3.) To formulate a brassica-based seed coating and compare its ability to control the pathogen against that of the commercial fungicide seed treatment product in a soil infested with *R. solani*. (4.) To investigate the potential of biofumigation with brassica plant material (fresh and frozen tissues) for control of *R. solani*. (5.) To compare the effect of *Trichoderma* strains and the brassica-based seed treatment as biocontrol agents for *R. solani* in a field trial.

## 1.3 Hypotheses

1. Soil-borne *Rhizoctonia solani* will decrease Pak Choi production by negatively affecting Pak Choi seedling emergence and plant growth.
2. *Trichoderma* seed coating and brassica-based seed and plant treatment will promote Asian cabbage growth and effectively control the pathogen.

## Chapter 2: Literature review

### 2.1 Background information on brassica crops

Brassicas are a common vegetable crops in most parts of the world, but only a few species are grown in the hot, humid tropics. They originated in the Mediterranean region and were edible vegetables in ancient Greece (approximately 2000 to 2500 BC). The main vegetable brassica species are *Brassica oleracea* L, *B. campestris* L., *B.napus* L. and *B. Juncea* L. (George, 1999). Brassica crops have various uses and are important around the world. They provide leaves, flowers and roots that can be eaten fresh, cooked, or processed. Their oils and proteins can be used as industrial lubricants. Some species are also used as forage crops for animals and as cover crops to suppress pests and pathogens (Dixon, 2017).

### 2.2 Economic importance

Asia has a high demand for vegetable brassica crops. In 2003 Asia consumed more than 70% of the worlds brassica supply which means around 540 million tonnes, followed by Europe with 85 million tonnes. However, Greece had the highest per capita consumption (275 kg per person per year), while China was close behind with 270 kg (FAO, 2009). Brassica crops are in high demand due to their nutritional value but also because they contain antioxidant compounds that are beneficial to pest and pathogen management in vegetable production (Cartea et al., 2011)

New Zealand is one of the world's leading producers of a diverse range of brassica seed. Brassica seed grown in New Zealand is recognized for its quality and is a key export crop for this country. New Zealand produces approximately 5,500 tonnes of brassica seed per year, 60% of which is vegetable and 40% forage species. Two-thirds of New Zealand's brassica seed production is exported to Asia and Europe (primarily vegetable seeds) as well as Australia, the United States of America, and the United Kingdom (primarily forage seeds) (Van Zijll De Jong et al., n.d.). The increase in brassica production has resulted in increased disease and pest pressures.

### 2.3 Brassica production in Papua New Guinea

Temperate vegetables are grown successfully in the highland regions of PNG. Cabbage is grown by a quarter of the rural population, with most production in the highland's provinces and in the mountains of Morobe Province. The annual production of brassica crops in PNG is approximately 100 tonnes (Bourke et al., 2009). The main varieties grown are round cabbage, broccoli, cauliflower, lettuce, and Chinese cabbages (*B. campestris* L. sub species *pekinensis*). Mustard is not grown very widely by farmers due to its bitterness in taste. Brassica crops are grown generally for own consumption and for generating income. According to Haguluha and Natera (2007) cabbages transported from the highlands region to urban markets are worth K42 (PNG Kina) per 60kg bag. They are sold on market shelves for K2-K3 per kilogram (Pacific Horticultural Market, 2016).

### 2.4 Pathogens of brassica crops in New Zealand

Plant diseases threaten both seed and vegetable Brassica crops in New Zealand. Common fungal diseases of Brassica include; Clubroot (*Plasmodiophora brassicae*), Alternaria leaf spot (*Alternaria brassicae*, *Alternaria brassicicola*), Black leg/Phoma rot (*Phoma lingam*), Downy mildew (*Peronospora parasitica*), Light leaf spot (*Pyrenopeziza brassicae*), Damping-off, (*Rhizoctonia solani*), Ring spot (*Mycosphaerella brassicicola*), Sclerotinia rot (*Sclerotinia sclerotiorum*, *Sclerotinia minor*). Bacterial diseases are Black rot (*Xanthomonas campestris* pv. *campestris*), Bacterial soft rot (*Erwinia carotovora*), Head rot (*Pseudomonas fluorescens*, *Pseudomonas marginalis*) and Peppery spot (*Pseudomonas syringae* pv. *maculicola*). Viral diseases are cauliflower mosaic virus, beet western yellow virus and turnip mosaic virus (Ekman et al., 2014; NZ-Plant&FoodResearch, 2016).

### 2.5 Pathogens of brassica crops in Papua New Guinea

In PNG pathogens also affect vegetable production. According to Price and Munro (2009) *Macrophomina phaseolina*, *Fusarium equiseti*, *F. semitectum* and *F. moniliforme* were the main pathogens found affecting vegetable crops mainly on tomatoes, bean and cabbages. In addition, current research at PNG University of Technology has also indicated the presence of *Rhizoctonia solani* and *F. oxysporum* in PNG (Ban et al., 2022). In 2018 leaf spot disease caused by *Alternaria brassicicola* on Broccoli was identified up in the highlands region of PNG (Britto de & Jogaiah, 2020). The pathogenicity of these organisms was shown to be dependent on soil type, depth of sowing and inoculum density (Gurr et al., 2016). However,

there is limited information on soil borne pathogens in PNG due to a lack of research and publications.

## **2.6 Biology of the pathogen *Rhizoctonia solani***

*Rhizoctonia solani* is a seed and soil borne plant pathogenic fungus with a wide host range. This fungus affects hundreds of vegetable, field, fruit and nut, and ornamental crops. The fungus's binomial name is derived from the Greek words rhiza (root) and ktonos (murder). *R. solani* was discovered on potato in 1858 and is currently found throughout the world (Ceresini, 1999). *Rhizoctonia* is the genus name that refers to a group or complex of fungi. Fungal species were placed in this *Rhizoctonia* group because they share certain features such as their reproductive phases.

*Rhizoctonia* species have two life cycles: anamorph( asexual state) and teleomorph (sexual state). *R. solani* has the basidiomycete *Thanatephorus cucumeris* as its sexual stage which produces basidiospores and does not produce conidia which are the asexual spores (Pegg & Manners, 2014). According to Koike & Subbarao (2015) the sexual stage of *T. cucumeris* is rarely found in the field and probably doesn't cause disease in plants. However, the asexual stage, infects plants and causes diseases.

*R. solani* is an important soil borne pathogen of horticultural and field crops. The host range includes cabbage, lettuce, bean, rice, radish, buckwheat, tomatoes, radish, onion, sugar beet, ginger, pea, corn, potatoes, and sorghum. Soil-borne *R. solani* can cause crop damage at various stages of growth, including seed rot, seedling damping off, wilting/stunting of leaves and root rot and cracking (Lee,2018).

### **2.6.1 Hyphal anastomosis**

*R. solani* species are extremely diverse and can be divided into distinct groups. Different isolates of *R. solani* fall into one of the many anastomosis groups or AGs. There are currently 14 AGs: AG1 to AG13 and AGB-1 which have been identified based on the fusion of hyphae, morphology, virulence (pathogenicity), physiology, and DNA homology (Koike & Subbarao, 2015). Seven of these AGs are further divided into subgroups based on anastomosis frequency, physiological and morphological features and pathogenic, bimolecular, biochemical, genetic, and DNA homology characteristics. For example, AG-1 has been divided into six subgroups: IA, IB, IC, ID, IE, and IF. Similarly, AG-4 has been divided into three subgroups: HGI, HGII, and HGIII and AG-2 has been divided into nine subgroups 1, 2, Nt,

2IIIB, 2IV, 2LP, 3 and 4. AG-2, AG-4, AG-5, AG-3, AG-7, and AG-11 cause damping-off and hypocotyls rot, whereas AG-1 is responsible for the foliar and web blight of soybean (Abbas et al., 2022). Das et al. (2014) reported three AGs from *R. solani* collected from infected potato tubers in New Zealand: AG-3PT, AG-2-1 and AG5.

### 2.6.2 Symptoms

Pairing of the same AG isolate types or same genotype can occasionally result in hyphal fusion. The following AGs are known to infect *Brassica* crops around the world ; AG-2-1, AG-2-2 and AG-4 (Budge et al., 2009). On *Brassica* crops, *Rhizoctonia* causes two types of disease symptoms: damping-off (or wirestem) and bottom rot.

Damping-off or wirestem occurs on emerged seedlings. It is also a serious seedling disease of other vegetable crops grown in glasshouses. In the soil *R. solani* attacks the hypocotyl or lower stem tissue of the plant. This causes browning and cracking of the epidermis and the formation of lesions. The outer stem decays as the infection spreads, leaving the fibrous inner xylem which is known as "wirestem". Plants that are affected wilt, turn purple and remain stunted. Some seedlings may break off at the soil line.

Bottom rot is most common on cabbage, Bok choy, and Chinese cabbage. Once head formation begins, *R. solani* can infect lower leaves that are in contact with soil. The first symptoms are irregular brown-black lesions on the leaves and sometimes a wet decay of the base of outer leaves. Lesions expand until entire heads rot. In 2007 an outbreak of head rot of cabbage (*B. oleracea*) occurred in China, reducing yields by 50% (Zhang et al., 2008).

### 2.6.3 Life cycle

*R. solani* is a fungal pathogen that survives in infected seeds or soil via sclerotia and mycelia. Infection occurs when sclerotia or hyphae are attracted to chemical exudates from brassica crops or any plant hosts. This stimulates the production of hyphae from overwintering mycelia. After contact, fungal hyphae spread over the surface developing infection cushions and pegs. The hyphae enter plant tissues and absorb nutrients for continued fungal development. Natural openings (lenticels, stomata) or wounds can also allow penetration (Pegg & Manners, 2014).

In the soil, the pathogen survives as resting structures called sclerotia or as hyphae on debris or weeds. *R. solani* can survive for long periods without a host on crop debris. The optimum

temperature for growth and production of sclerotia is between 20 to 30°C and the optimum relative humidity is 80 -100 %, whereas sclerotia germination is restricted at 5°C (Hemalatha & Singh, 2019; Senapati et al., 2022). Excess nitrogen application increases disease severity and yield loss. The pathogen spreads up to 20cm per day under favourable field conditions. Sclerotia and mycelia disperse through irrigation runoffs and rainfall, contaminated equipment, and footwear. Infected seeds can introduce the pathogen to new areas (Pegg & Manners, 2014).

## **2.7 Disease management practices applied in PNG**

Soil borne pathogens are common in warm environments including PNG. The vegetable crops in PNG are not well managed. Subsistence growers are still reliant on traditional practices. These cultural practices include 'slash and burn' production in which crops are established on newly cleared land (Core, 2006). However, vegetable crops are often grown in a small production unit (garden) as a series of consecutive crops for multiple years rather than as an annual crop rotated among multiple fields as in developed countries (Gurr et al., 2016). Village production of food and export crops uses negligible amounts of herbicides, insecticides, or fungicides. Small amounts of insecticide are applied by a few people to temperate-climate vegetables, particularly brassicas, and very small quantities of herbicide are used by some villagers (Bourke & Harwood, n.d.). However, recently experiments were conducted in the laboratory, greenhouse, and field at the Papua New Guinea University of Technology (PNGUT) to assess the efficacy of the biocontrol fungus *Trichoderma harzianum* against *Rhizoctonia solani* and *Fusarium oxysporum* on bean and tomato. The results were promising, and the outcomes of this study will form the basis for further investigation into the potential routine use of *Trichoderma* spp. as biological control agents against soil-borne pathogens in PNG (Ban et al., 2022).

## **2.8 Biological control**

It is difficult to control the pathogen *R. solani*. Overuse of chemicals in agriculture has a detrimental effect on both the environment and human health. There is also the risk of fungicide resistance build-up from overuse with consequent environmental damage. On the other hand, the demand for safe food production and sustainable farming is increasing. Therefore, introducing biocontrol agents for pathogens is one of the alternative methods that could be used to control soil borne pathogens.

### **2.8.1 Brassicas for biocontrol**

The use of brassica crop residues and seed meal to manage soilborne disease may be an alternative disease control strategy. Several studies have indicated that some Brassica species such as canola (*Brassica napus*) and mustard (*Brassica juncea*) have the potential to suppress soil borne pathogens including *R. solani* (Ascencion et al., 2015)

The bio fumigation effect of *B. juncea*, used as dry plants, seed meal, seed powder and fresh plants (at the vegetative and flowering stages), against *R. solani* on tomato plants was investigated. Seed meal was the most effective, followed by the seed powder, fresh plants at the flowering stage then fresh plants at the vegetative stage (Ascencion et al., 2015; Cochran & Rothrock, 2015). The results show that mustard (*B. juncea*) could be used to help control the fungus, not only as a common green manure but also as a defatted seed meal.

### **2.8.2 Chemical compounds in brassica crops**

The application of plant secondary metabolites is another aspect of current sustainable methods for managing soil-borne plant diseases. Studies using brassica crop residues and brassica seed meal treatments have shown good results in suppressing pathogens in the soil. The suppressive effects are attributed to compounds such as glucosinolates (GSLs) that are released from brassica crops. These compounds are hydrolysed by myrosine, an isoenzyme contained in the same plant producing numerous biological active compounds including isothiocyanates, ionic thiocyanate, organic cyanides and glucose (Ascencion et al., 2015; Chung et al., 2002).

Ally isothiocyanate was one of the active compounds identified to be toxic and mustard (*Brassica. juncea*) was identified as having a high amount of the compound (Chung et al., 2002; Cochran & Rothrock, 2015). During decomposition, isothiocyanates are degraded in the soil. This has a negative impact on soil borne pathogens and is known as bio fumigation. The field bio fumigation technique is now used in several countries e.g., USA, Australia, Italy and the Netherlands (Shahnaz et al., 2022).

### 2.8.3 *Trichoderma* spp

*Trichoderma* spp. have been shown to be effective biocontrol agents in agriculture and horticulture around the world. They are commonly found in both soil and inorganic matter. *Trichoderma* produces a range of secondary metabolites that can boost plant growth and suppress soil-borne pathogens (Caporale et al., 2019). *Trichoderma* species have been found to show five primary mechanisms of action. They are mycoparasitism through hydrolytic enzyme secretion, competition for nutrients and space, antibiosis by the production of secondary metabolites, stimulation of plant growth and plant systemic resistance reactions (Caporale et al., 2019).

There are about 340 species of *Trichoderma* described so far. *Trichoderma* species *T. atroviride*, *T. asperellum*, *T. polysporum*, and *T. harzianum* are the most frequently used as biological agents (Zin & Badaluddin, 2020). Lee (2018) assessed the ability of 21 strains of 8 New Zealand *Trichoderma* spp to control *R. solani* on radish and found that isolates LU132 (*T. atroviride*), LU785 (*T. hamatum*), LU1347 (*T. harzianum*) and LU1358 (*T. polysporum*) significantly reduced *R. solani*. These four selected *Trichoderma* strains were formulated into seed-coatings and their performance was evaluated in vitro and in vivo using soil naturally infested with *R. solani*. *Trichoderma* seed treatment significantly increased radish production by reducing the impact of *R. solani* (Lee,2018). This method of biocontrol of this pathogen could be readily adopted by small farmers in developing countries.

## **Chapter 3: Determining *Rhizoctonia solani* inoculum rate for glasshouse experiments.**

### **3.1 Introduction**

Pak Choi (*Brassica chinensis* L.) originated from China and is one of the most important Brassica vegetables grown in many areas of the world. It is an important vegetable crop in Papua New Guinea. A number of plant pathogens, including *Rhizoctonia solani*, attack Brassicas around the world. *R. solani* also attacks members of the Poaceae (e.g. maize, rice, wheat, barley, oat), Fabaceae (e.g. soybean, peanut, dry bean, alfalfa, chickpea, lentil, field pea) and Solanaceae (e.g. tobacco, potato) families (Ajayi & Bradley, 2018). Symptoms on diverse hosts include seed rot, root rot, hypocotyl rot, crown rot, stem rot, limb rot, pod rot, stem canker, black scurf, seedling blight, and pre- and post-emergence damping off (Ajayi & Bradley, 2018).

When evaluating the ability of biocontrol agents to protect seedlings from *R. solani* in glasshouse experiments, a decision needs to be made as to the amount of pathogen inoculum to be added to the growing medium. Kandula, et al. (2015) suggested that an inoculum rate which resulted in 50% diseased seedlings was an appropriate rate for this purpose. Two isolates of *R. solani* AG 2-1 (043-1 and 043-4) were kindly provided by Plant and Food Research, Lincoln from their culture collection. The inoculum rate required to produce approximately 50 % diseased seedlings was assessed in a pot trial. The effect of these pathogen rates on subsequent plant performance for two Asian cabbage varieties was also assessed.

### **3.2 Materials and methods**

#### **3.2.1 Culturing of *R. solani* isolates on PDA**

Potato Dextrose Agar (PDA) was used to culture colonies of *R. solani*. The PDA was prepared according to the manufacturer's instructions (Appendix 1). The PDA (18ml) was poured into petri plates and left to solidify for 20 – 25 minutes in a laminar flow cabinet before the lids were placed on top of the plates. The plates were then stored in plastic bags in the dark at 4 °C until required. From the two isolates of *R. solani* AG1 growing on agar petri plates, a colonized agar block (5mm diameter circle) was cut from each of the *R. solani* isolates using a

sterilized scalpel and placed in the centre of a fresh PDA plate (Figure 3.1). The lids were placed on top of the petri plates, sealed with parafilm, and the plates were placed in an Contherm Biosyn 6000CP incubator with a 12-hour light and dark cycle at 25°C for 7 days.

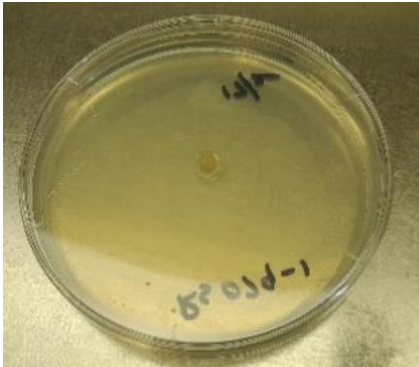


Figure 3.1 Colonized *R. solani* agar block placed on a petri plate.

### 3.2.2 Inoculation of wheat bran and peat media

Wheat bran - peat inoculum (Kandula et al., 2015) was prepared using the following method. One litre each of dried wheat bran and peat were mixed with one litre of tap water (ratio of wheat bran: peat: water = 1:1:1) and 200g of the medium was placed in six 1 litre bottles and autoclaved for 15 minutes at 121 °C, at 15 Psi. After autoclaving, five 5mm diameter circle agar pieces of each *R. solani* isolate were placed into each bottle (Figure 3.2) and sealed. *R. solani* was allowed to multiply in an Contherm Biosyn 6000CP incubator with a 12-hour light and dark cycle at 23°C for 13 days (Kandula, et al.,2015).



a



b

Figure 3.2 Wheat bran and peat mixed with distilled water before autoclaving (a) and *R. solani* inoculated into wheat bran peat media (b).

### 3.2.3 Selection of *R.solani* rate for future glasshouse experiments

#### Potting Mix

Lincoln University Nursery potting (Appendix 2) and seed raising mixes (Appendix 3) were used. A sheet of paper was placed on top of a tray (60 cm x 50 cm x 4 cm) which was filled with 1800 g of seed raising mix (Figure 3.3 a). Four *R. solani* inoculum rates were generated by mixing 0.1 g, 0.25g, 0.5 g and 1 g of *R. solani* inoculated wheat bran-peat media into each amount of 1800g seed raising mix respectively. A control treatment had no inoculum added to the seed raising mix. After mixing, 225g of the *R. solani* inoculated seed raising mix was applied to the top of each pot (Figure 3.3 b) which had been filled with 1000g potting mix. There was a total of 160 pots (2 *R. solani* isolates x 2 Asian cabbage varieties x 5 treatments x 8 replicates). The pots were arranged in a randomized complete block design (RCBD).



Figure 3.3 Seed raising mix was mixed with *R. solani* isolates (a) and 225g was applied to the top of each pot containing 1000g potting mix (b).

#### Seed sowing

Two varieties of Asian cabbage were used, Pak Choi Green Ace F1 and Pak Choi Mei Qing Choi (Figure 3.4). Ten seeds were placed in each pot using tweezers at a depth of 1 cm with approximately 2 to 3 cm between the seeds. The sown pots were placed in a glasshouse with a temperature range of between 10 to 25 °C and a relative humidity of > 40%. The pots were watered daily using a water spray gun.

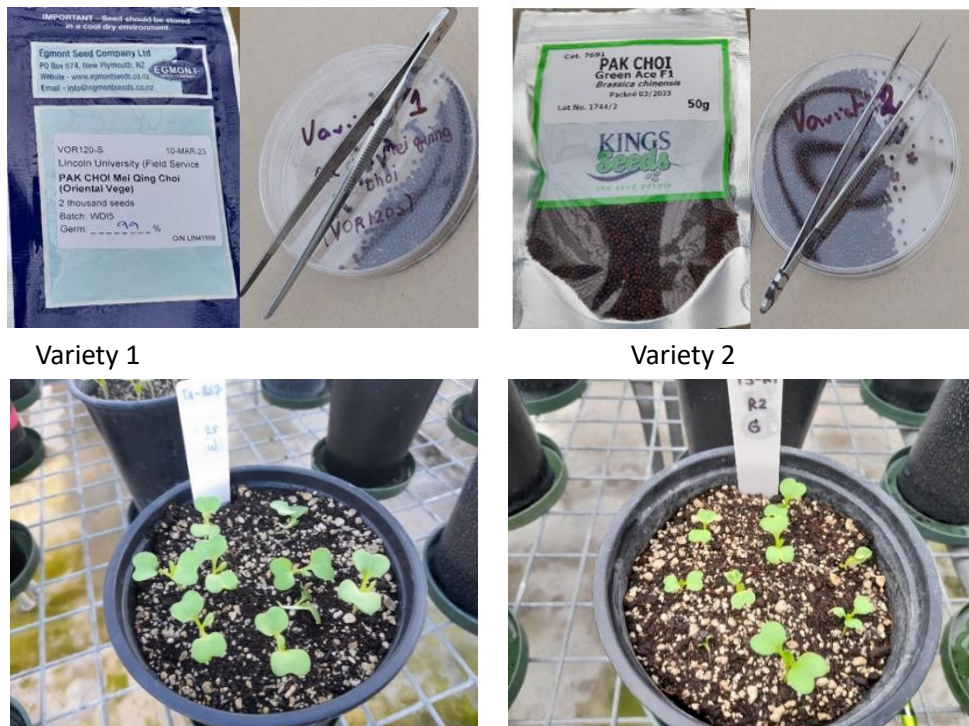


Figure 3.4 Two Asian cabbage varieties (1) Pak Choi Mei Qing Choi and (2) Pak Choi Green Ace F1 at 14 days after sowing in the Horticulture Research Glasshouse, Lincoln University in April 2023

### 3.2.4 Data collection at 20 and 33 days after sowing

The number of healthy seedlings, diseased seedlings, and dead seedlings were counted daily for 33 days after sowing.

#### i) At 20 DAYS

At 20 days after sowing, four of the replicates were used to observe the plant development and *R. solani* damage at the seedling stage (leaf size: approximately 5 cm x 5 cm). The number of live and dead seedlings per pot were counted. The seedlings were carefully extracted from the pots without removing their fibrous roots by gently shaking off the potting mix from the root surface. The seedlings were then scored using a disease score of 0 to 5 according to Rittmo et al. (2008), where 0 = no symptoms, 1 = lesion length < 2.5mm on leaf, hypocotyl or root, 2 = lesion length 2.5mm to 5mm on leaf, hypocotyl or root, 3 = lesions length > 5 mm on leaf, hypocotyl or root, 4 = post-emergence damping off and 5 = plant wilted or dead. (Figure 3.5). Shoot and root length (cm) were measured with a ruler. Shoot length was measured from the hypocotyl to the highest leaf tip and root length was measured from the base of the hypocotyl to the base of the radicle. Fresh weight (g) was recorded using an electronic scale (AND FX - 3000i). The seedlings were then put into a paper bag (size 90 x 205

mm) and dried in an oven at 65°C for three consecutive days and dry weight was recorded using an electronic scale.

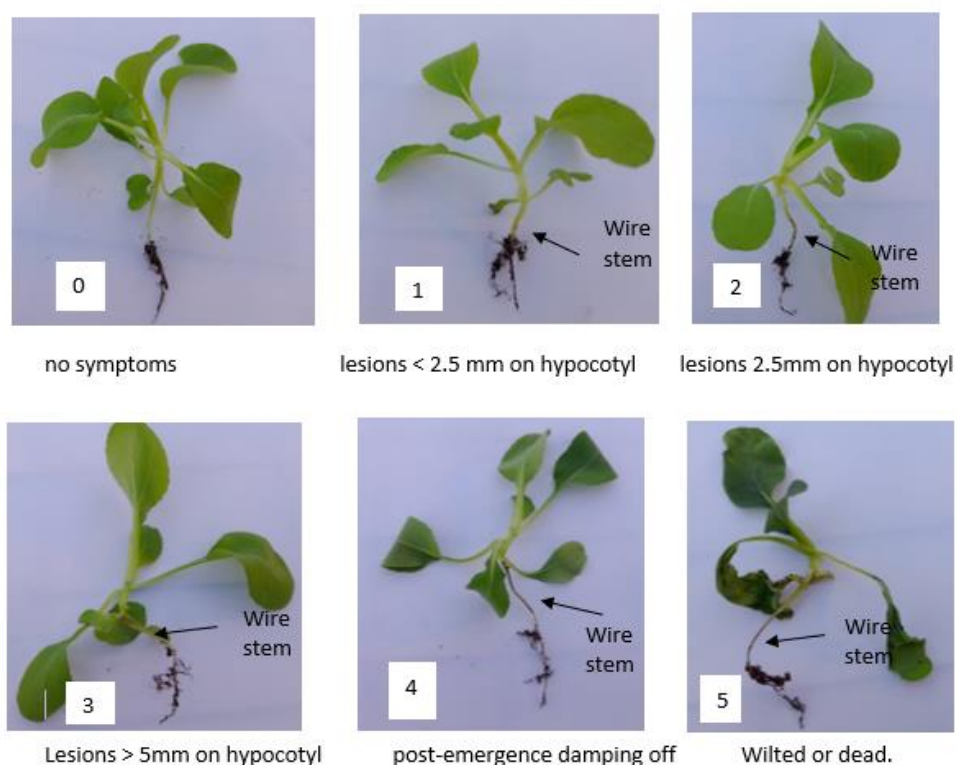


Figure 3.5 Disease score of 0 to 5 on seedlings at 20 days after sowing. Wire stem symptoms on the hypocotyl are indicated by arrows.

## ii) At 33 DAYS

Counts of the number of healthy seedlings, diseased seedlings, and dead seedlings were continued for a further 13 days in the remaining 4 replicates. After 33 days, the remaining seedlings were harvested and the pathogen impact on the harvestable vegetative plant parts was assessed (Figure 3.6). Data recorded were the same as those taken on day 20, with the addition that the leaf surface area was measured using a Li-Cor-3100C<sup>®</sup> leaf area meter.

Data for day 20 and 33 were processed by the One-way analysis of variance (ANOVA) using GenStat (v.22). Significance between treatments was tested using Fishers unprotected least significant difference (LSD) test at the 5% level.



Figure 3.6 Disease score of 0 to 5 on seedlings at 33 days after sowing.

### 3.3 Results

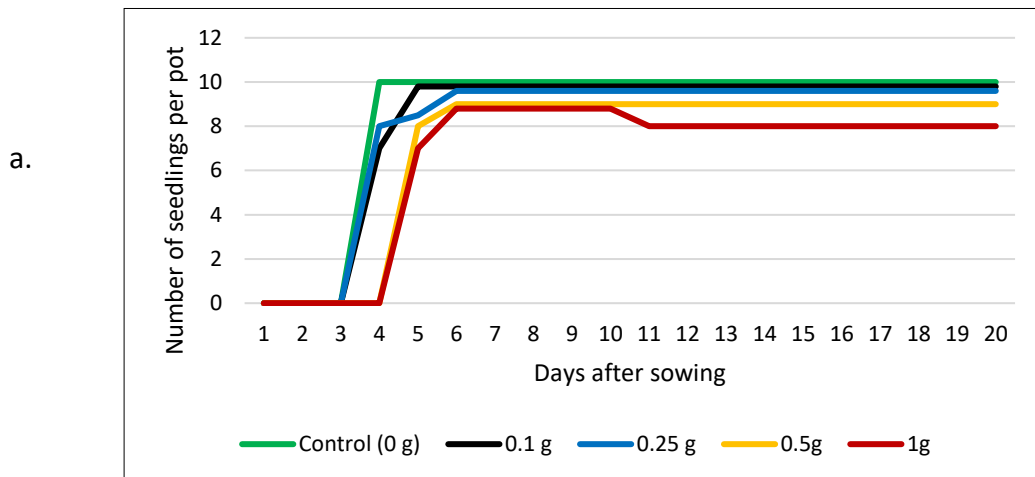
#### 3.3.1 Daily counts of seedling emergence

##### ***R.solani* strain (043-1) with variety Mei Qing Choi & Green Ace F1**

Seedling emergence in variety Mei Qing Choi in the presence of the *R. solani* strain (043-1) occurred between day 4 and 5 after sowing (Figure 3.7 a). By day 6, all treatments had between 8 to 10 seedlings per pot (Figure 3. 7 a). At day 10 the number of seedlings for the highest *R. solani* rate (1 g) was reduced by one, but no further losses occurred. For the control and the other three *R. solani* inoculum rates the number of seedlings remained constant at 9 to 10 per pot till day 20.

Seedling emergence in variety Green Ace F1 in the presence of the *R. solani* strain (043-1) also occurred between days 4 and 5 after sowing (Figure 3.7 b). The control had 10 seedlings per pot and there were no post emergence losses for the two lowest (0.1g and 0.25g) inoculum rates (Figure 3.7, Table 3.1). Seedling losses were greatest in the 0.5g inoculum rate (1 seedling) and 1g rate (4 seedlings) by day 20.

***R. solani* (043-4) & variety Mei Qing**



***R. solani* (043-4) & variety Green Ace F1**

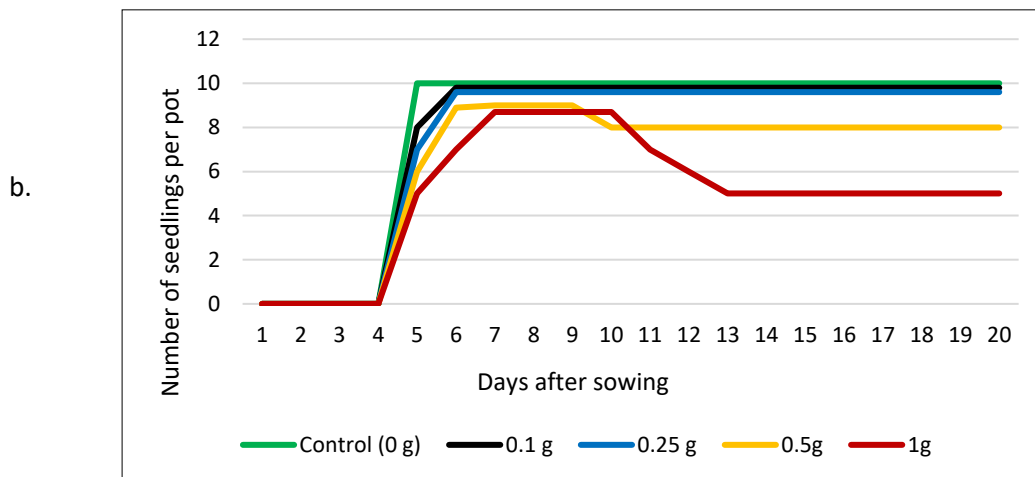


Figure 3.7 The effect of *R. solani* strain (043-1) inoculum rates on the number of seedlings (out of 10 seeds sown per pot) for variety (a) Mei Qing Choi and (b) Green Ace F1 between 1 and 20 days after sowing in the Horticultural Research Glasshouse, Lincoln University.

Table 3.1 Effect of *R. solani* strain (043-1) inoculum rates on Pak Choi seedling performance at 20 days after sowing.

<i>R. solani</i> strain (043-1) inoculum rates (g)	Variety	Maximum number of emerged seedlings	Mean number of diseased seedlings	Mean number of seedlings which died post emergence
0g	Mei Qing Choi	10.0 a	0.0	0.0 a
0.1g	Mei Qing Choi	9.8 a	0.0	0.0 a
0.25g	Mei Qing Choi	9.6 a	0.0	0.0 a
0.5g	Mei Qing Choi	9.0 a	0.0	0.0 a
1g	Mei Qing Choi	9.0 a	0.0	1.0 b
LSD (5%)		1.09	0.0	0.97
Significance		NS	NS	P<0.05
0g	Green Ace F1	10.0 a	0.0 a	0.0 a
0.1g	Green Ace F1	9.8 a	0.0 a	0.0 a
0.25g	Green Ace F1	9.6 ab	1.3 b	0.0 a
0.5g	Green Ace F1	9.2 ab	2.1 b	1.2 a
1g	Green Ace F1	8.6 b	3.4 c	3.6 b
LSD (5%)		1.11	0.83	1.81
Significance		P<0.05	P<0.001	P<0.05

Letters were assigned according to a Fishers 5% level unprotected LSD procedure. Means with the same letter within one variable are not significantly different at P=0.05

Signs of wire stem infection showed on the hypocotyl from day 7 and onwards but only in Green Ace F1 for the three highest inoculum rates. The number of seedlings which died post emergence was greatest at the highest inoculum rate of *R. solani* strain (043-1), but this was more pronounced in variety Green Ace F1 than variety Mei Qing Choi (Table 3.1).

#### ***R. solani* strain (043- 4) with variety Mei Qing Choi & Green Ace F1**

In variety Mei Qing Choi, the control treatment had ten seedlings emerged by day 5 (Figure 3.8 a) and no post emergence seedling death occurred (Table 3.2). Seedling emergence was lower for all *R. solani* inoculum rates (Figure 3.8 a) but only differed significantly from the control for the two highest inoculum rates (Table 3.2). Post emergence seedling death occurred in all four inoculum rates but only the 0.25 and 0.5g rates differed from the control (Table 3.2).

In variety Green Ace F1 the results were very similar to those for variety Mei Qing Choi (Figure 3.8 b, Table 3.2). For both varieties, the higher the inoculum rate of *R. solani* strain

(043-4), the lower the number of seedlings. Maximum seedling emergence decreased, and the number of diseased seedlings and post emergence seedling death increased as the inoculum rate of *R. solani* strain (043-4) increased (Table 3.2). Signs of infection (wire stem) were found on hypocotyls from day 7 onwards. Variety Green Ace F1 had a higher number of diseased and dead seedlings than variety Mei Qing Choi (Table 3.2). The two highest inoculum rates significantly reduced seedling emergence and increased post emergence death (Figure 3.8 b) (Table 3.2).

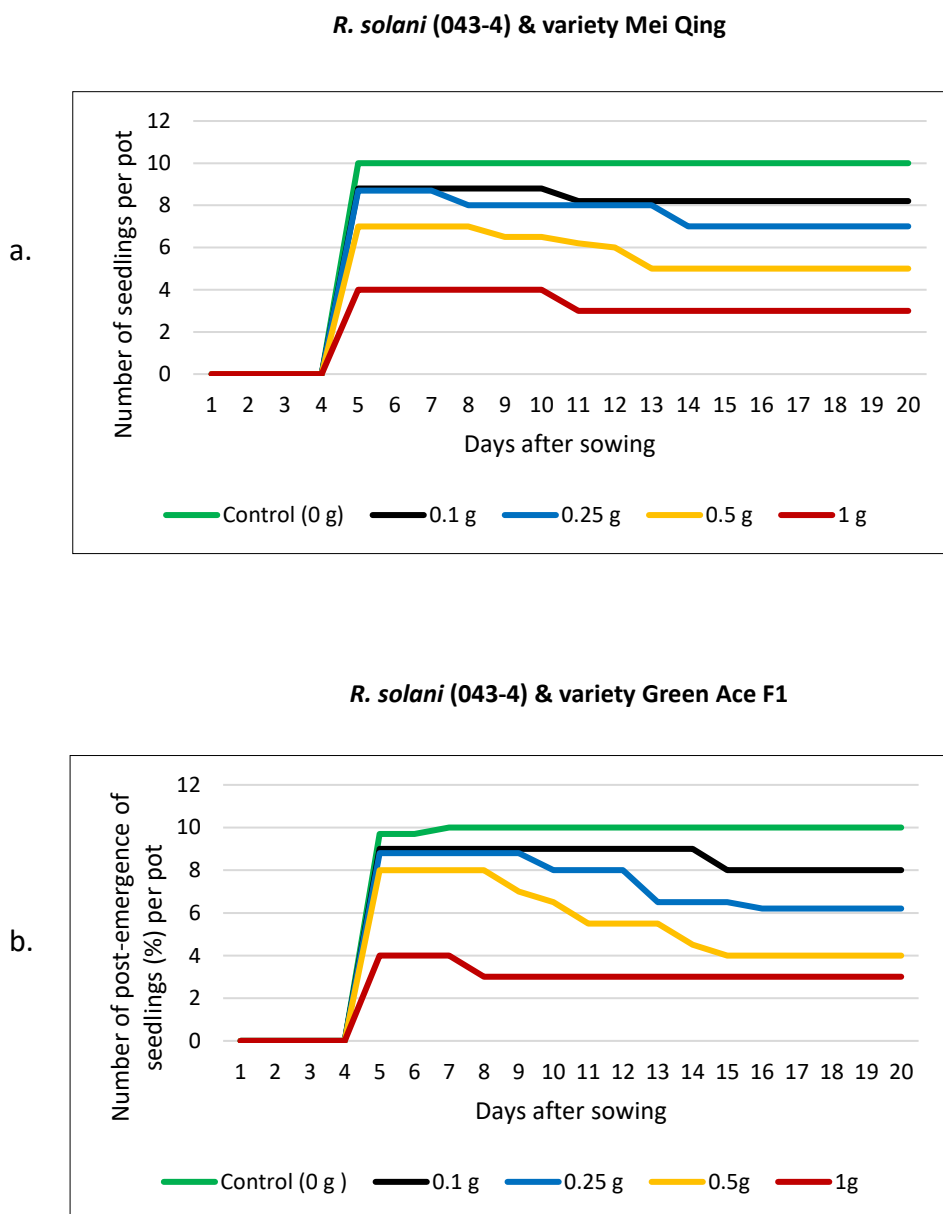


Figure 3.8 The effect of *R. solani* strain (043-4) inoculum rates on the number of seedlings (out of 10 seeds sown per pot) for variety Mei Qing and Green Ace F1 between 1 and 20 DAS after sowing in the Horticulture Research Glasshouse, Lincoln University

Table 3.2 Effect of *R. solani* strain (043-4) inoculum rates on Pak Choi seedling performance at 20 DAS after sowing.

<i>R. solani</i> strain (043-4) inoculum rates (g)	Variety	Maximum number of emerged seedlings	Mean number of diseased seedlings	Mean number of seedlings which died post emergence
0g	Mei Qing Choi	10 a	0.0 a	0.0 a
0.1g	Mei Qing Choi	9.0 ab	2.2 b	1.0 a
0.25g	Mei Qing Choi	9.0 ab	2.4 b	2.0 b
0.5g	Mei Qing Choi	7.2 b	3.3 b	2.2 b
1g	Mei Qing Choi	4.2 c	2.0 b	1.2 a
LSD (5%)		2.35	1.95	1.51
Significance		P<0.05	P<0.05	P<0.05
0g	Green Ace F1	10 a	0.0 a	0.0 a
0.1g	Green Ace F1	9.2 ab	2.1 a	1.2 a
0.25g	Green Ace F1	8.7 ab	4.3 b	2.7 b
0.5g	Green Ace F1	8.0 b	4.0 b	4.0 b
1g	Green Ace F1	4.0 c	1.0 a	1.0 a
LSD (5%)		1.91	2.79	1.80
Significance		P<0.001	P<0.05	P<0.05

Letters were assigned according to a Fisher's 5% level unprotected LSD procedure. Means with the same letter within one variable are not significantly different at  $P = 0.05$

### 3.2.5 Assessment at day 20

#### Number of surviving seedlings

There was a significant difference among the *R. solani* isolate (043-1) inoculum rates ( $P < 0.001$ ) with the 0.5g and 1.0 g inoculum rate having fewer surviving seedlings than all the other treatments (Figure 3.9 a). The mean number of surviving seedlings was significantly lower for Green Ace F1 than for Mei Qing Choi (Appendix 4). There was a significant difference among *R. solani* isolate (043-4) inoculum rates ( $P < 0.001$ ), with the 0.5g and 1.0g inoculum rate having fewer seedlings than all other treatments (Figure 3.9 b). The 1.0g rate had less than the 0.5g rate. The mean number of seedlings did not differ between the two Asian cabbage varieties (Appendix 4). However, there was a significant difference between the two *R. solani* isolates, ( $P < 0.001$ ) as the number of surviving seedlings for *R. solani* isolate (043-4) was lower than for the *R. solani* isolate (043-1) (Appendix 4).

For *R. solani* isolate (043-1) there was a significant interaction between inoculum rates and variety, as for the two highest inoculum rates, seedling survival was lower for Green Ace F1

than Mei Qing Choi and but there was no significant interaction for *R. solani* isolate (043-4) (Appendix 4).

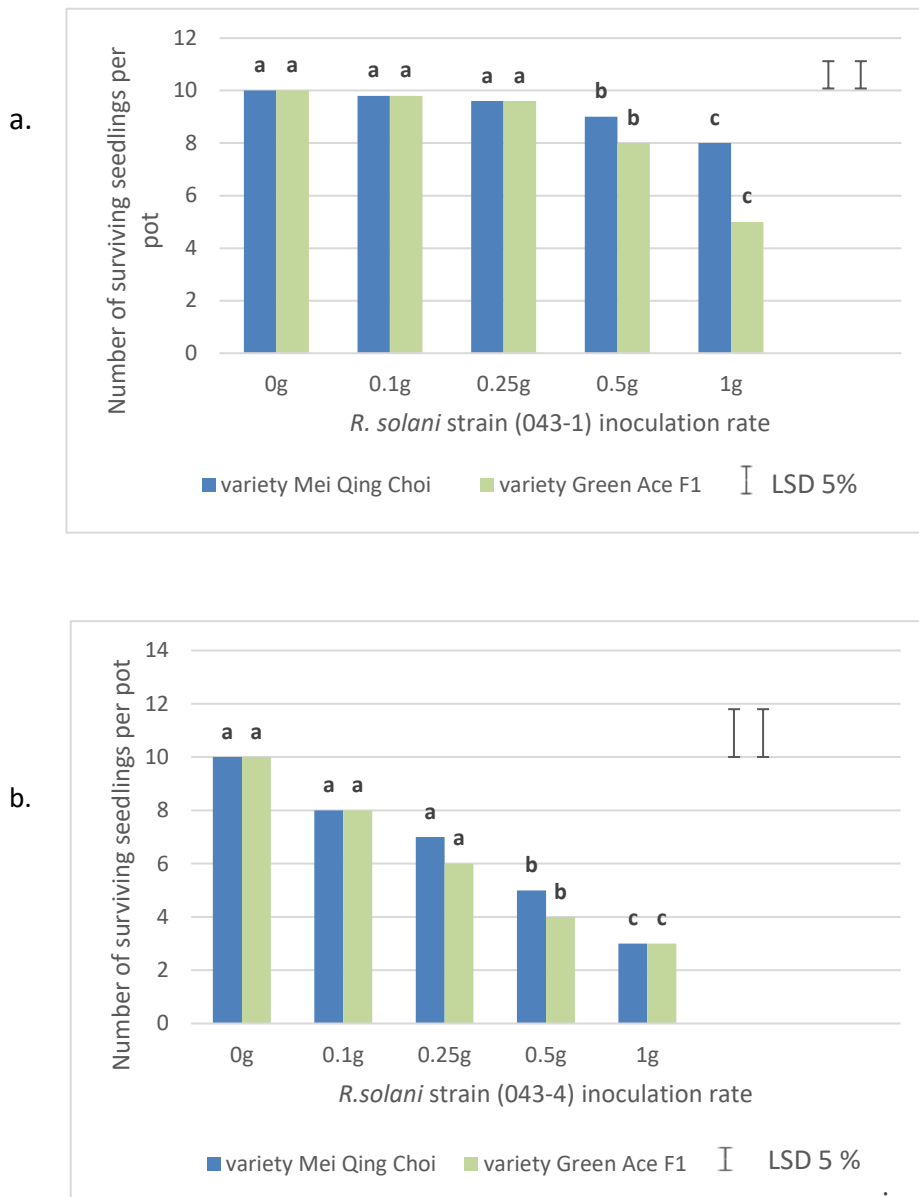


Figure 3.9 Effect of *R. solani* isolate (043-1) inoculum rates on the mean number of surviving seedlings of two Asian cabbage varieties at 20 days after sowing (a). Effect of *R. solani* isolate (043-4) inoculum rates on the number of seedlings of two Asian cabbage varieties at 20 days after sowing (b). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

### Number of seedlings which died post emergence

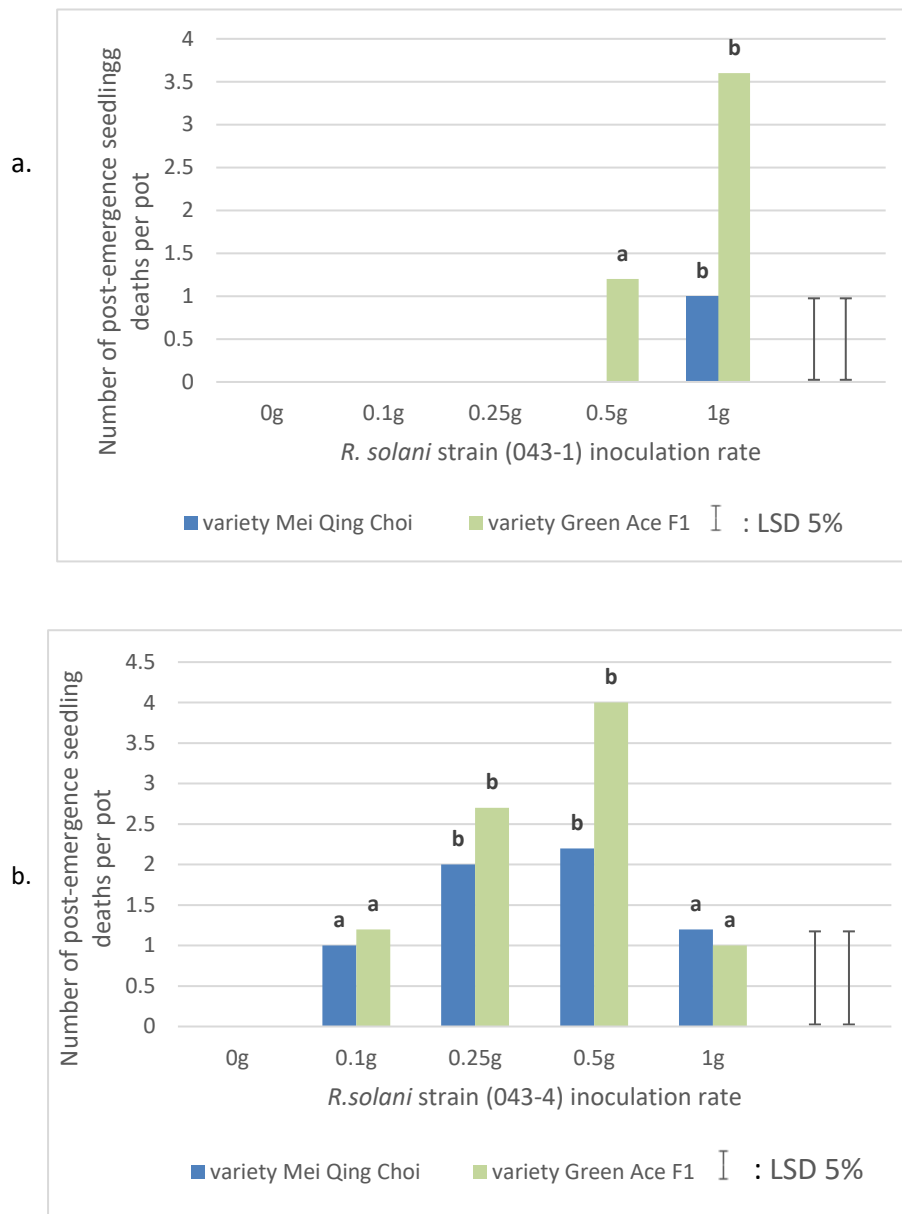


Figure 3.10 Effect of *R. solani* isolate (043-1) inoculum rates on the mean number of seedlings which died post-emergence seedlings of two Asian cabbage varieties at 20 days after sowing (a). Effect of *R. solani* isolate (043-4) inoculum rates on the number of post-emergence seedlings of two Asian cabbage varieties at 20 days after sowing (b). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

For *R. solani* isolate (043-1) seedling death only occurred at the 1.0 g inoculum rate for both varieties and 0.5g inoculum rate for Green Ace F1 (Figure 3.10 a). The number of post-emergence seedling deaths also differed between the two Asian cabbage varieties ( $P < 0.05$ ) (Appendix 5). There was a significant difference among *R. solani* isolate (043-4) inoculum rates ( $P < 0.001$ ), with the 0.25g and 0.5g inoculum rates having more post-emergence seedling deaths than the other treatments (Figure 3.10 b). The number of post emergence

seedling deaths did not differ between the two varieties (Appendix 5). However, there was a significant difference between the *R. solani* isolates (043-1 and 043-4) (Appendix 5) as the number of post-emergence seedling deaths for *R. solani* isolate (043-4) was higher than for the *R. solani* isolate (043-1) (Appendix 5). There was a significant interaction for *R. solani* isolate (043-1) between inoculum rate and variety because deaths were greater in Green Ace F1 than Mei Qing Choi (Appendix 5). There was no interaction for *R. solani* isolate 043-4.

### Number of Healthy Seedlings

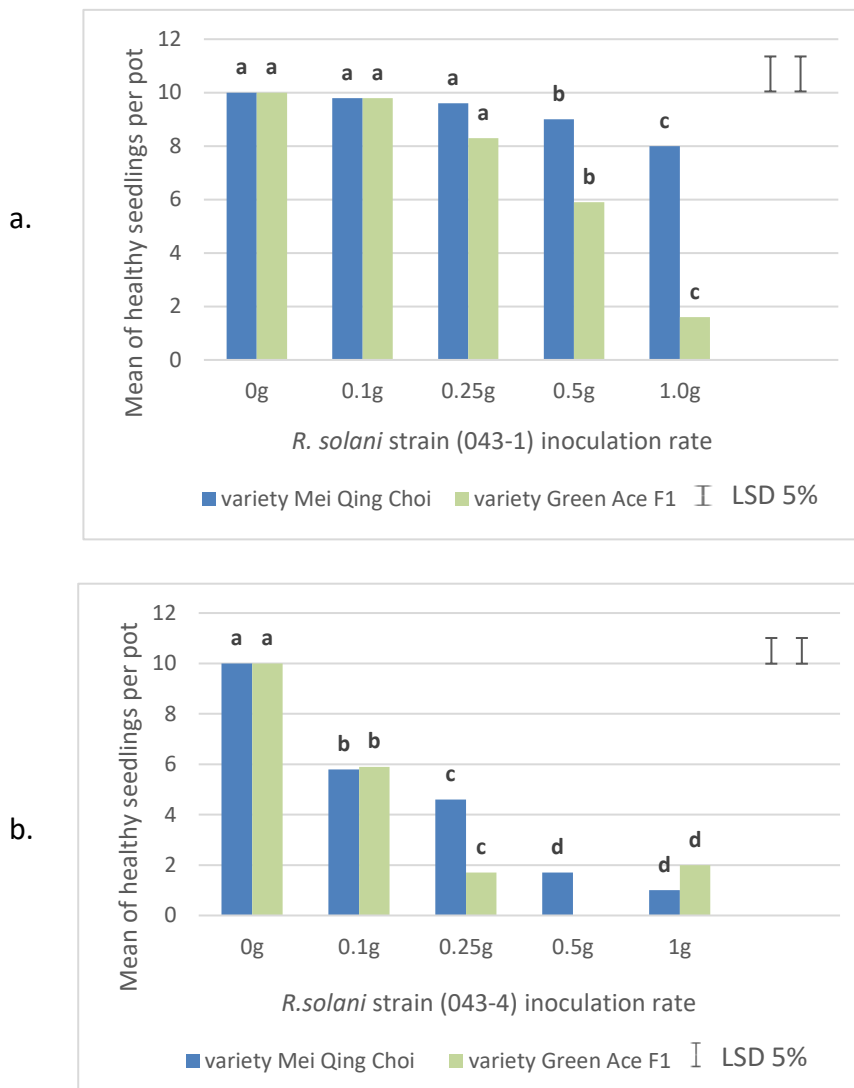
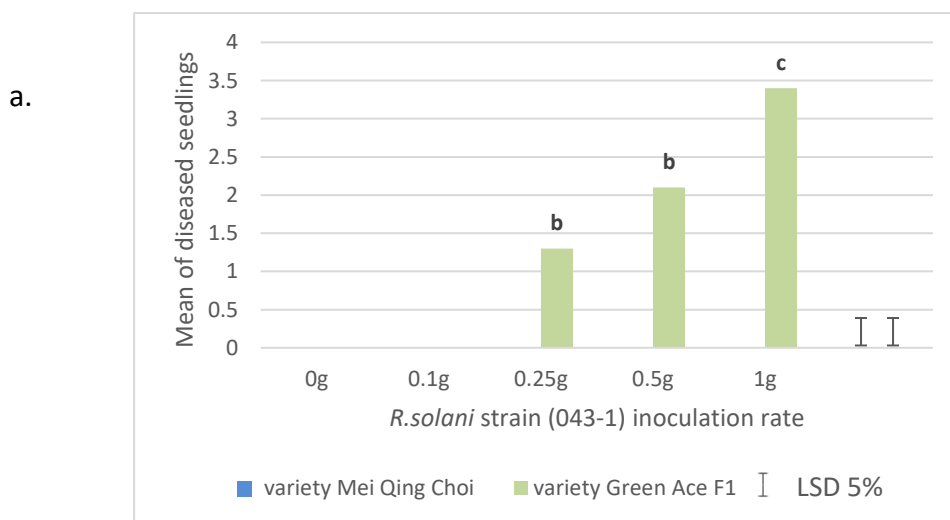


Figure 3.11 Effect of *R. solani* isolates (043-1) (a) and (043-4) (b) inoculum rates on the mean number of healthy seedlings of two Asian cabbage varieties at 20 days after sowing. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P=0.05.

There was a significant difference in the number of healthy seedlings for the *R. solani* isolate (043-1) inoculum rates ( $P < 0.001$ ), with both the 0.5g and 1.0g rates having fewer healthy seedlings than the other treatments, but these reductions were more severe in Green Ace F1 than Mei Qing Choi (Figure 3.11a). The number of healthy seedlings also differed between the two Asian cabbage varieties ( $P < 0.001$ ) (Appendix 6). There was also a significant difference among *R. solani* isolate (043-4) inoculum rates ( $P < 0.001$ ), with healthy seedlings reducing as inoculum rates increased (Figure 3.11 b). The number of healthy seedlings also differed between the two Asian cabbage varieties ( $P < 0.05$ ) (Appendix 6). There was a significant difference between the two *R. solani* isolates ( $P < 0.001$ ), as the number of healthy seedlings for *R. solani* isolate (043-4) was lower than for *R. solani* isolate (043-1) (Appendix 6). For both isolates there was a significant interaction between inoculum rate and variety with both isolates being more severe on Green Ace F1 than Mei Qing Choi (Appendix 6).

#### Number of diseased seedlings



b.

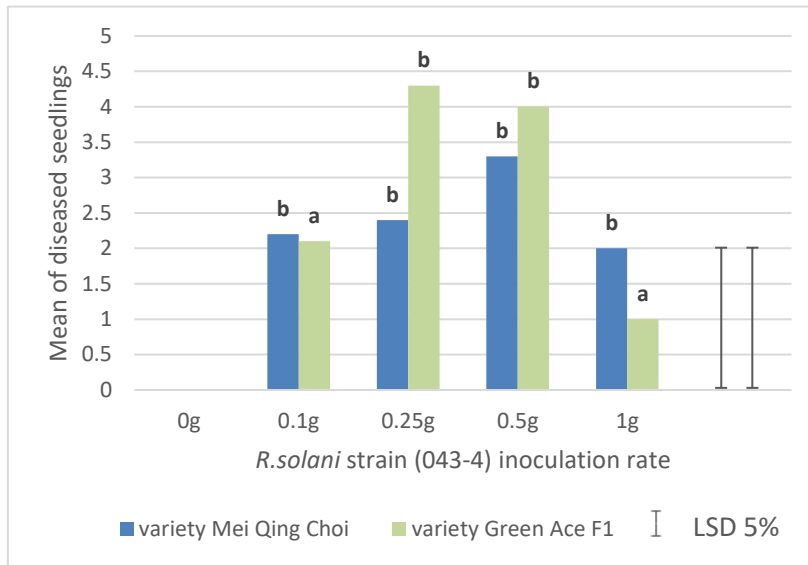


Figure 3.12 Effect of *R. solani* isolates (043-1) (a) and (043-4) (b) inoculum rates on the mean number of diseased seedlings of two Asian cabbage varieties at 20 days after sowing. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5 % level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$

For Mei Qing Choi there were no diseased seedlings for all the *R. solani* strain (043-1) inoculum rates (Figure 3.12a). For Green Ace the control and 0.1 g rate had no diseased seedlings but then the number increased as inoculum rate increased. The number of diseased seedlings differed between the two Asian cabbage varieties ( $P < 0.001$ ) (Appendix 7). There was a significant difference among treatments of *R. solani* isolate (043-4) for the number of diseased seedlings ( $P < 0.05$ ) (Figure 3.12 b). For Mei Qing Choi diseased seedlings did not differ among inoculum rates, but for Green Ace F1 the 0.25g and 0.5g inoculum rates had more diseased seedlings than the lower and high rates. The number of diseased seedlings did not differ between the two Asian cabbage varieties but there was a significant difference between the two *R. solani* isolates (Appendix 7), and a significant interaction between inoculum rate and variety for isolate 043-1, as Mei Qing Choi had no diseased seedlings (Appendix 7).

## Disease score of seedlings

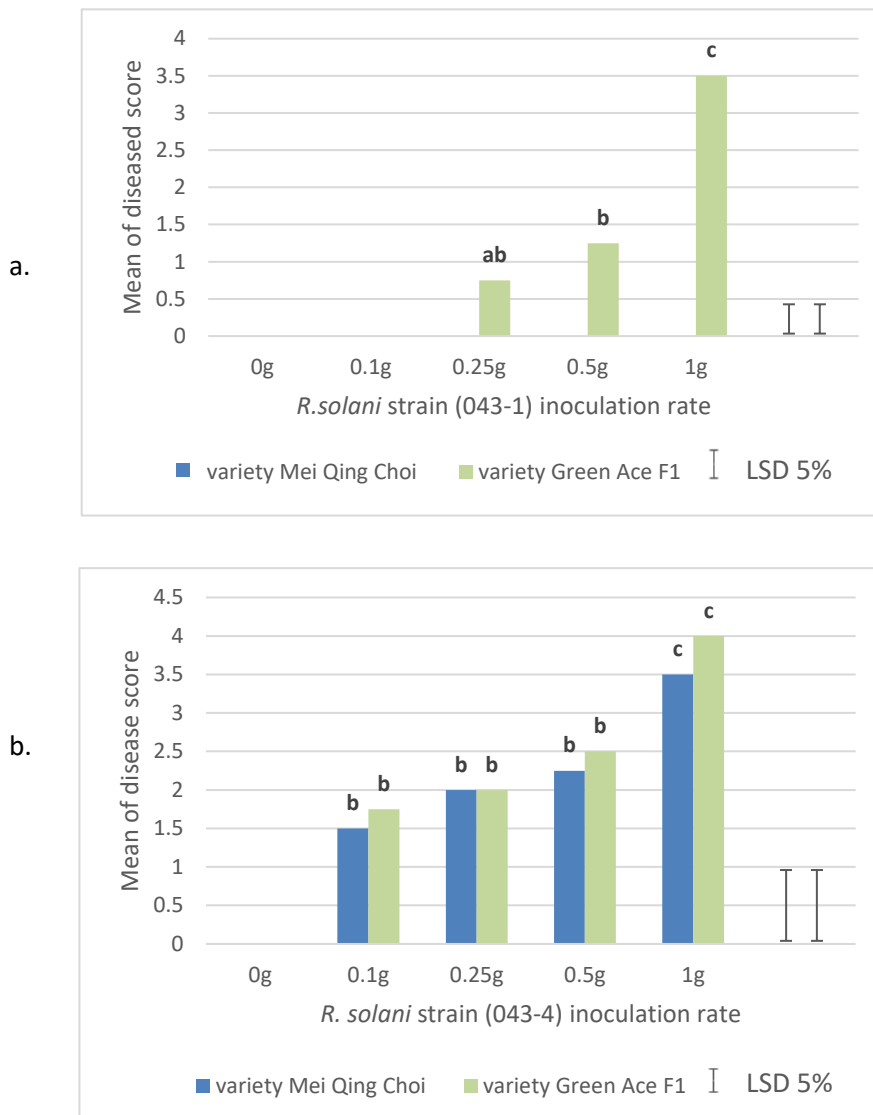


Figure 3.13 Effect of *R. solani* isolate (043-1) inoculum rates on the disease score for the two Asian cabbage varieties at 20 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the disease score for two Asian cabbage varieties at 20 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

For variety Green Ace F1 there was a significant difference for disease score among the *R. solani* isolate (043-1) inoculum rates ( $P < 0.001$ ) (Appendix 8) as the 1.0 g inoculum rate had a higher disease score higher than the other treatments (Figure 3.13 a). Disease score between the two Asian cabbage varieties differed ( $P < 0.001$ ) with Green Ace F1 more susceptible than Mei Qing Choi (Appendix 8). There was a statistically significant difference among treatments of *R. solani* isolate (043-4) ( $P < 0.001$ ) (Figure 3.13 b) with the 1.0g rate having a higher score than the other inoculum rates. Disease score between two Asian cabbage varieties did not

differ. There was a significant difference between the *R. solani* isolates ( $P < 0.001$ ) (Appendix 8) as 043-4 produced a higher score than 043-1, and for 043-1 a significant interaction between the inoculum rate and variety, because Mei Qing Choi had no infected plants.

### Root length

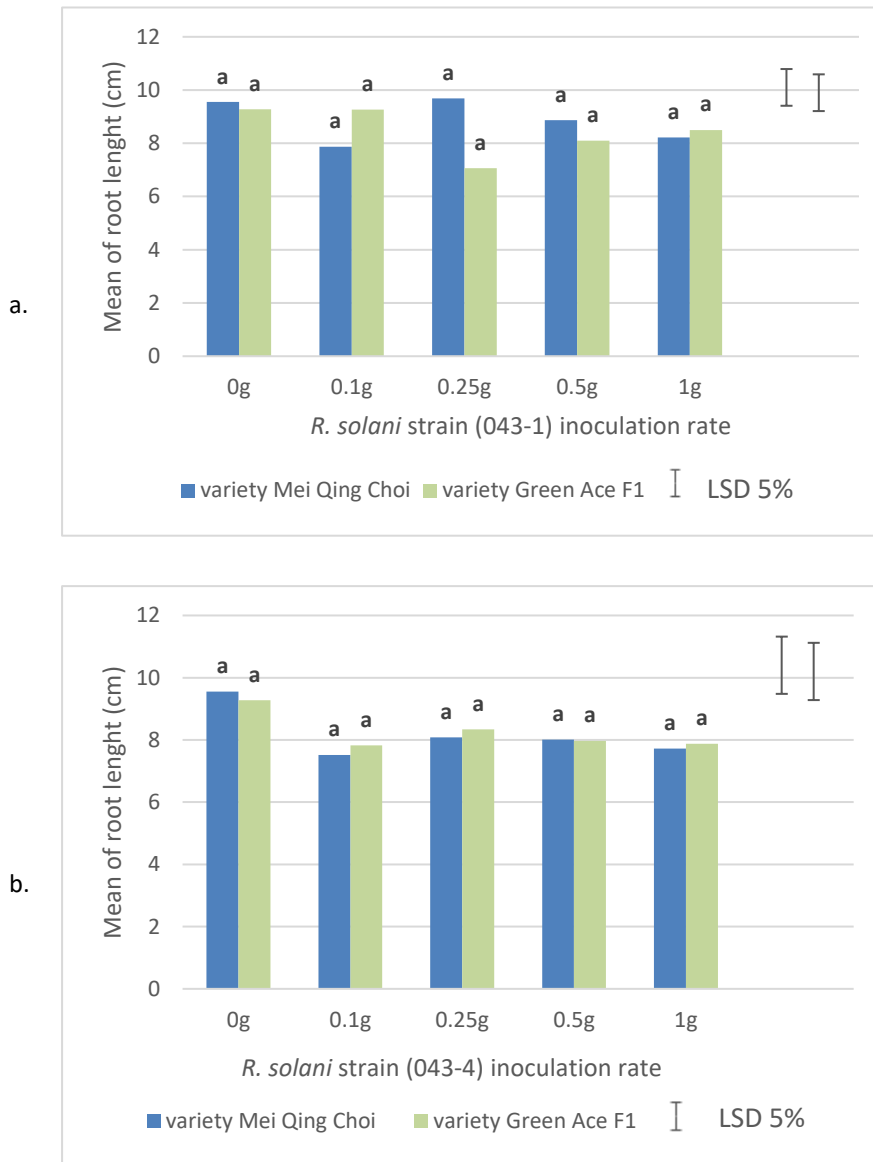


Figure 3.14 Effect of *R. solani* isolate (043-1) inoculum rates on the root length for the two Asian cabbage varieties at 20 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the root length for two Asian cabbage varieties at 20 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5 % level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

There was no significant difference in root length among treatments for *R. solani* isolate (043-1) Figure 3.14 a). Root length did not differ between the two Asian cabbage varieties (Appendix 9). There was also no significant difference in root length among treatments of *R. solani* isolate (043-4) (Figure 3.14 b) and again the root length did not differ between the two

Asian cabbage varieties (Appendix 9). There was a difference between the two *R. solani* isolates and the control ( $P < 0.05$ ) as the control had longer roots than the two *R. solani* isolates (Appendix 9). There was no significant interaction between inoculum rate and variety for either isolate (Appendix 9).

### Shoot length

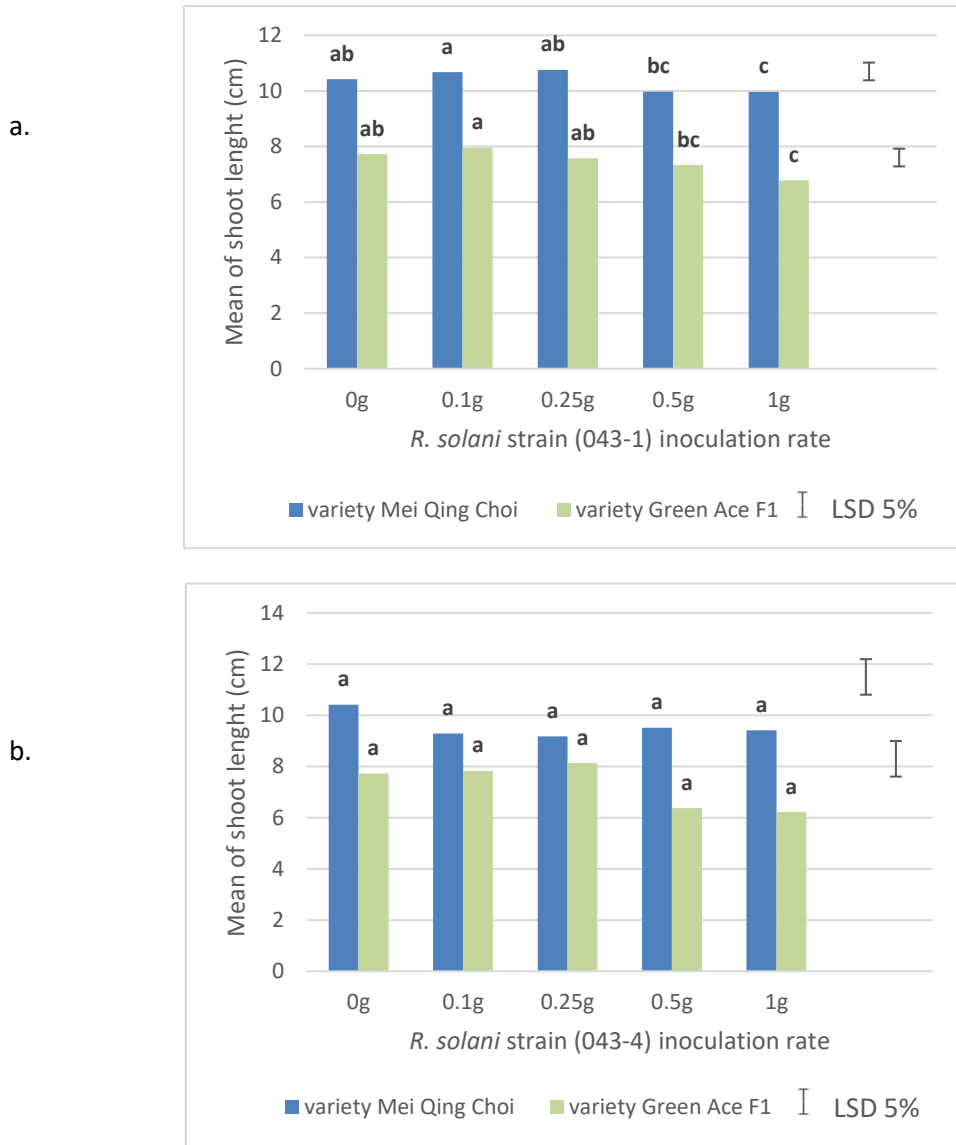


Figure 3.15 Effect of *R. solani* isolate (043-1) inoculum rates on the shoot length for the two Asian cabbage varieties at 20 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the shoot length for two Asian cabbage varieties at 20 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to a Fishers level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

Shoot length differed among the treatments for *R. solani* isolate (043-1) because the 1g inoculum rate had shorter shoots than the control and the 0.1g and 0.25g inoculum rates but did not differ from the 0.5g inoculum rate (Figure 3.15 a). There was a significant difference in shoot length between the two Asian cabbage varieties (Appendix 10) as Mei Qing Choi had longer shoots than Green Ace F1. There was no significant difference among the treatments for *R. solani* isolates (043-4) (Figure 3.15b). Shoot length did differ between the two Asian cabbage varieties, ( $P < 0.001$ ) but did not differ between the two *R. solani* isolates (Appendix 10), and there were no significant interactions.

### Seedling fresh weight

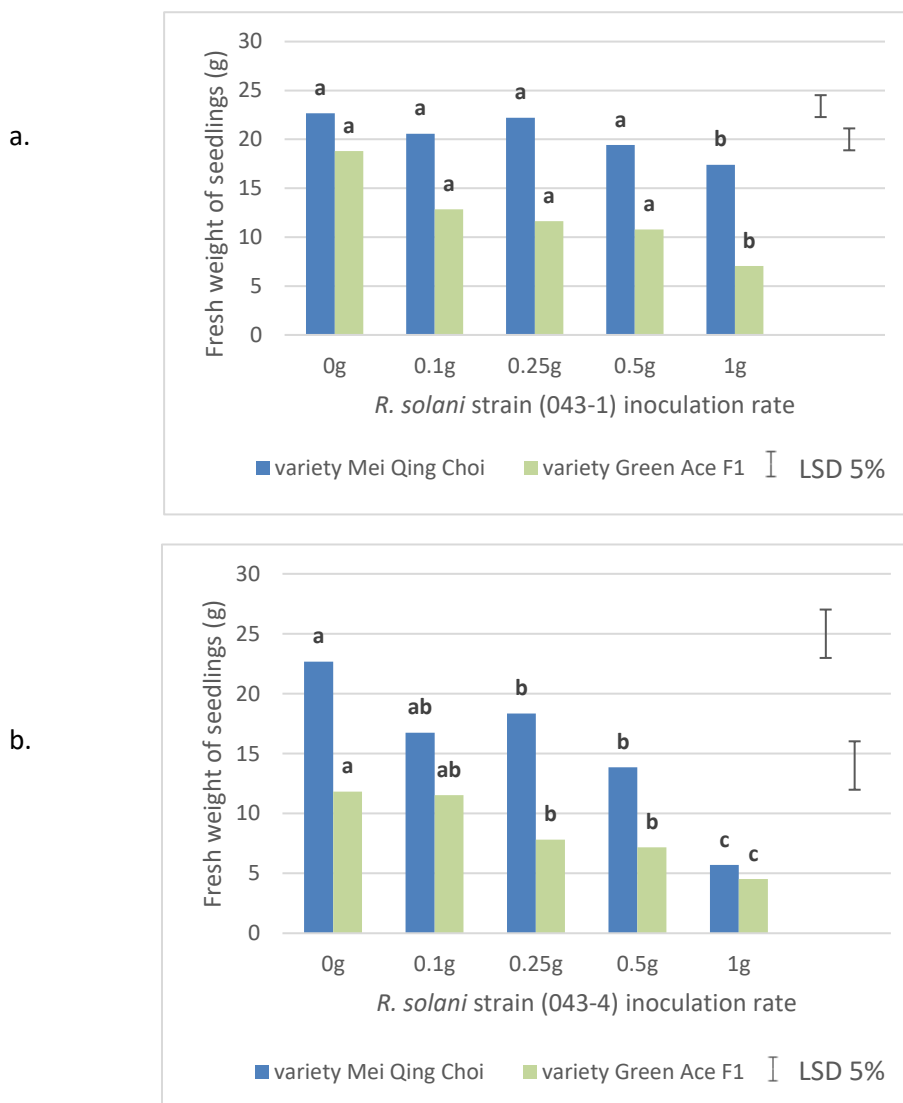


Figure 3.16 Effect of *R. solani* isolate (043-1) inoculum rates on the fresh weight of seedlings for the two Asian cabbage varieties at 20 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the fresh weight of seedlings for two Asian cabbage varieties at 20 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

There was a significant difference in fresh weight of seedlings among treatments of (043-1) *R. solani* isolate (043-1) (Figure 3.16 a) (Appendix 11). For both varieties the 1.0g rate had lower fresh weight than the other treatments. Fresh weight differed between the two Asian cabbage varieties for *R. solani* isolate (043-1) with Green Ace F1 being smaller than Mei Qing Choi (Appendix 11). There was a significant difference in fresh weight of seedlings among treatments of *R. solani* isolate (043-4) ( $P < 0.001$ ), (Figure 3.16 b). The inoculum rate of 1.0g had lower fresh weight compared to the other treatments while the 0.1g rate did not differ from the control. The fresh weight differed between the two Asian cabbage varieties (Appendix 11). There was a significant difference between the two *R. solani* isolates ( $P < 0.001$ ) with *R. solani* isolate (043-4) having lower fresh weight than the control and *R. solani* isolate (043-1) (Appendix 11). There were no significant interactions between inoculum rate and variety (Appendix 11).

### Seedling dry weight

a.



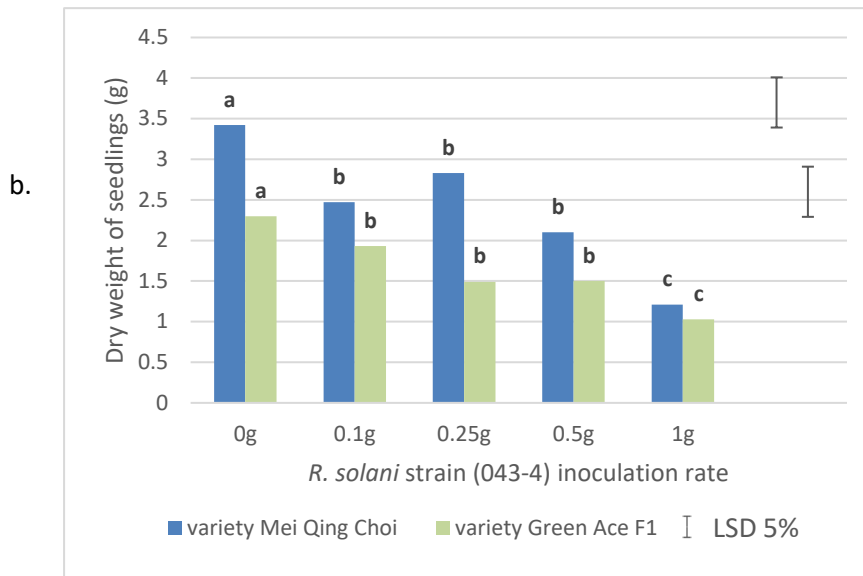


Figure 3.17 Effect of *R. solani* isolate (043-1) inoculum rates on the dry weight of seedlings for the two Asian cabbage varieties at 20 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the dry weight of seedlings for two Asian cabbage varieties at 20 days after sowing (b.) Vertical bar is the LSD (5%). Letters were assigned to Fisher's level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

There was a significant difference in seedling dry weights of *R. solani* isolate (043-1) inoculum rates ( $P < 0.001$ ), in that the 1.0g rate had a lower seedling dry than the control and 0.1g and 0.25g rates, but not the 0.25g and 0.5g rates for both varieties (Figure 3.17 a.). Dry weight between the two Asian cabbage varieties differed as the dry weights for Mei Qing Choi were higher than for Green Ace F1 (Appendix 12). There was also a significant difference among *R. solani* isolate (043-4) inoculum rates ( $P < 0.001$ ), (Figure 3.17 b) as for both varieties the control had a higher dry weight than the other inoculum rates and the 1.0g rate had the lowest dry weight. Dry weights between the two Asian cabbage varieties also differed. Dry weights of seedlings were lower for *R. solani* isolate (043-4) than for *R. solani* isolate (043-1) but there were no significant interactions (Appendix 12).

### 3.2.6 Assessment at 33 Days

#### Seedling Survival

The highest *R. solani* strain 043-1 inoculum rate had slightly reduced the number of surviving seedlings for variety Mei Qing Choi and Green Ace F1 by 33 DAS ( $P < 0.05$ ) (Table 3.3). The number of seedlings which died post-emergence (Mei Qing Choi only) and disease score for both varieties tended to have increased as inoculum rate increased (Table 3.3)

However, at 33DAS for *R. solani* strain (043-4) increasing inoculum rates had significantly reduced the number of surviving seedlings for the two varieties ( $P<0.001$ ) (Table 3.4). The control and the 0.1g inoculum rate had a higher number of seedlings than the other inoculum rates. At 33 DAS the number of diseased seedlings had increased for the 0.25g and 0.5g inoculum rates but oddly not the 1g inoculum rate (Table 3.4). All the inoculum rates had a higher disease score than the control (Table 3.4).

Table 3.3 Effect of *R. solani* strain (043-1) inoculum rates on maximum number of emerged seedlings, diseased seedlings, disease score, number of seedlings which died post emergence and number of surviving seedlings at 33 days after sowing.

<i>R. solani</i> (043-1)	Variety	Maximum number of emerged seedlings	Number of diseased seedlings	Disease score	Number of seedlings which died post-emergence	Number of surviving seedlings
Control (0g)	Mei Qing Choi	10 a	0.0 a	0.0 a	0.0 a	10.0 a
0.1g	Mei Qing Choi	9.75 a	0.25 a	1.0 ab	0.0 a	9.75 a
0.25g	Mei Qing choi	10 a	0.75 a	1.0 ab	0.25 a	9.75 a
0.5g	Mei Qing choi	9.75 a	1.0 a	2.0 b	0.5 ab	9.25 ab
1g	Mei Qing choi	10 a	1.0 a	2.0 b	1.25 b	8.75 b
LSD (5%)		0.50	1.11	1.77	0.90	0.79
Significance		NS	NS	$P<0.05$	$P<0.05$	$P<0.05$
Control (0g)	Green Ace F1	10 a	0.0 a	0.0 a	0.0 a	10.0 a
0.1g	Green Ace F1	10 a	0.0 a	0.0 a	0.25 a	9.75 a
0.25g	Green Ace F1	10 a	0.75 ab	1.0 ab	0.5 a	9.5 ab
0.5g	Green Ace F1	9.75 a	0.75 ab	2.0 bc	0.5 a	9.25 ab
1g	Green Ace F1	8.5 b	1.25 b	3.0 c	0.0 a	8.5 b
LSD (5%)		0.93	1.09	1.22	0.95	1.04
Significance		$P<0.05$	$P<0.05$	$P<0.001$	NS	$P<0.05$

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

Table 3.4 Effect of *R. solani* strain (043-4) inoculum rates on maximum number of emerged seedlings, diseased seedlings, disease score, number of seedlings which died post emergence and number of surviving seedlings at 33 days after sowing.

R. solani (043-4)	Variety	Maximum number of emerged seedlings	Number of diseased seedlings	Disease score	Number of seedlings which died post-emergence	Number of surviving seedlings
Control (0g)	Mei Qing Choi	10.0 a	0.0 a	0.0 a	0.0 a	10.0 a
0.1g	Mei Qing Choi	10.0 a	1.25 ab	3.0 b	0.25 a	9.75 a
0.25g	Mei Qing choi	9.0 a	2.75 bc	3.0 b	0.5 a	8.5 b
0.5g	Mei Qing choi	10.0 a	3.0 c	4.0 b	2.0 b	8.0 b
1g	Mei Qing choi	3.5 b	0.75 a	4.0 b	0.0 a	3.5 c
LSD (5%)		2.08	1.57	1.12	1.12	0.91
Significance		P<0.001	P<0.05	P<0.001	P<0.05	P<0.001
Control (0g)	Green Ace F1	9.75 a	0.0 a	0.0 a	0.0 a	9.75 a
0.1g	Green Ace F1	9.25 a	5.25 c	3.0 b	0.0 a	9.25 a
0.25g	Green Ace F1	7.25 ab	2.25 b	3.0 b	2.25 b	5.0 b
0.5	Green Ace F1	5.0 b	1.25 ab	4.0 b	1.75 b	3.25 bc
1g	Green Ace F1	4.75 b	0.75 ab	4.0 b	2.75 b	2.0 c
LSD (5%)		3.03	2.14	1.80	1.71	1.95
Significance		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at P= 0.05.

### Healthy plants

At 33 DAS the percentage of healthy plants for the two varieties for *R. solani* strain (043-1) was reduced for the three highest inoculum rates (Figure 3.18 a) (Appendix 13). The 1 g inoculum rate had the lowest healthy plant percentage (77.5 % for Mei Qing Choi and 72.5 % for Green Ace F1), while control had the highest healthy plant percentage (100 % for Mei Qing Choi and 100% for Green Ace F1 (Figure 3. 18 a).

All the inoculum rates for *R. solani* strain (043-4) significantly reduced the healthy plant percentages for both varieties (P<0.001) (Figure 3. 18 b). Healthy plant percentages decreased as the inoculum rates increased (Figure 3. 18 b). The healthy plant percentages did not differ among the two varieties for *R. solani* strain (043-1) but did differ for strain 043-4 as Green Ace F1 had fewer healthy plants than Mei Qing Choi (Appendix 13). There was a significant difference between the two *R. solani* strains which both differed from the control (P<0.001). The control and *R. solani* strain (043-1) had higher healthy plant percentages than *R. solani* strain (043-4) (Appendix 13). There were no significant interactions between inoculum rate and variety (Appendix 13).

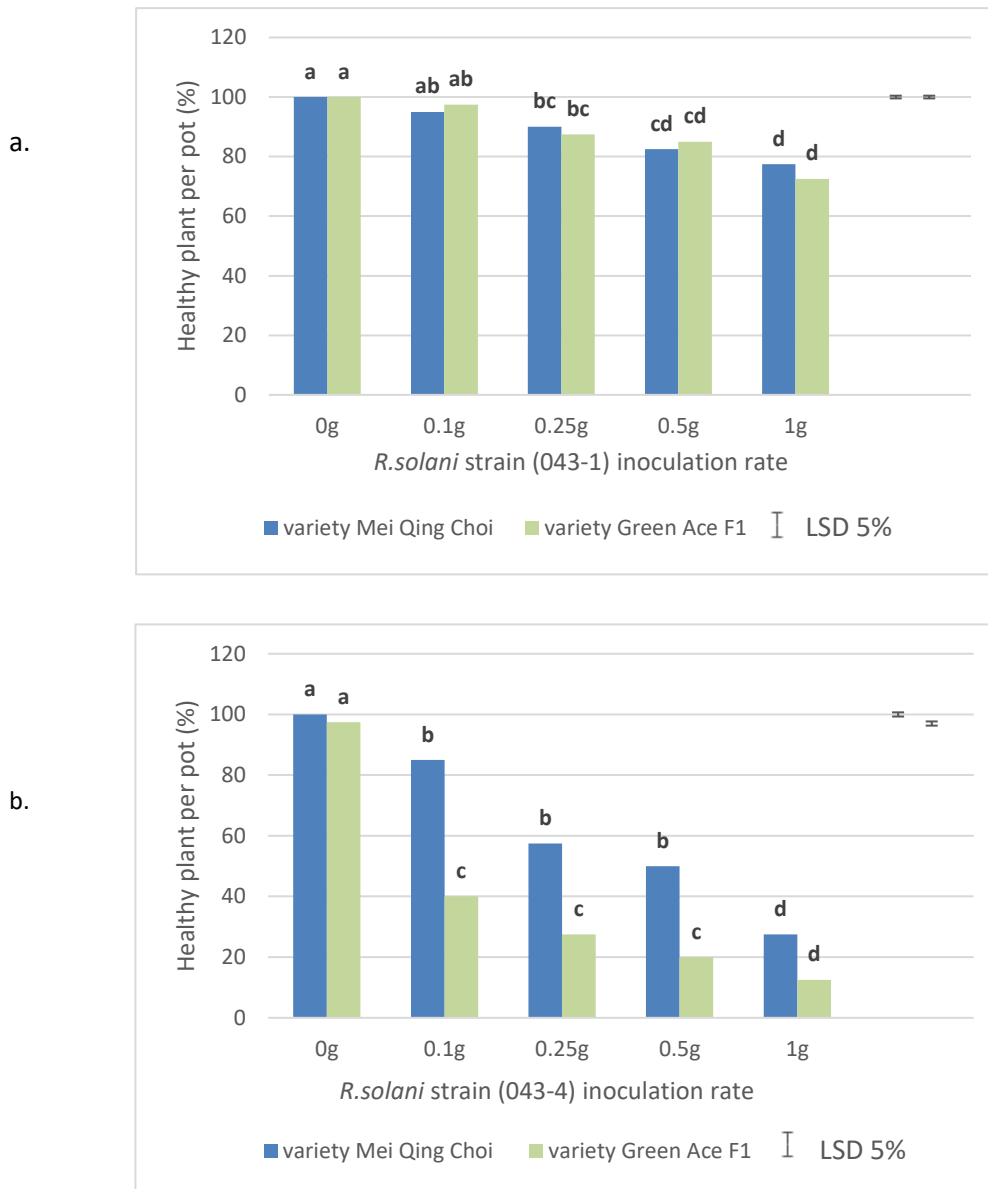


Figure 3.18 Effect of *R. solani* isolate (043-1) inoculum rates on percentage healthy plants for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on percentage healthy plants for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5 % level unprotected LSD procedure. Means with the same letter are not significantly different at  $P=0.05$ .

### Shoot length

Shoot length for the two varieties did not differ among the *R. solani* strain (043-1) inoculum rates (Figure 3.19a) (Appendix 14). There was a difference between the two varieties ( $P<0.001$ ) as variety Mei Qing Choi had a greater mean shoot length than Green Ace F1 (Appendix 14). Shoot length for the inoculum rates for *R. solani* strain (043-4) differed (Figure 3.19b), as shoot lengths for the 0.25g and 0.5g rates were smaller than the other treatments for both varieties. The shoot length differed between the two varieties ( $P<0.001$ ), with variety Mei Qing Choi have a greater shoot length than Green Ace F1

(Appendix 14). The shoot length for the two varieties did not differ between the two *R. solani* strains and there were no significant interactions between inoculum rate and variety (Appendix 14).

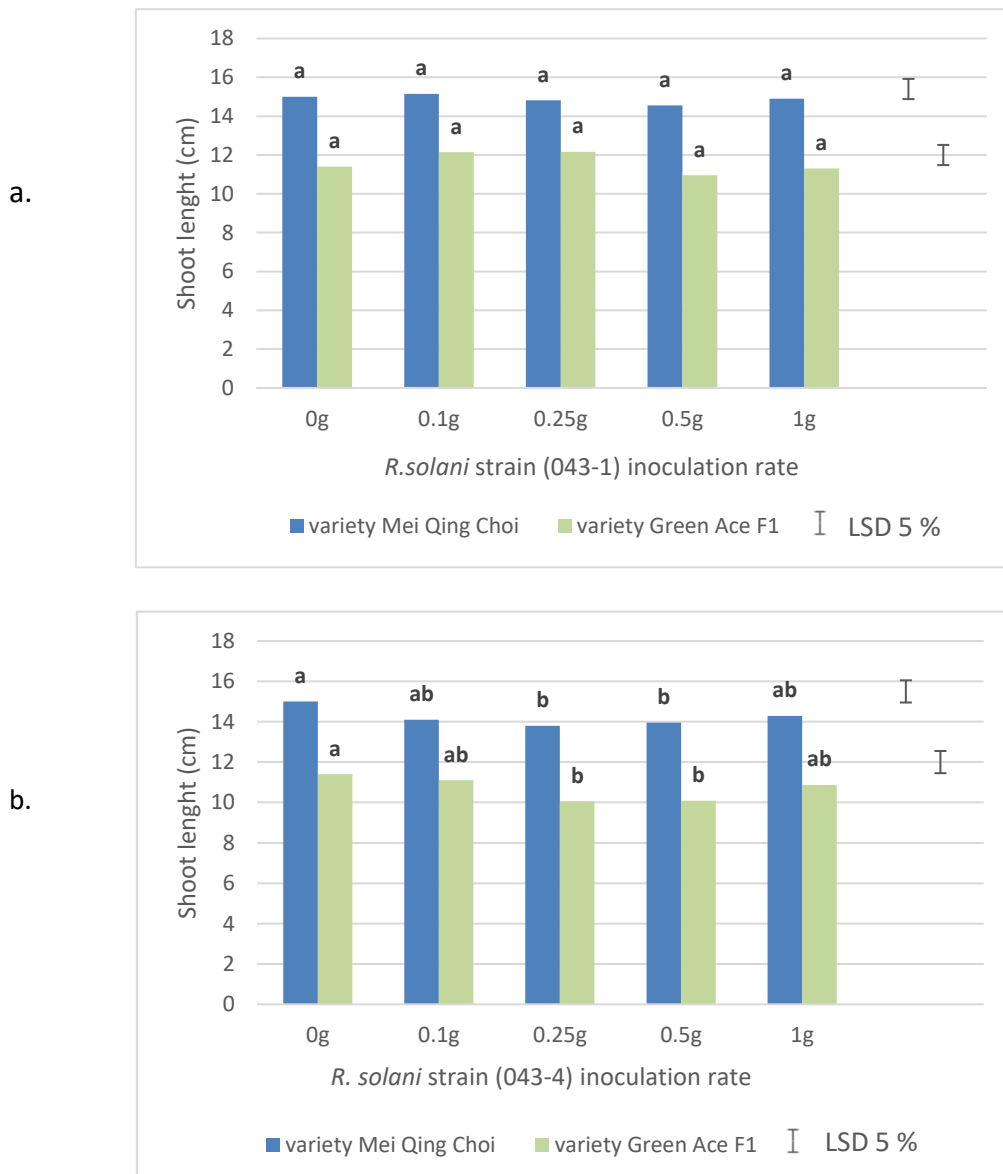


Figure 3.19 Effect of *R. solani* isolate (043-1) inoculum rates on shoot length for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the shoot length for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5%). Letters were assigned to Fisher's 5% level unprotectd LSD procedure. Means with the same letter are not significantly (NS) different at  $P=0.05$ .

### Root length

The root length for the two varieties did not differ among the *R. solani* strain (043-1) inoculum rates (Figure 3.20a). There was no difference between the two varieties. (Appendix 15). The inoculum rates for *R. solani* strain (043-4) also had no effect on root length for the two varieties (Figure 3.20b). The root length also did not differ between the two varieties.

The root length for the two varieties did not differ among the two *R. solani* strains and the control and there was no significant interaction (Appendix 15).

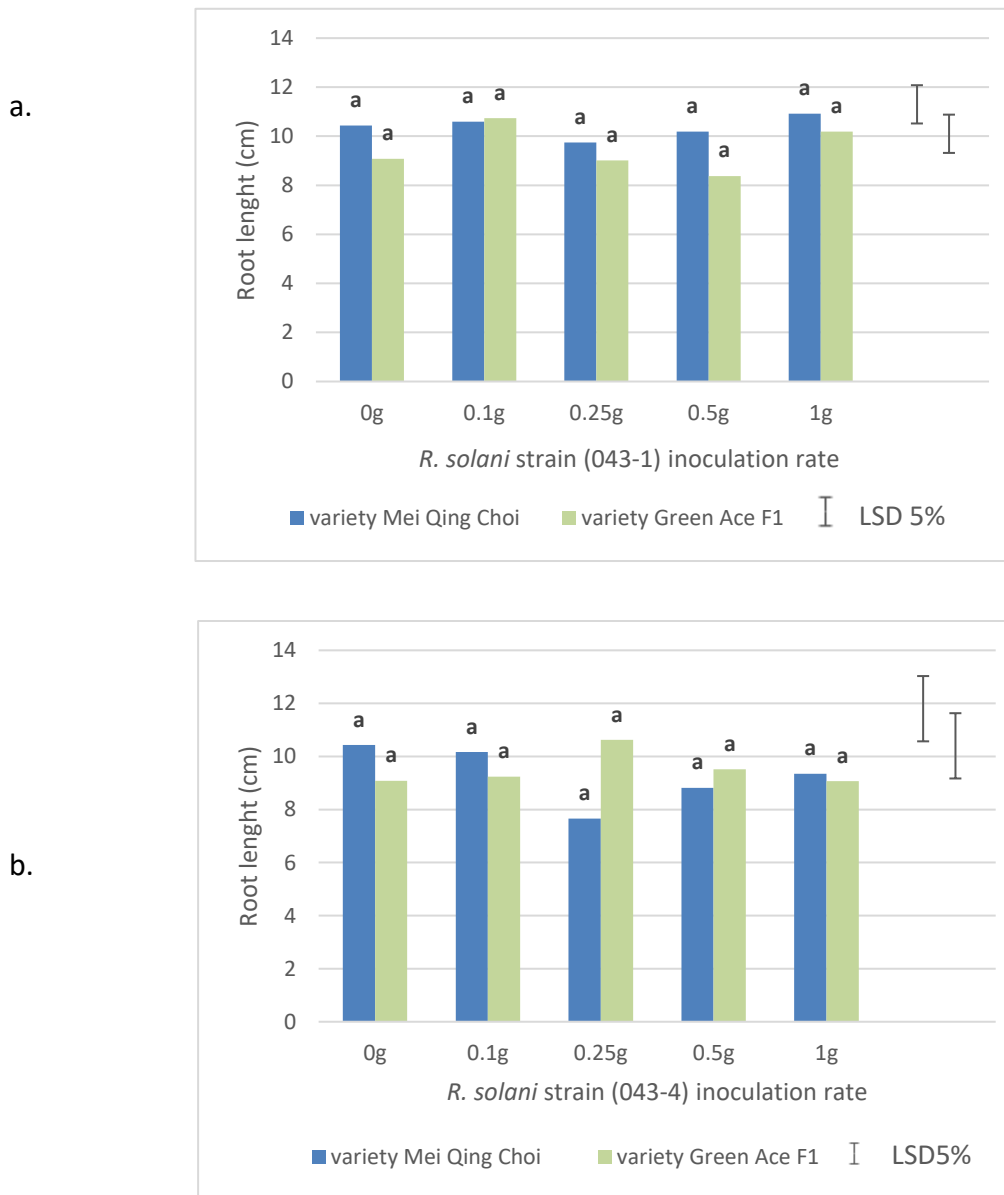


Figure 3.20 Effect of *R. solani* isolate (043-1) inoculum rates on root length for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on root length for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5 %). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at  $P=0.05$ .

### Leaf surface area per pot

Leaf surface area for the two varieties did not differ among *R. solani* strain (043-1) inoculum rates (Figure 3.21a) (Appendix 16). The leaf surface area differed significantly between the varieties (Appendix 16) as variety Mei Qing Choi had a larger leaf surface area than Mei Qing Choi (Figure 3.21a) (Appendix 16).

The leaf surface area for both varieties was significantly reduced among *R. solani* strain (043-4) inoculum rates ( $P < 0.001$ ) (Appendix 16). The control and 0.1g rate had a larger leaf surface area than the other rates. As the inoculum rates increased, the leaf surface area reduced (Figure 3.21 b). The leaf surface area differed between the two varieties, as Green Ace F1 had a smaller leaf surface area than Mei Qing Choi (Figure 3.21 b) (Appendix 16).

The leaf surface area for the two varieties also significantly differed between the two *R. solani* strains and the control. *R. solani* strain (043-4) had plants with smaller leaves than *R. solani* strain (043-1) and the control (Appendix 16). There were no significant interactions between inoculum rate and variety (Appendix 16).

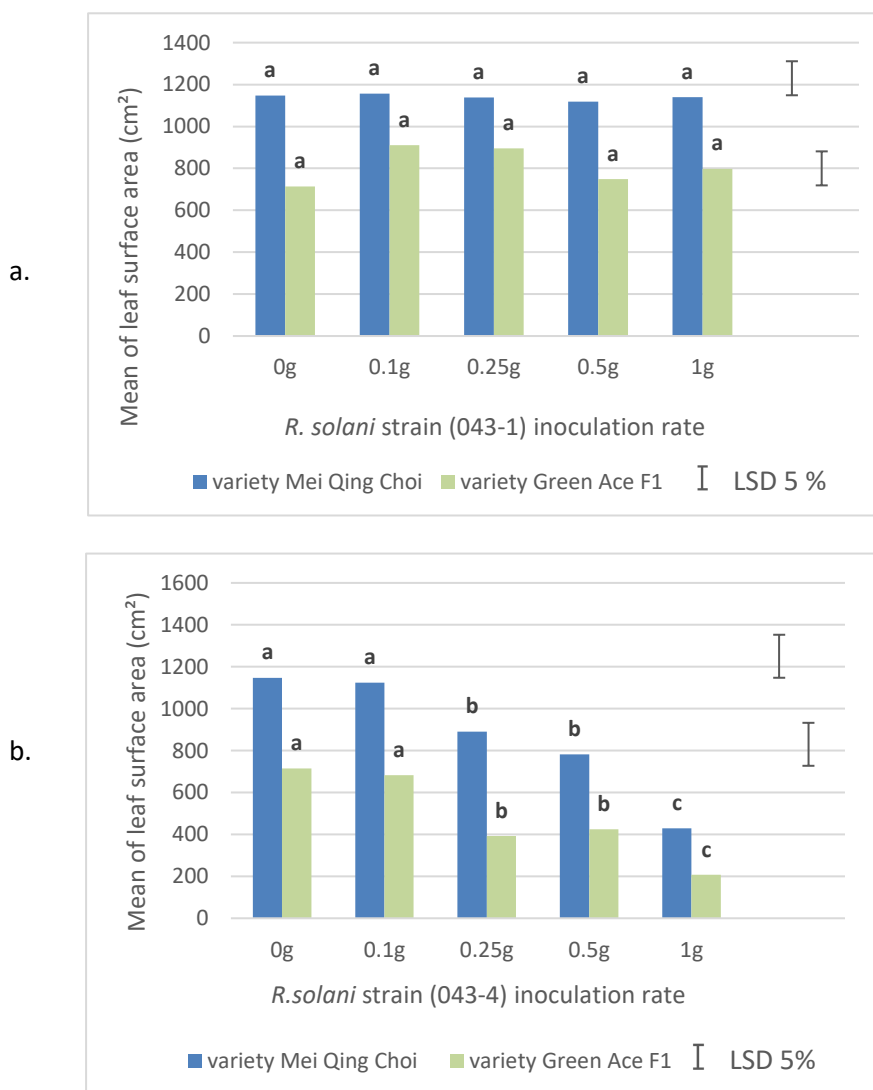


Figure 3.21 Effect of *R. solani* isolate (043-1) inoculum rates on leaf surface area for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on leaf surface area for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5 %). Letters were assigned to a Fisher's 5% level unpaired LSD procedure. Means with the same letter are not significantly (NS) different at  $P = 0.05$ .

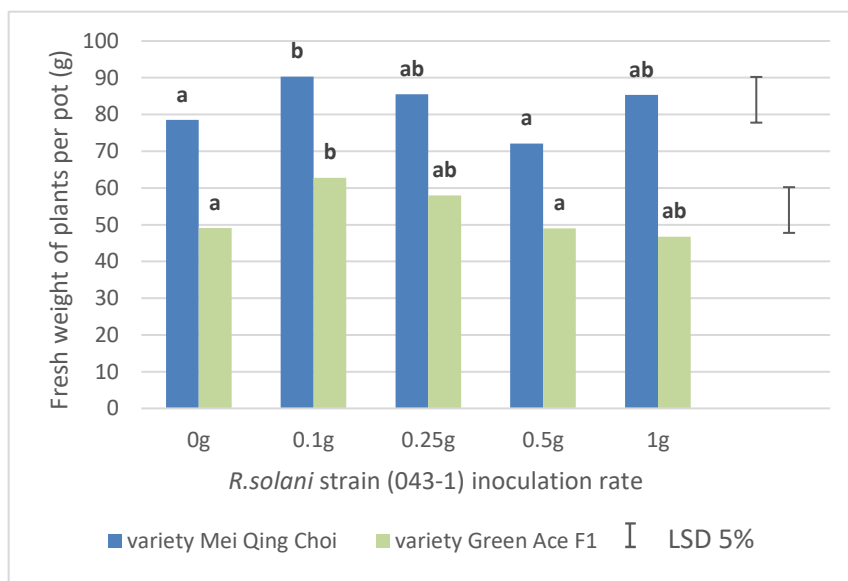
### Plant fresh weight per pot

*R. solani* strain (043-1) inoculum rates did not reduce plant fresh weight for either variety (Figure 3.22a) (Appendix 17) although for both varieties fresh weight for the 0.1g inoculum rate was greater than the control. There was a significant difference for fresh weight between the two varieties as fresh weight for Mei Qing Choi was higher than for Green Ace F1 (Appendix 17).

For *R. solani* strain (043-4) the fresh weight for the two varieties decreased significantly at inoculum rates greater than 0.1g (Figure 3.22 b) (Appendix 17). The fresh weight differed between the two varieties, as variety Green Ace F1 had a lower plant fresh weight than Mei Qing Choi (Appendix 17).

The plant fresh weight for the two varieties differed among the two *R. solani* strains and the control because the control and *R. solani* strain (043-1) had heavier plant fresh weights than *R. solani* strain (043-4) (Appendix 17). There were no significant interactions between inoculum rate and the variety (Appendix 17).

a.



b.

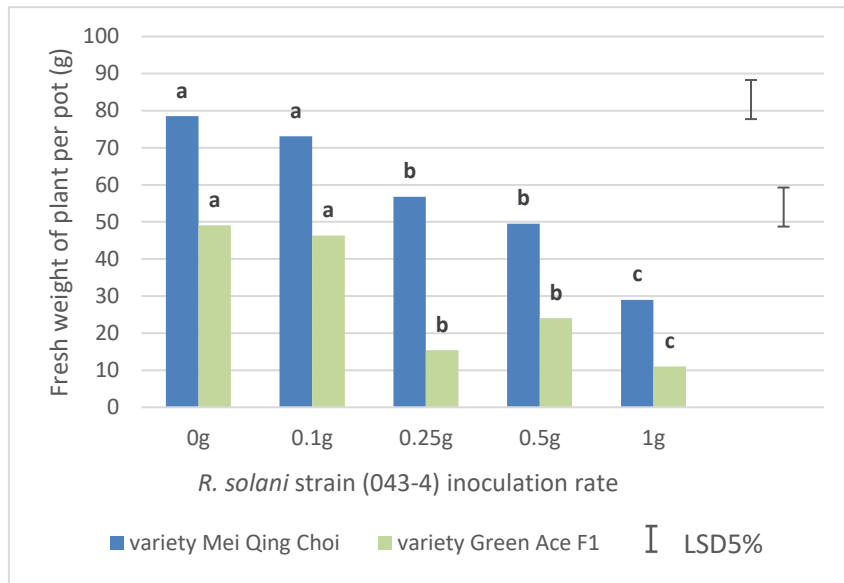


Figure 3.22 Effect of *R. solani* isolate (043-1) inoculum rates on the fresh weight of plants for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the fresh weight of plants for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5 %). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at  $P=0.05$ .

### Plant dry weight per pot

The plant dry weight for both varieties for the *R. solani* strain (043-1) inoculum rates did not differ from the control (Figure 3.23 a) (Appendix 18). The dry weights did differ between the two varieties, as variety Mei Qing Choi had heavier dry weights than Green Ace F1 (Appendix 18). There was a significant difference among *R. solani* strain (043-4) inoculum rates for the two varieties ( $P<0.001$ ) (Figure 3.25b). Apart from the 0.1g rate, the dry weights reduced as the inoculum rates increased (Figure 3.23b). The 1 g inoculum rate had the lowest plant dry weights (2.7 g for Mei Qing Choi and 1.5 g for Green Ace F1) (Appendix 18). The dry weights of plants differed between the two *R. solani* strains and the control ( $P < 0.001$ ), as the control and *R. solani* strain (043-1) had a heavy plant dry weight than *R. solani* strain (043-4) (Appendix 18). There were no significant interactions between inoculum rate and variety (Appendix 18).

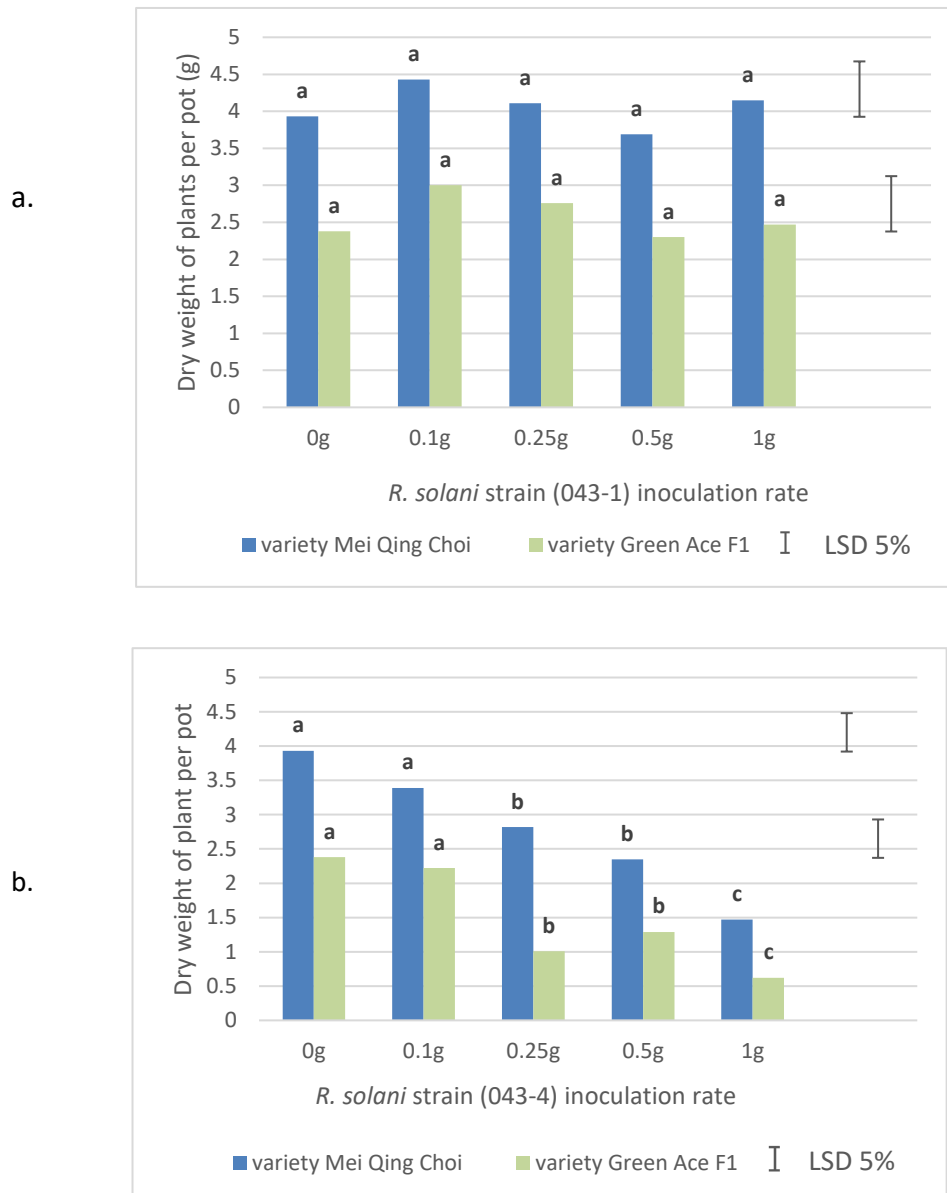


Figure 3.23 Effect of *R. solani* isolate (043-1) inoculum rates on the dry weight of plants for the two Asian cabbage varieties at 33 days after sowing (a.). Effect of *R. solani* isolate (043-4) inoculum rates on the dry weight of plants for two Asian cabbage varieties at 33 days after sowing (b.). Vertical bar is the LSD (5 %). Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at  $P=0.05$ .

### 3.5 Discussion

This glasshouse study examined the impact of two *R. solani* strains (043-1 and 043-4) on disease development and plant growth of two Asian cabbage varieties (Mei Qing Choi and Green Ace F1). The isolates had been sequenced for internal transcribed spacer (ITS) and AG classification by Plant and Food Research, Lincoln, and this confirmed that they belong to the subgroup or subset of AG-2-1 (Das et al., 2014). AG-2-1 is the predominant group of isolates

that are virulent to oilseed rape, canola, and other Brassica crops around the world (Gugel et al., 1987; Jayaweera & Ray, 2023; Sturrock et al., 2015).

The results in this study indicated that the differences in virulence recorded between the two isolates of *R. solani* were statistically significant ( $P < 0.001$ ). *R. solani* (043-4) reduced seedling emergence more and was more virulent than 043-1. In pathogenicity experiments using 11 AG-2-1 isolates on potato under glasshouse and shade house conditions. Das et al. (2014) reported the greater virulence of, *R. solani* 043-4 which reduced potato stem emergence and negatively affected stolon growth.

Both isolates used in this glasshouse study did not prevent seedling emergence but did cause post-emergence death of Asian cabbage seedlings. Post-emergence seedling death occurred due to a severe infection of wire stem on the seedlings. Seedlings have tender cuticles and epidermal cells that cannot resist the pathogen infection. A dark water-soaked lesion initially appears on the stem. The tissue then becomes constricted, wiry and slender at the site of the lesion (Figure 3.5). Seedlings may then break off at the soil line (Kataria & Verma, 1992). According to Das et al. (2014) and Woodhall et al. (2008), AG-2-1 isolates can instigate severe stem diseases and may cause yield losses on many hosts. Asian cabbage is one of these hosts.

However, isolate 043-1 did not cause disease in variety Mei Qing Choi and required inoculum rate of 0.25g before symptoms appeared in variety Green Ace F1, in contrast isolate 043-4 which caused disease symptoms to appear in both varieties at all inoculum rates. This again confirms the greater virulence of isolate 043-4. However, there is no published information on susceptibility to the pathogen for these two varieties.

The disease score increased with increasing inoculum rate as also report by Lee (2018), for radish. An increase in inoculum rate can provide more mycelium (propagules) leading to increased opportunity for infection (Yitbarek et al., 1988). Disease severity maybe partly influenced by abiotic factors such as temperature and soil moisture. The AG-2-1 subgroup of isolates are dominant during colder periods (Erper et al., 2021; Kataria & Verma, 1992) with Sims (2022) reporting that, AG-2-1 isolates were most virulent to brassica seedlings at cooler (7-12 °C ) night-day air temperatures. In the glasshouse, temperature did not go below 10 °C at night. Moderate soil moisture (60 to 65% soil moisture) conditions are favourable to hyphal growth and disease development (Moorman, 2023).

There were also differences in growth performance between the two varieties. This may have been due to the difference in seed sizes between the varieties. Seed size can be a factor contributing to the success of seedling emergence and subsequent crop performance (Hwang et al., 2013). Hwang et al. (2013) investigated the effect of seed size on *R. solani* AG-2-1 seedling blight of canola. They showed that in the presence of the pathogen, seed lots with larger seed sizes had higher seedling emergence, and greater plant height and shoot dry weight than seed lots with smaller seed sizes. However, for the Asian cabbage varieties used in the study, it is possible that the different responses to the pathogen were associated with plant genetics, with the larger seedlings of Mei Qing Choi better able to withstand pathogen attack. This requires investigation.

Although the number of seedlings per pot which had survived at 33 DAS was reduced slightly by isolate 043-1, and majorly by isolate 043-4, there were often no significant differences in plant growth parameters. For example, plant shoot and root length did not differ among treatments, and for isolate 043-1 this also occurred for plant fresh and dry weight. However, for isolate 043-4, leaf surface area and plant fresh and dry weight were reduced particularly at the highest inoculum rate. For isolate 043-1, the greatest mean loss of plants post-emergence was 1.5 per pot out of 10, so that it could be expected that all the remaining plants were competing equally for resources and that growth would not differ. For isolate 043-4 however, at the highest inoculum rate, only 3.5 (variety Mei Qing Choi) and 2 (variety Green Ace F1) plants per pot had survived at 33 DAS. It would therefore be expected that these surviving plants, because of the reduced inter-plant competition, would have been larger than the control (Thapa & Katwal, 2024). Fewer plants should also have provided the opportunity for increased light interception and greater radiation use efficiency (Thapa & Katwal, 2024; Weraduwanage et al., 2015). Leaf area determines the light the leaves intercept, and the efficiency of that use determines plant biomass (Gardner et al., 2017). The plant growth data presented are on a per-pot basis, and therefore on a per plant basis might have been expected to be larger for fewer plants per pot, but this was not the case for leaf area and fresh and dry weight (data not presented). These plants did have a mean disease score of 4 (Rittmo et al., 2008), indicating the presence of wire stem but prior to plant wilting or dying (Figure 3.6). It is possible therefore that the pathogen was restricting the supply of water and nutrients to the plants, thus impeding growth.

This study showed that *R. solani* strain (043-4) was more virulent than *R. solani* (043-1). Woodhall et al. (2008), reported that subsets or subgroups of AG-2-1 with different virulence have previously been defined using IGS1 fragment analysis. Those with shorter IGS1 lengths and similar cultural appearance were less virulent than those with longer IGS sequences. However, there were limitations to the IGS1 sequencing as multiple IGS1 sequences were found in a single AG-2-1 isolate (RS043-1). This suggested that an alternative method is required to identify different virulence types in AG-2-1 (Das et al., 2014).

In conclusion, the damage caused to Asian cabbage production by *R. solani* in this glasshouse experiment was similar to that reported by Lee (2018) for radish. However, *R. solani* isolate (043-4) was more damaging than *R. solani* isolate (043-1). The inoculum rate of 0.25g of *R. solani* isolate (043-4) resulted in a 50% survival of seedlings in variety Green Ace F1, and this was the rate selected to be used in Experiment 2.

## Chapter 4: Seed treatment with Trichoderma

### 4.1 Introduction

Integrated pest management (IPM) is a sustainable approach developed for reducing pests or diseases. It involves integrating different types of control options ( e.g. cultural, biological, chemical, mechanical and physical controls) (Paul & Page, 2008; Shamsudin et al., 2010). In many production systems, fungicides are used to sustain quality food production and are effective at minimizing pathogen attacks in the short term. They are primarily used as seed treatments or foliar sprays onto growing crops (Hideo, 2006). However, pathogens may become resistant to agrichemicals and agrichemicals can cause damage to the environment when used excessively. The use of bio-control agents may be an alternative to the use of synthetic chemical compounds.

Seed coating with plant beneficial microbes (PBM) may promote crop growth and yield and protect *against* pathogens. Seed coating has been studied using various species of plant growth-promoting bacteria (e.g. Rhizobia) and mycorrhizal fungi ( e.g. *Trichoderma* (Rocha et al., 2019). *Trichoderma* isolates were identified as effective bio-control agents (BAC) against several common phytopathogens because of their characteristics, antagonism and plant growth stimulation (Cavalcante et al., 2007; Shahnaz et al., 2022).

Elad and Ilan (1982) reported that coating cotton seeds with *T. hamatum* and *T. harzianum* reduced the incidence of disease caused by *R. solani* by 83 % in the green house and 47 – 60 % in field experiments. In addition, Lee (2018) selected four potential *Trichoderma* strains for control of *R. solani* in radish. They were found to promote plant growth and enhance plant health in glasshouse and field experiments.

For this experiment, the four *Trichoderma* strains (LU785, LU132, LU1347 and LU1358) selected by Lee (2018) were formulated into seed coats and tested for their biocontrol ability against *R. solani* in potting mix. Seeds of the two Asian cabbage varieties, Pak Choi: Green Ace F1 and Pak Choi: Mei Qing Choi were coated and grown in Lincoln University's glasshouse.

## 4.2 Materials and methods

### 4.2.1 Culturing of Trichoderma isolates

The four Trichoderma isolates (LU132: *T. atroviride*, LU785: *T. hamatum*, LU1347: *T. harzianum* and LU1358: *T. polysporum*) were sub-cultured from cultures in the Lincoln University microbial collection, on Malt Yeast Extract agar (MYE) as outlined in Appendix 19. In the laminar flow cabinet, a sterilized hockey stick was used to spread spores from each Trichoderma treatment across the MYE agar in a zig zag pattern. Six petri dishes of each isolate were sealed with parafilm and enclosed in a sterile zip lock bag and placed in a growth room for 14 days at approximately 25 °C to produce spores.

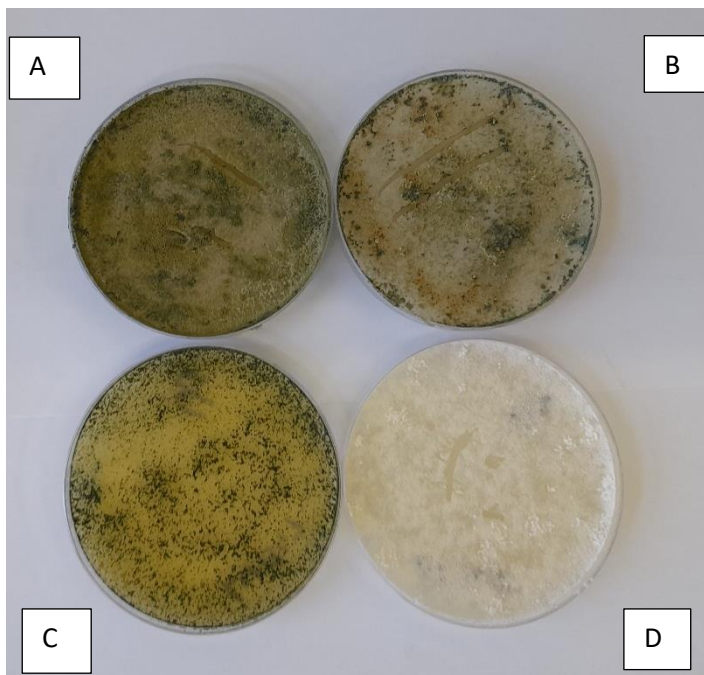


Figure 4.1 The four Trichoderma isolates on MYE after 14 days in the growth room. A: *T. atroviride* ( LU132), B: *T. hamatum* (LU785), C: *T. harzianum* (LU1347), and D: *T. polysporum* (LU1358)

### 4.2.2 Harvesting Trichoderma spores

Spores were harvested by pouring approximately 5ml of sterile water on top of the MYE plates and scraping the surface with a sterilized hockey stick to dislodge the spores. The spore suspension was then poured into 15ml falcon tubes. This procedure was repeated two more times. The falcon tubes were labelled and centrifuged ('Centrifuge 5810R') at 21 °C for 10 minutes at 3500 RPM, to separate the water and spores. The centrifuged falcon tubes were placed in ice for 20 minutes for the spores to settle to the base of the tubes. The top

layer of water was decanted off by tipping the tube. Sterilized water (0.5ml) was then added to each tube using a pipette and the tube vortexed for 10 seconds to mix.

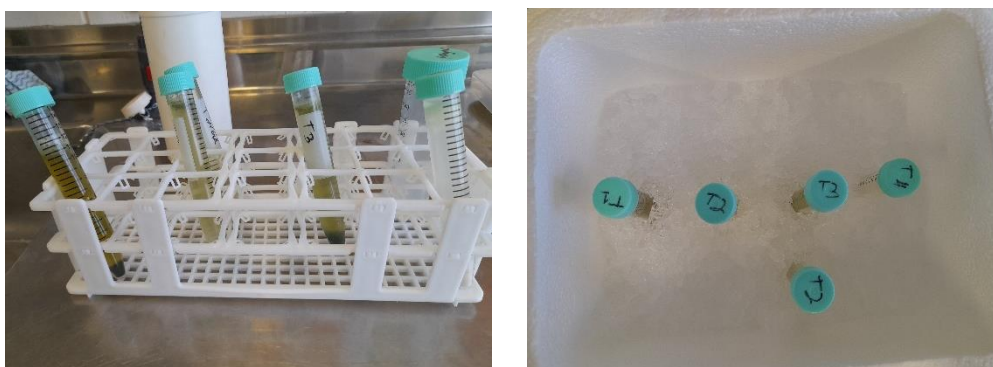


Figure 4.2 The four *Trichoderma* isolates in the 10ml falcon tubes after centrifuging (left) and the tubes were placed in ice for 20 minutes (right).

#### 4.2.3 Seed coating with *Trichoderma*

The seeds of the two Asian cabbage varieties (Mei Qing Choi and Green Ace F1) were coated with each of the four *Trichoderma* isolates and a seed coating polymer (Seedworx-Biofreindly-1) according to the procedure of Lee (2018). Due to the smaller amount of polymer needed for this experiment, a stock solution was produced by mixing 7  $\mu$ l of polymer with 12.5mL water in a 15ml Falcon tube, then vortexing for 1 minute. Seeds (0.5g) were placed in four 15ml falcon tubes, followed by each *Trichoderma* spore suspension, then the tubes were vortexed for 3 minutes. Then 12.5  $\mu$ l of polymer stock solution was added to the seed and vortexed for another 3 minutes. The seeds were tipped into petri plates in the laminar flow and stirred periodically as the coat dried over two hours. Then the plates were sealed with parafilm and placed in a sterilized plastic container and stored at 4°C until sowing the next day.

#### 4.2.4 Spore concentrations

Ten seeds of both varieties coated with each *Trichoderma* treatment were put into 15ml falcon tubes with 10ml sterilized water and mixed using a vortex for 1 minute. The spore concentration for each treatment was determined using a Neuman-Boyer haemocytometer as outlined in Appendix 20. *Trichoderma* spore concentration ranged between  $1.66 \times 10^7$  and  $6.28 \times 10^7$  (Table 4.1 and 4.2). For cv. Mei Qing Choi, the number of *Trichoderma* spores per

seed ranged from  $1.66 \times 10^6$  to  $6.28 \times 10^6$  while for cv. Green Ace F1 they ranged from  $1.65 \times 10^6$  to  $5.27 \times 10^6$  (Table 4.1 and 4.2).

Table 4.1 Haemocytometer counts, spore concentration ( $\text{ml}^{-1}$ ) and spores per seed of the four *Trichoderma* isolates on Mei Qing Choi seeds.

Mei Qing Choi	Haemocytometer counts	Spore concentration ( $\text{ml}^{-1}$ )	Spores per seed
Treatments			
T1 (LU132)	22	5.52E+07	5.52E+06
T2 (LU785)	25	6.28E+07	6.28E+06
T3 (LU1347)	15.8	3.97E+07	3.97E+06
T4 (LU1358)	6.6	1.66E+07	1.66E+06

Table 4.2 Haemocytometer counts, spore concentrations ( $\text{ml}^{-1}$ ) and spores per seed of the four *Trichoderma* isolates on Green Ace F1 seeds.

Green Ace F1	Haemocytometer counts	Spore concentration ( $\text{ml}^{-1}$ )	Spores per seed
Treatments			
T1 (LU132)	18.4	4.61E+07	4.61E+06
T2 (LU785)	21	5.27E+07	5.27E+06
T3 (LU1347)	12.4	3.11E+07	3.11E+06
T4 (LU1358)	6.6	1.65E+07	1.65E+06

#### 4.2.5 Apron XL<sup>®</sup> fungicide seed coating and control treatments

'Apron<sup>®</sup> XL', a fungicide seed treatment was used to compare chemical verses biological control of *R. solani*. This product contains 350g / litre metalaxyl - M in the form of a suspension concentrate. It was applied as a seed coating at the recommended rate of 75ml in 500ml water per 100kg seed (Holden, 2022). The required amount of Apron<sup>®</sup> XL was added to 12.5ml water, then vortexed for 13 minutes. Seed (0.5g) was placed in one 15ml falcon tubes, followed by 0.01288mL of Apron<sup>®</sup> XL stock suspension and vortexed for 3 minutes. Finally, 12.51  $\mu\text{l}$  of polymer stock solution was added to the seed and vortexed for 1 minute. The fungicide treated seeds were tipped into petri plates in the laminar flow cabinet and stirred periodically for two hours to dry the seeds. The plate was then sealed with parafilm and stored at 4°C until sowing the next day. For the control treatment, 12.51  $\mu\text{l}$  of polymer

stock solution was added to 0.5g bare seeds and vortexed for 1 minute then dried in the laminar flow for two hours.

#### 4.2.6 Glass house experiment (Trichoderma seed coating)

The second glass house experiment was conducted using the method described in Experiment 1. *R. solani* isolate (043-4) at the rate of 0.25g inoculated wheat bran-peat per 100g seed raising mix was chosen because it killed approximately 50% of seedlings in Experiment 1. Each pot (1.9L) was filled with 1000g of growing medium (Appendix 2), then 300g of *R. solani* inoculated seed raising mix was applied to the top (Figure 4.3). Seven seeds were sown per pot, at a depth of 1cm and with 2 to 3 cm between the seeds which were arranged in a circle. The sown pots were arranged in a randomized complete block design (RCBD) in the Lincoln University glasshouse, maintained at a temperature range between 10 to 25 °C and watered as required.

There was a total of 144 pots (2 Asian cabbage varieties x 6 treatments x 6 replicates). The treatments were four Trichoderma coated seed treatments; LU132, LU785, LU1347 and LU1358, one bare seed treatment and one Apron ® XL fungicide seed treatment all  $\pm$  *R. solani*.



Figure 4.3 Seed raising mix containing *R. solani* inoculated wheat bran - peat (left) and 1.9L pots filled with 1000g potting mix and topped with 300g of the *R. solani* inoculated seed raising mix (right).

#### 4.2.7 Data collection

##### Seedling emergence and chlorophyll concentrations.

Seedling emergence was counted every second day for 30 days and the number of healthy seedlings, diseased seedlings, post emergence seedling deaths and surviving seedlings were

recorded. At 20 and 50 days after planting, healthy leaves with a size greater than 30 x 30mm were randomly selected and the chlorophyll concentration was measured with a 'KONICA MINOLTA' SPAD-502 meter (Figure 4.4).



Figure 4.4 Ten randomly leaves were selected per pot for chlorophyll measurement. Indicative leaves are marked with yellow stars (left). A SPAD-502 meter was used to measure the chlorophyll concentration (right).

### **Diseased seedlings and surviving plants**

At 60 days after planting, disease was scored using the disease score categories of 0 to 5 described in Experiment One. Each plant was then harvested, and the roots washed under running water for approximately one minute to remove the potting mix material. The roots were dried with paper towels. Plant parameters recorded included shoot length (cm), root length (cm) and hypocotyl diameter (mm) which was measured using a 'Carbon Fibre Composite' digital calliper. The number of leaves on the surviving plants were counted and the leaf area (cm<sup>2</sup>) was measured using a Li-Cor-3100C<sup>®</sup> leaf area meter. Plant shoots, hypocotyls, and fibrous roots were cut using scissors and the fresh weight of each part was obtained using an 'FX-3000i' electronic scale. The separate plant parts were then dried in the oven at 65 °C for three days and the dry weights recorded.

### **Trichoderma root colonization**

Six plants from all the treatments except Apron were selected for Trichoderma root colonization measurements after the fresh weights were recorded. Thirty randomly selected pieces of root (7 to 10 mm long) were harvested using scissors. Root samples were sealed in zip lock bags and stored at 4°C until tested the next day. In a laminar flow cabinet root pieces were soaked in 1% (v/v) Virkon (50mL) for 10 minutes in petri dishes then removed

and rinsed with sterilized water. Root samples were dried on filter papers (Whatman1 diameter 90mm) for 5 minutes and 6 root pieces were placed onto five prepared Trichoderma selective medium (TSM) plates (Appendix 21). The plates were put into sterile plastic trays, sealed with autoclave bags, and stored in the Trichoderma growth room at 25°C. The plates were assessed for growth of Trichoderma colonies between 7 and 14 days later (Figure 4.5).

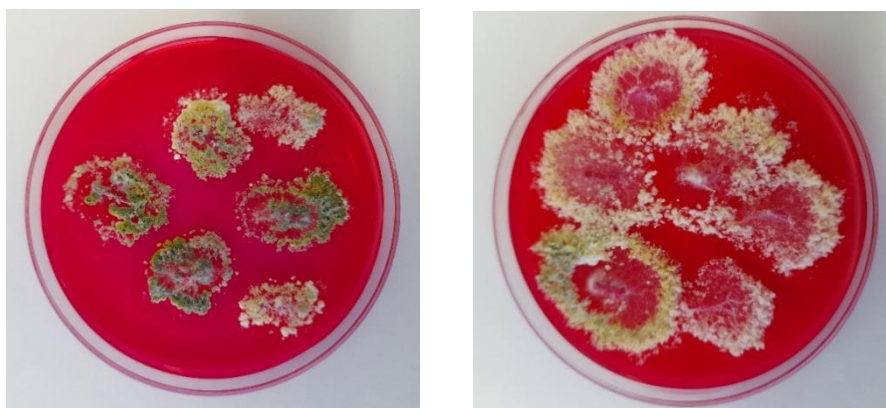


Figure 4.5 Colony growth of T1: LU132 (*T. atroviride*) (left) and T3: LU1347 (*T. harzianum*) (right) on root pieces of variety Mei Qing Choi at 14 days after plating on TSM media.

## 4.3 Results

### 4.3.1 Seedling emergence

#### Variety Mei Qing Choi

Seedling emergence in variety Mei Qing Choi in the presence of *R. solani* occurred between day 4 and 10 after sowing (Figure 4.6). Initially all the seed treatments except LU1347 had greater emergence than the control (Figure 4.6). Some seedling deaths occurred in all the treatments beginning at day 17 (Figure 4.6).

Seedling emergence in variety Mei Qing Choi with no *R. solani* also occurred between day 4 and 10 after sowing, with again the control and LU1347 having slightly less rapid emergence. There were no post-emergence seedling deaths (Figure 4.7)

Variety Mei Qing Choi in the presence of *R. solani*

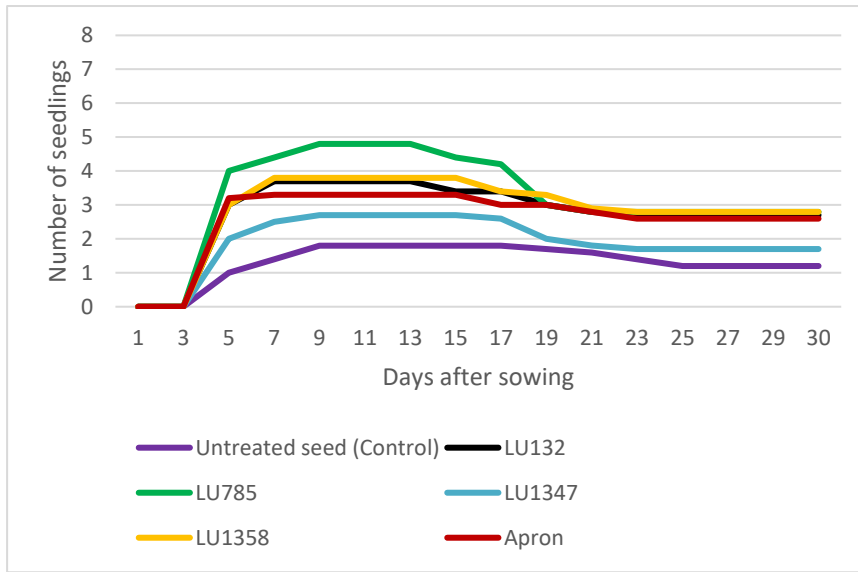


Figure 4.6 Effect of seed treatment on the number of seedlings per pot (out of 7 seeds sown) between 1 and 30 DAS for variety Mei Qing Choi in the presence of *R. solani* at the Horticulture Research Glasshouse, Lincoln University.

Variety Mei Qing Choi with no *R. solani*

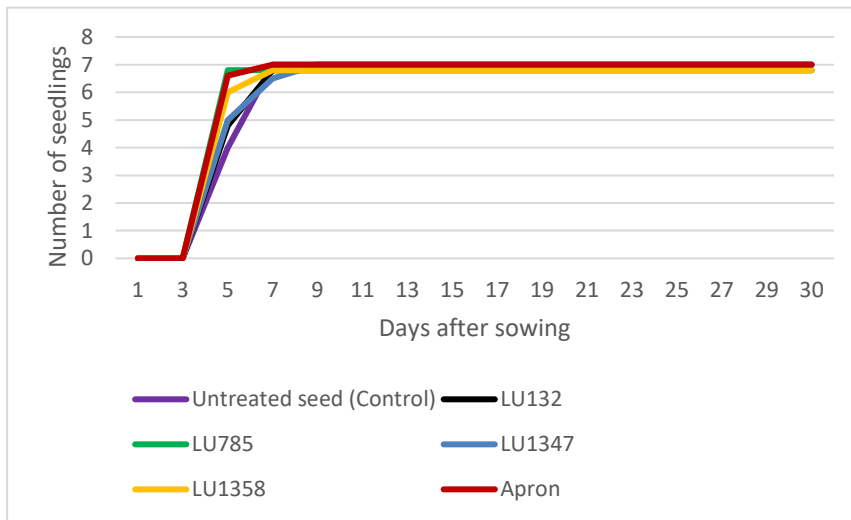


Figure 4.7 Effects of seed treatment on the number of seedlings per pot (out of 7 seeds sown) between 1 and 30 DAS for variety Mei Qing Choi with no *R. solani* after sowing at the Horticulture Research Glasshouse, Lincoln University.

### Variety Green Ace F1

Seedling emergence in variety Green Ace F1 in the presence of *R. solani* occurred between day 4 and 9 after sowing (Figure 4.8). Some seedling death occurred after day 15 for all the treatments except LU132 (Figure 4.8).

Seedling emergence in variety Green Ace F1 with no *R. solani* also occurred between day 4 and 9 after sowing, and there were no seedling losses once maximum emergence had been reached (Figure 4.9).

Variety Green Ace F1 in the presence of *R. solani*

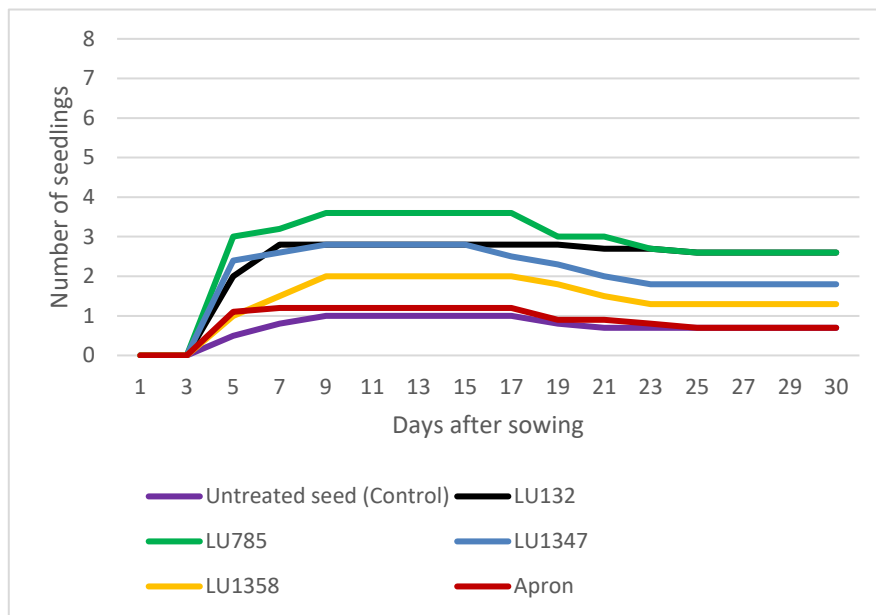


Figure 4.8 Effect of seed treatment on the number of seedlings per pot (out of 7 seeds sown) between 1 and 30 DAS for variety Green Ace F1 in the presence of *R. solani* at the Horticulture Research Glasshouse, Lincoln University.

### Variety Green Ace F1 with no *R. solani*

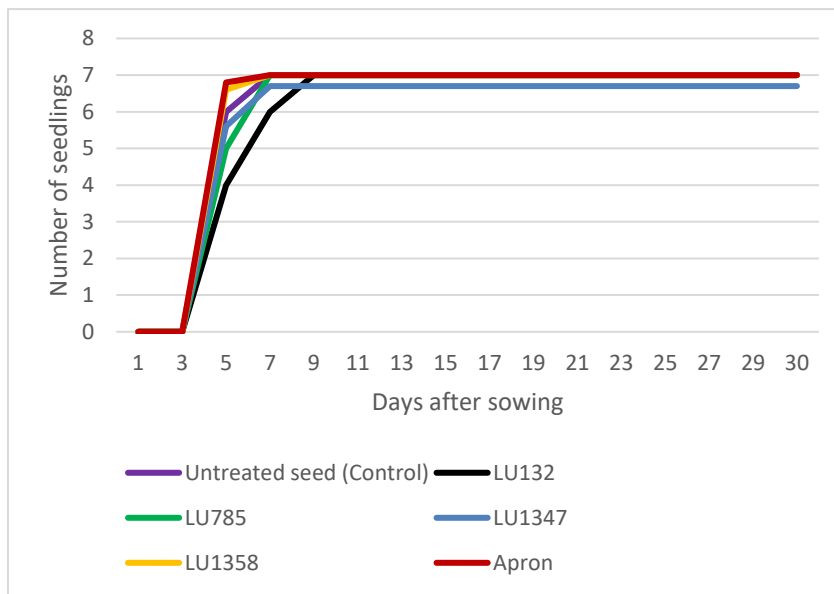


Figure 4.9 Effect of seed treatment on the number of seedlings per pot (out of 7 seeds sown) between 1 and 30 DAS for variety Green Ace F1 with no *R. solani* at the Horticulture Research Glasshouse, Lincoln University.

#### 4.3.2 Assessment at 30 DAS

##### Maximum seedling emergence, seedlings which died post emergence, surviving seedlings, diseased seedlings and healthy seedlings

In the presence of the pathogen maximum seedling emergence for variety Mei Qing Choi was only 1.8 per pot for the control, and this did not differ from that of LU1347 (Table 4.3). LU785 had a greater emergence than the control, Apron and LU1347, but did not differ from LU132 and LU1358 (Table 4.3).

At 30 DAS the number of seedlings which had died post emergence in the presence of the pathogen also differed ( $P < 0.05$ ) among the treatments for variety Mei Qing Choi (Appendix 23). LU785 had more post emergence seedling deaths than the control and Apron, but this did not differ from the other treatments (Table 4.3).

There was a significant difference ( $P < 0.05$ ) among the treatments for variety Mei Qing Choi with the number of surviving seedlings at 30 DAS. LU785, LU1358, LU132 and Apron had a significantly greater number of surviving seedlings per pot than the control, but LU1347 did not differ from the control (Table 4.3).

The number of diseased and healthy seedlings did not differ among the treatments with *R. solani* for variety Mei Qing Choi at 30 DAS (Table 4.3).

Table 4.3 Effect of seed treatment on maximum seedling emergence per pot, and at 30 DAS seedlings which died post emergence, surviving seedlings and diseased and healthy seedlings per pot for variety Mei Qing Choi in the presence of *R. solani*

Treatments	Maximum seedling emergence	Seedlings which died post emergence	Surviving seedlings	Diseased seedlings	Healthy seedlings
Control	1.8 c	0.6 b	1.2 b	0.7 a	0.5 a
LU132	3.7 ab	1.0 ab	2.7 a	1.4 a	1.3 a
LU785	4.8 a	2.0 a	2.8 a	1.0 a	1.8 a
LU1347	2.7 bc	1.0 ab	1.7 ab	0.7 a	1.0 a
LU1358	3.8 ab	1.0 ab	2.8 a	0.8 a	2.0 a
Apron	3.3 b	0.7 b	2.6 a	1.0 a	1.6 a
LSD 5%	1.3	1.2	1.2	0.9	1.5
Significance	P<0.001	P<0.05	P<0.05	NS	NS

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

In the absence of the pathogen for variety Mei Qing Choi there were no significant differences for maximum seedling emergence, and at 30 DAS for post emergence seedling death, seedling survival or diseased and healthy seedlings among all the treatments (Table 4.4). There was a significant difference (P<0.001) between pathogen and no pathogen for variety Mei Qing Choi for the maximum number of emerged seedlings (Appendix 22). In the presence of the pathogen there was a mean of 3.4 emerged seedlings and with no pathogen, a mean of 6.9 emerged seedlings. There was also a significant interaction (P<0.001) between seed treatments and pathogen for the maximum number of emerged seedlings for variety Mei Qing Choi. Seed treatments with pathogen had a lower mean number of emerged seedlings than seed treatments with no pathogen (Appendix 22).

The number of seedlings which died post emergence differed (P<0.001) between pathogen and no pathogen for variety Mei Qing Choi (Appendix 23). A mean of 1.0 seedling was lost in the presence of the pathogen, but with no pathogen there were no seedling losses.

There was a significant interaction (P<0.001) between seed treatments and the pathogen for the mean number of seedlings which died post emergence for Mei Qing Choi. Seed

treatments with the pathogen had a higher number of seedling losses than seed treatments without the pathogen (Appendix 23).

There was a significant difference ( $P < 0.001$ ) between pathogen and no pathogen for the number of surviving seedlings for variety Mei Qing Choi (Appendix 24). A mean of 2.3 seedlings survived in the presence of the pathogen but with no pathogen, a mean of 6.9 seedlings survived (Appendix 24). There was also a significant ( $P < 0.05$ ) interaction between the seed treatments and the pathogen for the number of surviving seedlings (Appendix 24). Seed treatments with no pathogen had a higher number of surviving seedlings than the treatments with the pathogen (Appendix 24).

The number of diseased seedlings differed ( $P < 0.001$ ) between pathogen and no pathogen (Appendix 25). There was a mean of 0.9 diseased seedlings in the presence of the pathogen, but no disease occurred on the seedlings in the absence of pathogen (Appendix 25). There was no significant interaction between seed treatments and pathogen for the number of diseased seedlings for variety Mei Qing Choi (Appendix 25).

The number of healthy seedlings differed between the pathogen and no pathogen (Appendix 26). In the presence of the pathogen, there was a mean of 1.4 healthy seedlings and with no pathogen a mean of 6.9 healthy seedlings for variety Mei Qing Choi (Appendix 26). There was no significant interaction between seed treatments and pathogen for the number of healthy seedlings (Appendix 26).

Table 4.4 Effect of seed treatment on maximum seedling emergence per pot, and at 30 DAS, seedlings which died post emergence, surviving and, diseased and healthy seedlings per pot for variety Mei Qing Choi with no *R. solani*.

Treatments	Maximum seedling emergence	Seedlings which died post emergence	Surviving seedlings	Diseased seedlings	Healthy seedlings
Control	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU132	6.8 a	0.0 a	6.8 a	0.0 a	6.8 a
LU785	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU1347	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU1358	6.8 a	0.0 a	6.8 a	0.0 a	6.8 a
Apron	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LSD 5%	0.3	0.0	0.3	0.0	0.3
Significance	NS	NS	NS	NS	NS

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

There was a significant difference ( $P < 0.05$ ) among the treatments in the presence of *R. solani* for variety Green Ace F1 for the maximum number of emerged seedlings (Table 4.5). LU785 had the highest number of seedlings emerged but this did not differ from the number for the other three Trichoderma isolates; however, LU785 had a greater maximum seedling emergence than the control and Apron (Table 4.5).

The number of seedlings which died post emergence and the number of diseased seedlings did not differ among the treatments with *R. solani* for variety Green Ace F1 (Table 4.5). However, the number of surviving seedlings differed among the treatments ( $P < 0.05$ ) at 30 DAS (Table 4.5). The four Trichoderma isolates had higher number of surviving seedlings than the control and Apron (Table 4.5). LU132 and LU785 had a higher number of surviving seedlings at 30 DAS than the control, Apron and LU1358 (Table 4.5). The number of healthy seedlings differed among the treatments (Table 4.5). LU132 and LU785 had a higher number of healthy seedlings than the control and Apron at 30 DAS (Table 4.5).

Table 4.5 Effect of seed treatment on maximum seedling emergence per pot, and at 30 DAS seedlings which died post emergence, surviving seedlings and diseased and healthy seedlings per pot for variety Green Ace F1 in the presence of *R. solani*

Treatments	Maximum seedling emergence	Seedlings which died post emergence	Surviving seedlings	Diseased seedlings	Healthy seedlings
Control	1.0 b	0.3 a	0.7 b	0.5 a	0.2 b
LU132	2.8 ab	0.2 a	2.6 a	0.6 a	2.0 a
LU785	3.6 a	1.0 a	2.6 a	0.8 a	1.8 a
LU1347	2.8 ab	1.0 a	1.8 ab	0.8 a	1.0 ab
LU1358	2.0 ab	0.7 a	1.3 b	0.0 a	1.3 ab
Apron	1.2 b	0.5 a	0.7 b	0.2 a	0.5 b
LSD 5%	1.8	1.6	1.2	0.9	1.1
Significance	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0.05$

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

In the absence of the pathogen there were no significant differences for maximum seedling emergence among the treatments for variety Green Ace F1 (Table 4.6). The number of seedlings which died post emergence, surviving seedlings and diseased seedlings and healthy also did not differ among the treatments for variety Green Ace F1 at 30 DAS (Table

4.6). There was a significant difference ( $P < 0.001$ ) between pathogen and no pathogen for the maximum number of emerged seedlings for variety Green Ace F1 (Appendix 22). There was a mean of 2.2 emerged seedlings in the presence of the pathogen, and with no pathogen a mean of 6.9 emerged seedlings (Appendix 22). There was also a significant ( $P < 0.05$ ) interaction between the seed treatments and pathogen for the number of emerged seedlings (Appendix 22). Seed treatments with no pathogen had a higher mean number of emerged seedlings than the treatments with the pathogen (Appendix 22.).

The number of seedlings which died post emergence differed ( $P < 0.001$ ) between pathogen and no pathogen for variety Green Ace F1 (Appendix 23). A mean of 0.6 seedlings were lost in the presence of the pathogen, but with no pathogen there were no seedlings lost (Appendix 23). There was no significant interaction between the seed treatments and pathogen for the seedlings which died post emergence (Appendix 23).

There was a significant difference ( $P < 0.001$ ) for the number of surviving seedlings between pathogen and no pathogen for variety Green Ace F1 (Appendix 24). A mean of 1.6 seedlings survived in the presence of the pathogen, but with no pathogen a mean of 6.9 seedlings survived (Appendix 24). There was also a significant interaction ( $P < 0.05$ ) between the seed treatments and pathogen for the surviving seedlings for variety Green Ace F1 (Appendix 24). Seed treatments with the pathogen had a lower mean number of surviving seedlings than the treatments with no pathogen (Appendix 24).

The number of diseased seedlings differed ( $P < 0.001$ ) between pathogen and no pathogen for variety Green Ace F1 (Appendix 25). A mean of 0.5 seedlings were diseased in the presence of the pathogen but with no pathogen there were nil diseased seedlings (Appendix 25).

There was no significant interaction between seed treatments and pathogen for diseased seedlings for variety Green Ace F1 (Appendix 25). There was a significant difference in the number of healthy seedlings for variety Green Ace F1 between pathogen and no pathogen (Appendix 26). There was a mean of 1.1 healthy seedlings in the presence of the pathogen and a mean of 6.9 healthy seedlings in the absence of the pathogen (Appendix 2.6). There also a significant ( $P < 0.05$ ) interaction between the seed treatments and pathogen. The seed treatments with no pathogen had a higher mean number of healthy seedlings than the treatments with the pathogen (Appendix 26).

Table 4.6 Effect of seed treatment on maximum seedling emergence per pot, and at 30 DAS, seedlings which died post emergence, surviving seedlings and, diseased and healthy seedlings per pot for variety Green Ace F1 with no *R. solani*

Treatments	Maximum seedling emergence	Seedlings which died post emergence	Surviving seedlings	Diseased seedlings	Healthy seedlings
Control	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU132	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU785	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LU1347	6.7 ab	0.0 a	6.7 b	0.0 a	6.7 b
LU1358	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
Apron	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a
LSD 5%	0.3	0.0	0.3	0.0	0.3
Significance	P<0.05	NS	P<0.05	NS	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

#### **SPAD value (Chlorophyll concentration) at 20 DAS**

In the presence of the pathogen there were no significant differences among the treatments in SPAD value for variety Mei Qing Choi at 20 DAS (Figure 4.10). However, there was a significant difference in SPAD value for variety Green Ace F1 (Figure 4.10). LU132 had a higher SPAD value than the control, but not the other four treatments which did not differ from the control (Figure 4.10).

The SPAD values did not differ among the treatments in the absence of the pathogen in either variety at 20 DAS (Figure 4.11).

The SPAD value differed between pathogen and no pathogen for both varieties (Appendix 27). In both varieties in the absence of the pathogen the SPAD value was higher than in the presence of pathogen (Appendix 27). However, there was no significant interaction between seed treatments and pathogen within each variety (Appendix 27).

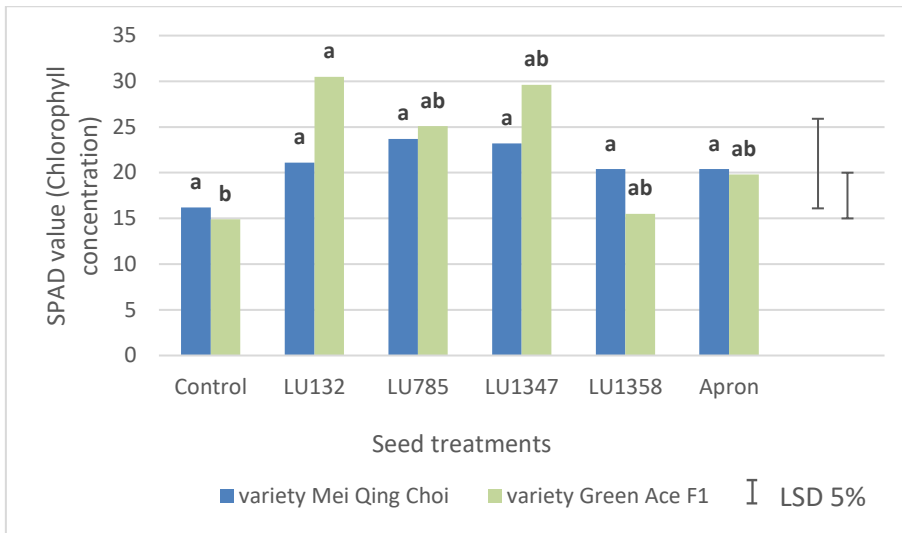


Figure 4.10 Effect of seed treatment on SPAD value (leaf chlorophyll concentration) at 20 DAS for varieties Mei Qing Choi and Green Ace F1 in the presence of *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a significantly different at P=0.05

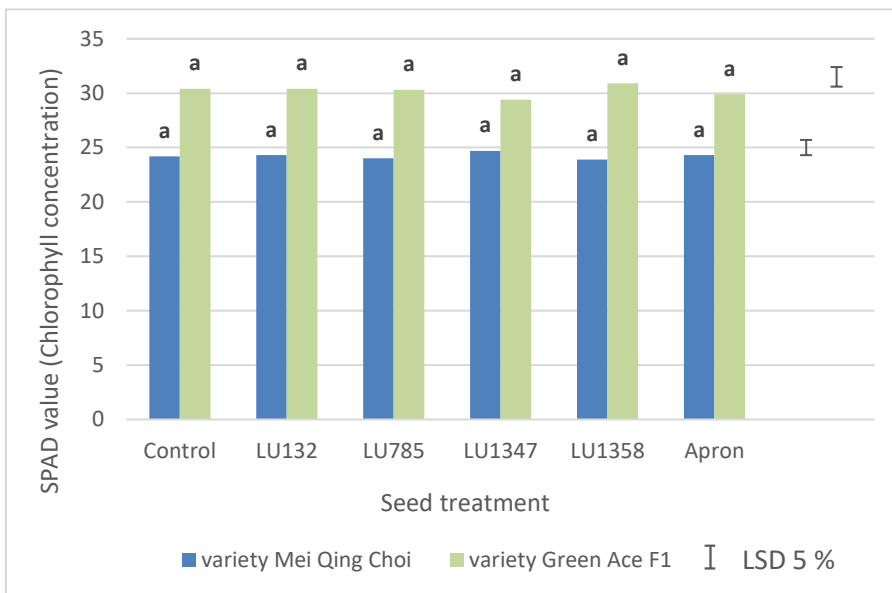


Figure 4.11 Effect of seed treatment on SPAD value (chlorophyll concentration) at 20 DAS for varieties Mei Qing Choi and Green Ace F1 with no *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level uncorrected LSD procedure within each variety. Means with the same letter are not significantly different at P= 0.05.

### 4.3.2 Assessment at 60 DAS

#### Number of surviving plants, dead plants, diseased plants, healthy plants and disease score.

In the presence of the pathogen the number of surviving plants for variety Mei Qing Choi differed ( $P < 0.05$ ) among the treatments at 60 DAS (Table 4.7). LU785 had more surviving plants than the control and LU1347, but the other three treatments did not differ from the control (Table 4.7). There was a difference ( $P < 0.05$ ) among the treatments for the number of

dead plants at 60 DAS (Table 4.7). LU132 had more dead plants than the control and LU785 but did not differ from that of the other treatments (Table 4.7). The number of diseased plants and disease score did not differ among the treatments at 60 DAS (Table 4.7). However, the healthy plants differed among the treatments at 60 DAS (Table 4.7) with LU785 and LU1358 having more healthy plants than the control and other treatments (Table 4.7).

Table 4.7 Effect of seed treatment on the number of surviving plants, dead plants, diseased plants, healthy plants, and disease score per pot at 60 DAS for variety Mei Qing Choi in the presence of *R. solani*.

Treatments	Number of surviving plants	Number of dead plants	Number of diseased plants	Number of healthy plants	Disease Score
Control	1.2 b	0.0 b	0.7 a	0.5 b	2.0 a
LU132	1.8 ab	0.9 a	0.5 a	1.3 ab	2.0 a
LU785	2.8 a	0.0 b	1.0 a	1.8 a	2.0 a
LU1347	1.3 b	0.4 ab	0.3 a	1.0 ab	1.0 a
LU1358	2.5 ab	0.3 ab	0.5 a	2.0 a	1.0 a
Apron	2.3 ab	0.3 ab	0.7 a	1.6 ab	2.0 a
LSD 5%	1.4	0.8	0.9	1.1	1.8
Significance	P<0.05	P<0.05	NS	P<0.05	NS

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05. The disease score was assessed using a disease score of 0 to 5.

In the absence of the pathogen the number of surviving plants, dead, diseased, and healthy plants as well as disease score for variety Mei Qing Choi did not differ among the treatments at 60 DAS (Table 4.8).

The number of surviving plants differed (P<0.001) between pathogen and no pathogen for variety Mei Qing Choi (Appendix 28). A mean of 2.0 plants survived in the presence of the pathogen and a mean of 6.9 survived with no pathogen (Appendix 28). There was a significant interaction between seed treatments and pathogen. Seed treatments with no pathogen had a higher number of surviving plants per pot than the treatments with the pathogen (Appendix 28).

There was a difference (P<0.05) between pathogen and no pathogen for the number of dead plants (Appendix 29). Plant losses occurred in the presence of the pathogen but with no pathogen losses did not occur. There was an interaction between the seed treatments and the pathogen as plant deaths were higher for seed treatments with the pathogen than

without the pathogen (Appendix 29). There were significant differences in the number of diseased plants and disease score between pathogen and no pathogen (Appendix 30 and Appendix 32). The number of diseased plants and disease score were higher in the presence than in the absence of the pathogen. There were no interactions between the seed treatments and the pathogen (Appendix 30 and Appendix 32). There was a significant difference between pathogen and no pathogen for the number of healthy plants (Appendix 31). With no pathogen, the number of healthy plants was higher than with the pathogen. There was also an interaction between the seed treatments and the pathogen. Treatments with no pathogen had a higher number of healthy plants than treatments in the presence of the pathogen (Appendix 31).

Table 4.8 Effect of seed treatment on the number of surviving plants, dead plants, diseased plants, healthy plants and disease scores per pot at 60 DAS for variety Mei Qing Choi with no *R. solani*.

Treatments	Number of surviving plants	Number of dead plants	Number of diseased plants	Number of healthy plants	Disease score
(Control)	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU132	6.8 a	0.0 a	0.0 a	6.8 a	0.0 a
LU785	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU1347	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU1358	6.8 a	0.0 a	0.0 a	6.8 a	0.0 a
Apron	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LSD 5%	0.3	0.0	0.0	0.3	0.0
Significance	NS	NS	NS	NS	NS

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05. The disease score was assessed using a disease score of 0 to 5.

The number of surviving plants for variety Green Ace F1 differed ( $P < 0.05$ ) among the treatments with the presence of pathogen at 60 DAS (Table 4.9). LU132 and LU785 had more surviving plants than all the other treatments while LU1347 and LU1358 had more surviving plants than the control and Apron at 60 DAS (Table 4.9). There was also a significant difference in the number of dead plants among the treatments (Table 4.9). LU1358, control and Apron had no plant loss at 60 DAS while LU1347 had significantly more than for those three treatments (Table 4.9). There were no differences in the number of diseased plants among the treatments at 60 DAS (Table 4.9), but the disease score did differ among the treatments. The control had the highest disease score of 2 at 60 DAS and this differed from

Apron and LU1347 which had a zero-disease score. Trichoderma isolates LU132, LU785 and LU1358 had a disease score between 0 to 1 at 60 DAS which did not differ from the control (Table 4.9).

The number of healthy plants differed among the treatments at 60 DAS (Table 4.9). LU132 and LU785 had a higher number of healthy plants than the control and the other three treatments (Table 4.9).

Table 4.9 Effect of seed treatment on the number of surviving plants, dead plants, diseased plants, healthy plants, and disease score per pot at 60 DAS for variety Green Ace F1 in the presence of *R. solani*.

Treatments	Number of surviving plants	Number of dead plants	Number of diseased plants	Number of healthy plants	Disease score
Control	0.7 c	0.0 b	0.5 a	0.2b	2.0 a
LU132	2.3 a	0.3 ab	0.3 a	2.0 a	1.0 ab
LU785	2.0 a	0.6 ab	0.2 a	1.8 a	1.0 ab
LU1347	1.0 b	0.8 a	0.0 a	1.0 b	0.0 b
LU1358	1.3 b	0.0 b	0.3 a	1.0 b	0.7 ab
Apron	0.5 c	0.0 b	0.0 a	0.5 b	0.0 b
LSD 5%	1.3	0.7	0.7	1.4	1.8
Significance	P<0.05	P<0.05	NS	P<0.05	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05. The disease score was assessed using a disease score of 0 to 5.

There were no significant differences for the number of surviving, dead, diseased, and healthy plants, and disease score among the treatments in the absence of the pathogen for variety Green Ace F1 at 60 DAS (Table 4.10).

The number of surviving plants differed between pathogen and no pathogen for variety Green Ace F1 (Appendix 28). A mean of 2.0 plants survived in the presence of the pathogen and a mean of 6.9 plants survived in the absence of the pathogen (Appendix 28). There was significant interaction between the seed treatments and pathogen as treatments with the pathogen had a lower number of surviving plants than treatments without the pathogen (Appendix 28).

There was a difference among the pathogen and no pathogen for the number of dead plants (Appendix 29). Plant losses occurred in the presence of the pathogen but without the pathogen, there was no plant loss (Appendix 29). There was a significant interaction between the seed treatments and the pathogen. Seed treatments with the pathogen had more dead plants than treatments without the pathogen (Appendix 29).

There was a significant interaction between pathogen and no pathogen for the number of diseased plants at 60 DAS (Appendix 30). Diseased developed in the presence of the pathogen, but with no pathogen, no disease developed on the plants (Appendix 30). There was no significant interaction between seed treatments and the pathogen (Appendix 30). The number of healthy plants differed between pathogen and no pathogen (Appendix 31). In the presence of the pathogen there was a mean of 1.1 healthy plants but without the pathogen the mean was 6.9 healthy plants (Appendix 31). There was an interaction between seed treatments and pathogen. Seed treatments with the pathogen had a lower number of healthy plants than treatments without pathogen (Appendix 31). The disease scores differed between pathogen and no pathogen (Appendix 32). All the seed treatments in the presence of the pathogen had diseased plants but the treatments with no pathogen had no diseased plants (Appendix 32). There was a significant interaction between seed treatments and the pathogen (Appendix 32) as treatments in the presence of the pathogen had plants with higher disease scores than treatments without the pathogen (Appendix 32).

Table 4.10 Effect of seed treatment on the number of surviving plants, dead plants, diseased plants, healthy plants, and disease scores per pot at 60 DAS for variety Green Ace F1 with no *R. solani*

Treatments	Number of surviving plants	Number of dead plants	Number of diseased plants	Number of healthy plants	Disease score
Control	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU132	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU785	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LU1347	6.7 a	0.0 a	0.0 a	6.7 a	0.0 a
LU1358	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
Apron	7.0 a	0.0 a	0.0 a	7.0 a	0.0 a
LSD 5%	0.3	0.0	0.0	0.3	0.3
Significance	NS	NS	NS	NS	NS

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05. The disease score was assessed using a disease score of 0 to 5.

### 4.3.2 SPAD value (Chlorophyll concentration) at 50 DAS

The SPAD value among the treatments did not differ in the presence of the pathogen for variety Mei Qing Choi or Green Ace F1 at 50 DAS (Figure 4.12). There was also no significant difference on the SPAD value among the treatments for both varieties in the absence of the pathogen at 50 DAS (Figure 4.13).

The SPAD value differed between pathogen and no pathogen for the two varieties (Appendix 33). For variety Mei Qing Choi in the presence of the pathogen the SPAD value was 27.8 and with no pathogen the SPAD value was 33.9. Variety Green Ace F1 in the presence of the pathogen had a SPAD value of 23.4 and with no pathogen it was 36.2 (Appendix 33). There was no significant interaction between the seed treatments and the pathogen (Appendix 33).

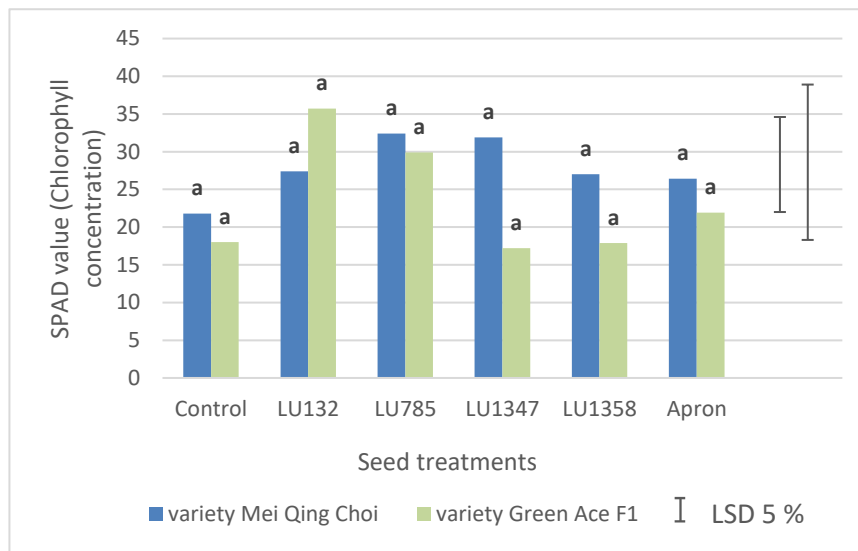


Figure 4.12 Effect of seed treatment on SPAD value (chlorophyll concentration) at 50 DAS for varieties Mei Qing Choi and Green Ace F1 in the presence of *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at  $P=0.05$ .

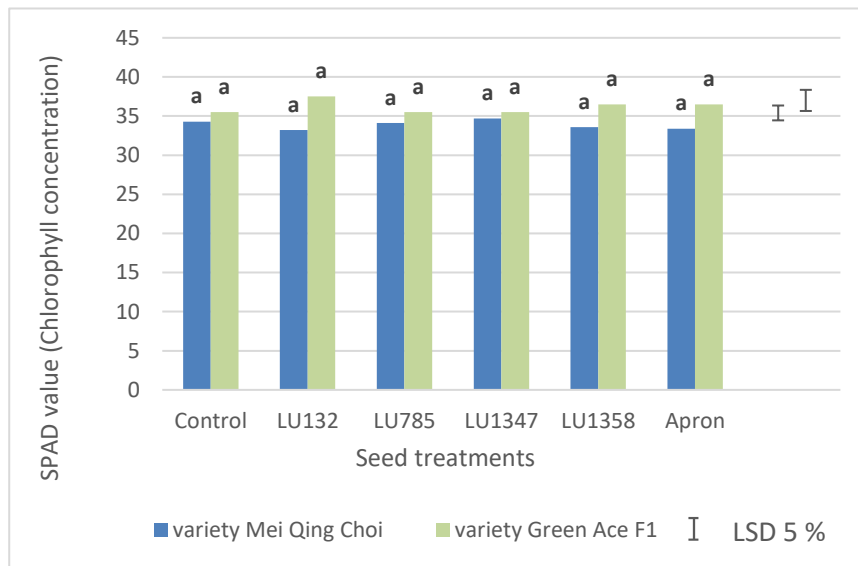


Figure 4.13 Effect of seed treatment on SPAD value ( chlorophyll concentration) at 50 DAS for varieties Mei Qing Choi and Green Ace F1 with no *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at P= 0.05.

#### 4.3.3 Shoot and root length, hypocotyl diameter, number of leaves and leaf surface area.

There were no significant differences for shoot and root length among the treatments in the presence of the pathogen for variety Mei Qing Choi at 60 DAS (Table 4.11). The hypocotyl diameter, number of leaves and leaf surface area did not differ among the treatments (Table 4.11).

There was also no significant difference in shoot and root length among the treatments in the absence of the pathogen for variety Mei Qing Choi at 60 DAS (Table 4.12). The hypocotyl diameter, number of leaves and leaf surface area did not differ among the treatments (Table 4.12). There was a significant difference between pathogen and no pathogen for shoot length for variety Mei Qing Choi (Appendix 34). The shoot and root lengths were longer in the absence of the pathogen than in the presence of the pathogen for both varieties. However, there was no significant interaction between seed treatments and pathogen (Appendix 34 and 35).

The hypocotyl diameter did not differ between pathogen and no pathogen for the two varieties (Appendix 36). There was no significant interaction between seed treatments and the pathogen (Appendix 36).

The number of leaves differed between the pathogen and no pathogen for variety Mei Qing Choi (Appendix 37). In the presence of the pathogen, the number of leaves was lower than with no pathogen; with the pathogen there was a mean of 12.8 leaves and without the pathogen the mean was 45.5 leaves per pot (Appendix 37). There was also a significant interaction between seed treatments and the pathogen as seed treatments with no pathogen had plants with a higher number of leaves than plants with the pathogen (Appendix 37).

There was a significant difference between pathogen and no pathogen for the leaf surface area for variety Mei Qing Choi (Appendix 38). The leaf surface area was higher in the absence of the pathogen. Plants without the pathogen had a mean of 2669 leaves per pot while those in the presence of the pathogen had a mean of 522 leaves per pot (Appendix 38). There was no significant interaction between seed treatments and the pathogen (Appendix 38).

Table 4.11 Effect of seed treatment on shoot and root length, hypocotyl diameter, number of leaves and leaf surface area per pot at 60 DAS for variety Mei Qing Choi in the presence of *R. solani*

Treatments	Shoot length (cm)	Root length (cm)	Hypocotyl diameter (mm)	Number of leaves	Leaf surface area (cm <sup>2</sup> )
Control	11.4 a	11.2 a	2.2 a	9.7 a	410.2 a
LU132	12.5 a	12.3 a	2.6 a	10.5 a	460.2 a
LU785	14.9 a	10.9 a	2.8 a	17.0 a	628.5 a
LU1347	15.4 a	16.4 a	3.6 a	10.0 a	443.1 a
LU1358	12.8 a	11.8 a	2.6 a	16.3 a	592.8 a
Apron	11.3 a	11.6 a	2.2 a	13.3 a	599.5 a
LSD 5%	8.0	7.4	1.6	7.7	350.5
Significance	NS	NS	NS	NS	NS

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

Table 4.12 Effect of seed treatment on shoot and root length, hypocotyl diameter, number of leaves and leaf surface area per pot at 60 DAS for variety Mei Qing Choi with no *R. solani*.

Treatments	Shoot length (cm)	Root length (cm)	Hypocotyl diameter (mm)	Number of leaves	Leaf surface area (cm <sup>2</sup> )
Control	16.7 a	12.6 a	3.1 a	46.5 a	903 a
LU132	16.7 a	13.8 a	3.0 a	47.7 a	2788 a
LU785	16.6 a	13.4 a	2.7 a	47.5 a	2893 a
LU1347	15.6 a	13.1 a	3.0 a	48.6 a	3353 a
LU1358	16.4 a	13.9 a	2.9 a	35.7 a	2936 a
Apron	15.6 a	13.3 a	3.2 a	46.8 a	3143 a
LSD 5%	1.8	2.5	0.5	9.0	2623
Significance	NS	NS	NS	NS	NS

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

There was no significant difference among the treatments in the presence of the pathogen for variety Green Ace F1 for shoot, and root length or hypocotyl diameter (Table 4.13). However, there was a significant difference in the number of leaves. LU132 had a higher number of leaves than the other three Trichoderma isolates but they did not differ from the control and Apron (Table 4.13).

The leaf surface area differed among the treatments in the presence of pathogen for variety Green Ace F1 at 60 DAS (Table 4.13). LU132 had the highest leaf surface area per pot than the other three Trichoderma isolates, control, and Apron (Table 4.13).

There was no significant difference among the treatments with no pathogen for variety Green Ace F1 for shoot and root length and number of leaves at 60 DAS (Table 4.14). However, the hypocotyl diameter differed among the treatments. LU785 had a greater hypocotyl diameter than Apron but did not differ from the control or other treatments (Table 4.14). There was also a significant difference among the treatments for leaf surface area for variety Green Ace F1 at 60 DAS. LU785 and LU1347 had a lower leaf surface area per pot than LU1358 but did not differ from the control or the other treatments (Table 4.14).

There was a difference between pathogen and no pathogen for shoot and root length for variety Green Ace F1. Plants in the presence of the pathogen had shorter shoot and root

lengths but with no pathogen they had longer shoots and roots (Appendix 34 and 35). However, there was no significant interaction between seed treatments and the pathogen for shoot and root length (Appendix 34).

The hypocotyl diameter did not differ between pathogen and no pathogen and there was no significant interaction between seed treatments and the pathogen (Appendix 36). However, the number of leaves and the leaf surface area differed between pathogen and no pathogen for variety Green Ace F1 at 60 DAS (Appendix 37 and 38). The number of leaves and leaf surface area were higher in the absence of the pathogen than in the presence of the pathogen (Appendix 37 and 38). There was no significant interaction between the seed treatments and the pathogen.

Table 4.13 Effect of seed treatment on shoot and root length, hypocotyl diameter, number of leaves and leaf surface area per pot at 60 DAS for variety Green Ace F1 in the presence of *R. solani*

Treatments	Shoot length (cm)	Root length (cm)	Hypocotyl diameter (mm)	Number of leaves	Leaf surface area (cm <sup>2</sup> )
Control	8.3 a	9.0 a	2.1 a	7.5 b	281.5 b
LU132	14.6 a	18.5 a	4.0 a	20.5 a	770.5 a
LU785	11.5 a	13.9 a	3.2 a	15.0 ab	359.8 b
LU1347	10.4 a	13.3 a	3.1 a	8.8 ab	346.7 b
LU1358	10.5 a	9.8 a	2.8 a	11.5 ab	386.3 b
Apron	8.6 a	9.0 a	2.0 a	5.7 b	213 b
LSD 5%	9.3	10.6	2.5	12.2	374.7
Significance	NS	NS	NS	P<0.05	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

Table 4.14 Effect of seed treatment on shoot and root length, hypocotyl diameter, number of leaves and leaf surface area per pot at 60 DAS for variety Green Ace F1 with no *R. solani*

Treatments	Shoot length (cm)	Root length (cm)	Hypocotyl diameter (mm)	Number of leaves	Leaf surface area (cm <sup>2</sup> )
Control	15.4 a	13.5 a	3.4 ab	57.7 a	1371 ab
LU132	15.2 a	13.3 a	3.2 ab	57.5 a	1369 ab
LU785	14.2 a	13.2 a	3.5 a	54.8 a	1343 b
LU1347	14.6 a	12.9 a	3.4 ab	52.3 a	1227 b
LU1358	15.0 a	14.5 a	3.4 ab	59.3 a	1768 a
Apron	14.9 a	13.3 a	2.9 b	57.3 a	1211 b
LSD 5%	1.9	2.5	0.5	8.7	409
Significance	NS	NS	P<0.05	NS	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

#### 4.3.4 Plant fresh weight (g) per pot

In the presence of the pathogen there was a significant difference among the treatments for plant fresh weight for variety Mei Qing Choi at 60 DAS (Figure 4.14). None of the treatments differed from the control, but LU1358 had a higher plant fresh weight than LU1347 (Figure 4.14). The fresh weights did not differ among the treatments in the absence of the pathogen for variety Mei Qing Choi (Figure 4.15). There was a significant difference between the pathogen and no pathogen for plant fresh weight for variety Mei Qing Choi (Appendix 39). In the presence of the pathogen the mean fresh weight was 54.2 g but with no pathogen the mean fresh weight was 165.9 g (Appendix 39). There was no significant interaction between the seed treatments and the pathogen for plant fresh weight (Appendix 39).

There was a significant difference among the treatments in the presence of the pathogen for plant fresh weight per pot for variety Green Ace F1 at 60 DAS (Figure 4.14). LU132 had higher fresh weight than all the other treatments (Figure 4.14).

The plant fresh weight did not differ among the treatments in the absence of the pathogen for variety Green Ace F1 at 60 DAS (Figure 4.15). The plant fresh weight did differ between the pathogen and no pathogen (Appendix 39) as it was higher with no pathogen than in the

presence of the pathogen. There was a significant interaction between the seed treatments and the pathogen (Appendix 39).

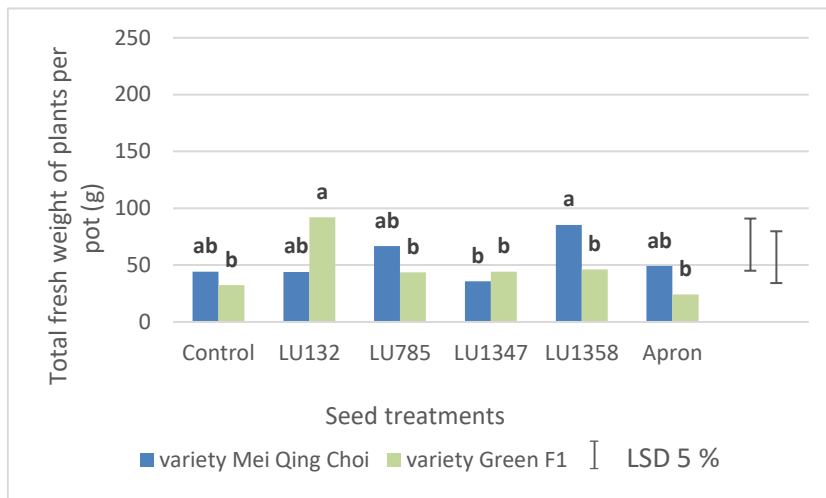


Figure 4.14 Effect of seed treatment on total fresh weight of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1 in the presence of *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at  $P=0.05$ .

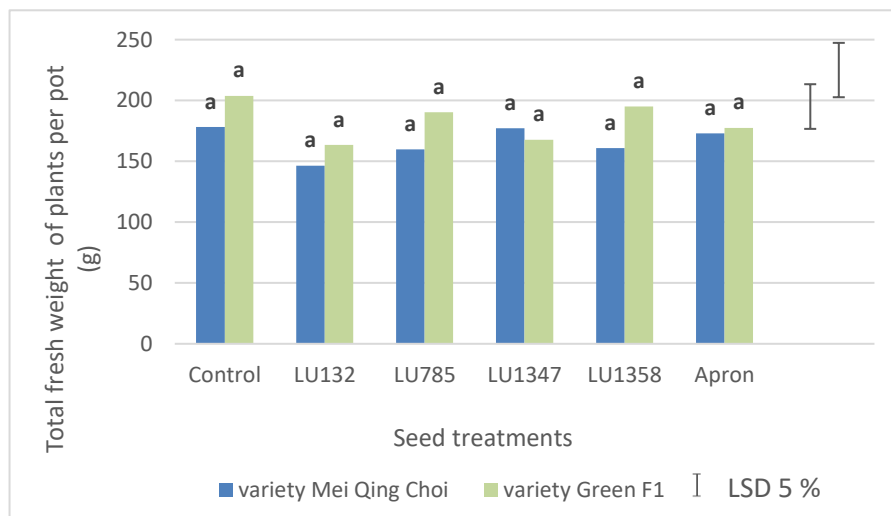


Figure 4.15 Effect of seed treatment on total fresh weights of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1 with no *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at  $P=0.05$ .

### 4.3.5 Plant dry weight per pot (g)

There was no significant difference among the treatments in the presence of the pathogen for plant dry weight per pot for variety Mei Qing Choi at 60 DAS (Figure 4.16) or in the absence of the pathogen ( Figure 4.17).

Plant dry weight differed between pathogen and no pathogen (Appendix 40). With no pathogen the mean dry weight was 18.9 g per pot but in the presence of the pathogen the mean dry weight was 11.9 g per pot. There was no significant interaction between seed treatments and the pathogen (Appendix 40).

The plant dry weight per pot differed among the treatments in the presence of the pathogen for variety Green Ace F1 at 60 DAS (Figure 4.16). LU132 had a significantly higher plant dry weight than the control and Apron (Figure 4.16).

There was no significant difference among the treatments in the absence of the pathogen in plant dry weight per pot for variety Green Ace F1 (Figure 4.17). There was a significant difference in plant dry weight per pot between the pathogen and no pathogen. In the presence of pathogen the mean dry weight was 9.1 g per pot, while with no pathogen the mean was 24.8 g per pot (Appendix 40). There was also a significant interaction between the seed treatments and the pathogen (Appendix 40).

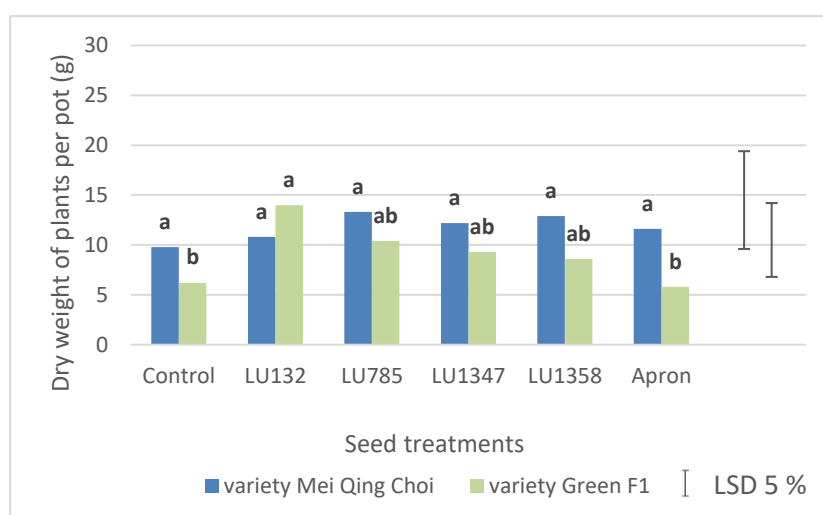


Figure 4.16 Effect of seed treatment on total dry weight of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1 in the presence of *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's 5% level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at  $P=0.05$ .

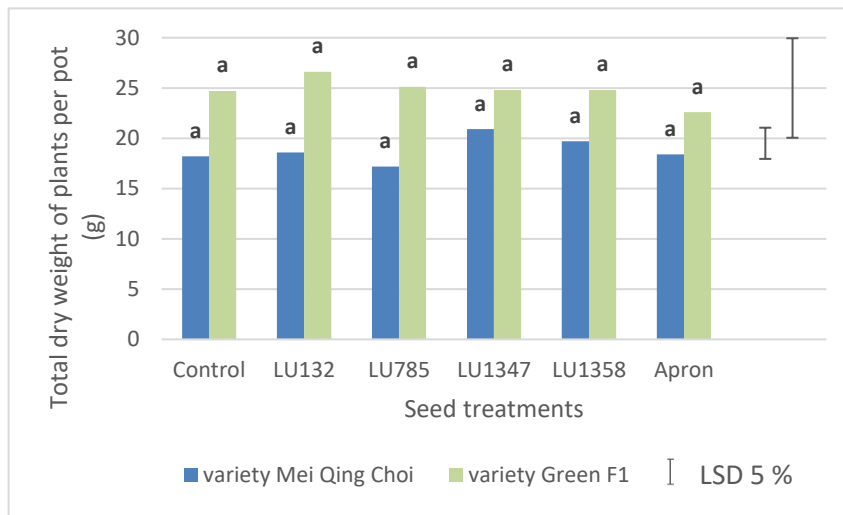


Figure 4.17 Effect of seed treatment on total dry weight of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1 with no *R. solani*. Vertical bar is the LSD (5%). Letters were assigned to a Fisher's (5%) level unprotected LSD procedure within each variety. Means with the same letter are not significantly different at  $P=0.05$ .

#### 4.3.6 Trichoderma colony growth

There was a significant difference ( $P<0.001$ ) in the number of *Trichoderma* colonies isolated from plant roots among the four *Trichoderma* isolates and the control in the presence of the pathogen for variety Mei Qing Choi (Table 4.15). LU132, LU785 and LU1347 had more roots colonised than LU1358 and the control (Table 4.15). Plants from LU132, LU785 and LU1347 had between 50 to 60 % of the roots colonized at 60 DAS (Table 4.15). The number of *Trichoderma* colonies isolated differed ( $P<0.001$ ) among the *Trichoderma* isolates and the control in the absence of the pathogen for variety Mei Qing Choi at 60 DAS (Table 4.15). LU132, LU785 and LU1347 had a higher number of *Trichoderma* colonies isolated than LU1358 and the control (Table 4.15). Plants from LU132, LU785 and LU1347 had between 86 to 93 % root colonization at 60 DAS (Table 4.16). There was a significant difference between pathogen and no pathogen among the four *Trichoderma* isolates and the control for the number of *Trichoderma* colonies isolated from roots for variety Mei Qing Choi (Appendix 41) as in the presence of the pathogen fewer *Trichoderma* colonies were isolated than in the absence of pathogen (Appendix 41). There was also a significant interaction between the treatments and the pathogen as treatments in the absence of pathogen had a higher number of *Trichoderma* colonies than treatments in the presence of the pathogen (Appendix 41).

Table 4.15: Number of Trichoderma colonies isolated from variety Mei Qing roots in the presence and absence of *R. solani* and the percentage of roots with colonies at 60 DAS.

Pathogen		
Treatments	Mean number of colonies isolated	Root colonisation (%)
Control	1.4 b	23.3
LU132	3.2 a	53.3
LU785	3.6 a	60.0
LU1347	3.2 a	53.3
LU1358	2.6 ab	43.3
LSD (5%)	1.4	
Significance	P<0.05	
No pathogen		
Treatments	Mean number of colonies isolated	Root colonisation (%)
Control	1.4 c	23.3
LU132	5.2 a	86.7
LU785	5.6 a	93.3
LU1347	5.4 a	90.0
LU1358	3.0 b	50.0
LSD (5%)	1.3	
Significance	P<0.001	

Letters were assigned to a Fishers 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at P=0.05.

There was a significance difference (P<0.001) in the number of Trichoderma colonies isolated among the four Trichoderma isolates and the control in the presence of the pathogen for variety Green Ace F1 (Table 4.16). LU785 had a higher number of colonies isolated than the control but did not differ from LU132, LU1347 or LU1358 (Table 4.16). All the Trichoderma treatments had 50% or more of the roots colonized at 60 DAS (Table 4.17). The number of Trichoderma colonies isolated differed (P<0.001) among the Trichoderma isolates and the control with no pathogen for variety Green Ace F1 at 60 DAS (Table 4.16). LU132, LU785 and LU1347 had a higher number of Trichoderma colonies than the other Trichoderma isolate and the control (Table 4.16). Trichoderma isolates LU132, LU785 and LU1347 had between 76 to 80 % of roots colonized at 60 DAS (Table 4.16). There was a significant difference between pathogen and no pathogen among the four Trichoderma isolates and the control for the number of Trichoderma colonies isolated for variety Green Ace F1 (Appendix 41) as in the presence of the pathogen fewer Trichoderma colonies were

isolated than in the absence of the pathogen (Appendix 41). There was no significant interaction between the treatments and the pathogen (Appendix 41).

Table 4.16: Number of *Trichoderma* colonies isolated from variety Green Ace F1 roots in the presence and absence of *R. solani* and the percentage of roots with colonies at 60 DAS.

Pathogen		
Treatments	Mean number of colonies isolated	Root colonisation (%)
Control	1.2 b	20.0
LU132	3.0 a	50.0
LU785	3.8 a	63.6
LU1347	3.4 a	56.7
LU1358	3.2 a	53.3
LSD (5%)	1.3	
Significance	P<0.05	

No pathogen		
Treatments	Mean number of colonies isolated	Root colonisation (%)
Control	1.2 c	20.0
LU132	4.8 a	80.0
LU785	5.0 a	83.0
LU1347	4.6 a	76.7
LU1358	3.2 b	53.3
LSD (5%)	1.1	
Significance	P<0.001	

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly (NS) different at P=0.05.

#### 4.4 Discussion

*Rhizoctonia solani* is a pathogen of economic important to crops worldwide, causing damage to plant growth with a resulting reduction in yield (Ajayi & Bradley, 2018; Aydin, 2022).

*Trichoderma* spp. are known as effective biocontrol agents, with the ability to promote plant growth and induce plant resistance against pathogens in the agriculture and horticulture industry (Alizadeh et al., 2024; Daryaei et al., 2016). These fungi have also been shown to promote plant growth in the absence of pathogens (Daryaei et al., 2016; Harman, 2000). *Trichoderma* can be applied as a seed treatment (Chliyeh et al., 2020). This glasshouse experiment was conducted to test the ability of the four selected *Trichoderma* isolates to control soil-borne *R. solani*.

The negative effects of *R. solani* on Pak Choi seedling emergence and growth were confirmed in Chapter 3 (Experiment 1). A similar result was previously reported by Kandular, et al. (2015) for forage species and by Lee (2018) for radish. In this glasshouse study *Trichoderma* spp. when applied as a seed treatment, were expected to promote Pak Choi seedling growth, and effectively control the pathogen as reported by Lee (2018) for radish.

Strains of the selected *Trichoderma* spp. *T. atroviride* LU32, *T. hamatum* LU785, *T. harzianum* LU1347 and *T. polysporum* LU1358 were previously identified as efficient strains for *R. solani* disease control and promotion of radish plant growth (Lee, 2018). Isolates of *T. atroviride* and *T. harzianum* are capable of controlling *R. solani* through mycoparasitism, antibiosis, competition and inducing plant defence responses (Abbas et al., 2017; Chaudhary et al., 2020).

For variety Mei Qing Choi, maximum emergence for the control was only 25% while for Green Ace F1 it was even lower at 14% in the presence of the pathogen. These results were much lower than the 50% achieved from the same inoculum rate in Experiment 1, but the reason for this is not known. For both varieties, LU785 increased maximum emergence to 69% and 51% respectively. For variety Mei Qing Choi, all the other treatments except LU1347 increased maximum emergence to around 50%, while for variety Green Ace F1 only LU785 increased maximum emergence. For Mei Qing Choi maximum emergence for the Apron seed treatment was around 25% greater than that of the control, but for Green Ace F1 it did not differ from the control. Lee (2018) reported that radish seedling emergence did not differ among the *Trichoderma* isolates and the fungicide thiram applied as a seed treatment. Kandula, et al. (2015) reported that in the presence of *R. solani*, perennial ryegrass emergence was increased by between 60- 150% by *T. atroviride*. *Trichoderma* spp. are endophytic plant symbionts that are widely used as seed treatments for controlling seed and soil-borne pathogens and enhancing plant growth and yield (Xue et al., 2017).

By 30 DAS the number of seedlings which had died post emergence ranged from 0.6 (control) to 2.0 (LU785) per pot for variety Mei Qing Choi and from 0.2 (LU132) to 1.0 (LU1347) per pot for variety Green Ace F1 in the presence of *R. solani*. These losses occurred as a result of post-emergence damping off caused by wire stem (Yang et al., 1992). By 60 DAS further plant losses had occurred for both varieties in the presence of *R. solani*. For the control, surviving plants had reduced to 17% (Mei Qing Choi) and 10% (Green Ace F1), while

for LU785 they had fallen to 40% and 31% respectively. Ajayi and Bradley (2018) reported that seedlings are more susceptible to *R. solani* infection because resistance generally increases with matured plants having thicker cell walls. Moreover, to establish a successful infection, pathogens need to pass through the plant cell wall, which is one of the most important physical barriers against invasion (Underwood, 2012). Further plant deaths would most probably not have occurred after 60 DAS because for both varieties, the number of diseased plants (<1 per pot) did not differ between the seed treatments.

At 60 DAS *T. hamatum* LU785 (both varieties and) *T. atroviride* LU132 (Green Ace F1 only) had a higher number of surviving and healthy plants than the controls in the presence of *R. solani*. *T. hamatum* is known to have beneficial properties (Lodi et al., 2023) including biocontrol and, plant growth promotion. *T. hamatum*, when used as a biofertilizer for *Brassica campestris* was reported to significantly increase seedling emergence (Ji et al., 2020). According to Daryaei et al. (2016) *T. atroviride* LU132 suppressed *R. solani* infection of ryegrass, and promoted growth of ryegrass plants in the absence of the pathogen, and this strain has also improved growth and establishment of ryegrass in the presence of *R. solani* (Kandula, et al.,2015).

According to Xue et al. (2017) there were no differences among strains of *Trichoderma* spp. and their relative disease control effectiveness when compared with a fungicide seed treatment. Lee (2018) also reported no differences between the four *Trichoderma* isolates and thiram used as seed treatment as all these treatments had significantly increased the number of surviving and healthy radish seedlings at 40 DAS. Multiple studies have indicated biocontrol capabilities of *Trichoderma* spp. against various plant fungal pathogens (Alizadeh et al., 2024) through inhibiting mycelial growth, reducing infection rates, and disease incidence and enhancing plant growth parameters (Abbas et al., 2017; Asad et al., 2014). However, the control mechanisms used by *Trichoderma*. were not determined in this experiment.

Chlorophyll is a photosynthetic pigment found in plants that plays a key role in photosynthesis. *Trichoderma* isolates in this experiment and the Apron treatment, had significantly higher SPAD values in the absence of *R. solani* at 20 DAS. But in the presence of the pathogen in variety Green Ace F1. *T. atroviride* 132 had a higher SPAD value than the control but not the other four treatments which did not differ from the control. The results

were consistent with previous studies at 20 DAS (Lee, 2018). Other studies have indicated that *Trichoderma* spp are associated with an increase in plant chlorophyll content (Gulzar et al., 2023; Swain et al., 2021; Vukelić et al., 2021). Harman et al. (2021) reported that, increases in photosynthetic capability in plants, induced by various *Trichoderma* spp., occurs because of an increase in the number of photosynthetic pigments or the expression of genes regulating the biosynthesis of chlorophyll. *Trichoderma* volatile organic compounds (e.g., 6-pentyl-2H-pyran-2-one from *T. atroviride*) have also been reported to promote chlorophyll content (Garnica-Vergara et al., 2016).

For variety Green Ace F1 in the presence of *R. solani*, LU132 had more leaves and a greater leaf surface area per pot than the control, but this was a result of differences in plant numbers. When the data were converted to a per plant basis, there was no difference from the control. However, other studies have demonstrated that these fungi stimulate plant development in the absence of pathogens (Harman, 2000; Harman & Bjorkman, 1998; Harman et al., 2004). Studies also reported that some *Trichoderma* spp such as *T. harzianum* T-22 and *T. viride* stimulated the elongation of shoots and the development of leaves (Prisa, 2020; Yahya et al., 2021).

Hypocotyl diameter was not increased significantly by *Trichoderma* isolates in either variety in the presence or absence of *R. solani*. Lee (2018) reported that in the presence of the pathogen isolates LU785, LU1347 and LU1358 produced higher hypocotyl lengths for radish variety Red Round than the control. Mayo-Prieto et al. (2020) also reported that *Trichoderma* isolated from soil increased hypocotyl diameter and root system of bean plants. *Trichoderma* can not only prevent diseases but also promote plant growth (Yao et al., 2023b).

Root and shoot length for both varieties did not differ among the treatments in the presence and absence of *R. solani* at 60 DAS. However, treatments without the pathogen had longer root and shoot length than those with the pathogen. The increased growth response of plants, caused by *Trichoderma*, depend mainly on the ability of *Trichoderma* to survive and develop in the rhizosphere (Harman, 2006; Harman et al., 2004). *Trichoderma* regulates phytohormones including auxins (Contreras-Cornejo et al., 2009). According to Casimiro et al. (2001) increases in lateral root growth are related to increased levels of auxin or auxin-like compounds. However, these compounds have an optimum activity at low

concentrations, they may become toxic to plants and have inhibitory effects on root development at high concentrations (Cutler et al., 1986; 1989; Vinale et al., 2008a).

In this experiment the plant fresh weight per pot did not differ from the control for variety Mei Qing Choi in the presence or absence of the pathogen at 60 DAS but *T. polysporum* LU1358 had a higher plant fresh weight than *T. harzianum* LU1347 in the presence of the pathogen. Furthermore, *T. atroviride* LU132 had a higher plant fresh and dry weight per pot than the control and Apron in the presence of the pathogen for Green Ace F1. Lee (2018) reported that for two radish varieties, all *Trichoderma* isolates produced significantly higher fresh and dry shoot weights per pot in the presence of the pathogen. But in this glasshouse experiment the whole plant was weighed. Similar results of plant biomass increase resulting from *Trichoderma* spp. inoculations were also reported by Yedidia et al. (2001) and Naseby et al. (2000) for cucumber and peas and by Kandula, et al. (2015) for perennial ryegrass. *Trichoderma* establish symbiotic interactions that enhance nutrient uptake and plant growth (Harman et al., 2004; Shores et al., 2010).

Roots of plants produced following *Trichoderma* seed treatment with isolate LU132, LU785 and LU1347 had a greater percentage root colonisation than those from LU1358 and the control in the presence of pathogen for variety Mei Qing Choi. LU785 also had a higher percentage of *Trichoderma* colonized roots in the presence of the pathogen for Green Ace F1 but this did not differ from that for the other three isolates. These results were consistent with the spore concentrations on seed determined that was assessed in the laboratory for each of the isolates before sowing, with LU785 having a greater number of spores adhering to the seeds of both varieties. According to Lee (2018), seed treatment of *Trichoderma* spp. allows establishment in the plant rhizosphere more quickly than wheat-bran inoculation. In addition, the quality of conidia is also important. Daryaei et al. (2016) stated that, *Trichoderma* conidia quality may influence rhizosphere colonization, since conidia are required to germinate, grow, and multiply in the rhizosphere, and this ability is potentially influenced by the incubation conditions and media composition during their production.

At 60 DAS roots from plants grown from the *Trichoderma* isolates in the absence of *R. solani* had slightly more *Trichoderma* colony forming units than those from isolates in the presence of *R. solani* for both varieties. This was associated with the strong saprotrophic and microparasitic behaviour of *Trichoderma* spp. in colonizing the root epidermis (Harman et

al., 2004). The presence of *Trichoderma* on roots is associated with increased nutrient uptake, hormone production, disease resistance and overall growth and development of the plant (Alizadeh et al., 2024).

*Trichoderma* can control a wide range of pathogens and interrupt pathogen growth thus improving plant health (Harman, 2000; Harman et al., 2004; Swain et al., 2021) and these fungi have also been shown to promote plant growth in the absence of pathogens (Daryaei et al., 2016). The application of *Trichoderma* spp. contributed positively to Pak Choi development (e.g. seedling emergence and survival). Harman (2000) reported that most *Trichoderma* isolates for disease control and growth promotion are rhizosphere-competent isolates, which can colonise plant roots and grow in them.

In this glasshouse experiment, the Apron fungicide treatment in the presence of *R. solani* did not really provide many beneficial effects as a seed coating when compared to seeds coated with *Trichoderma* isolates. Apron is a systemic seed treatment fungicide containing metalaxyl-M. In New Zealand it has a label claim for control of seedling damping-off in brassicas caused by *Pythium* spp. (Holden, 2022), but *R. solani* is not included. However, Panozzo et al. (2023) reported that metalaxyl-M is effective against both *Fusarium* and *R. solani* in oil seed rape. The results indicated that generally, *Trichoderma* seed treatment provided either the equivalent or better seedling emergence and control of *R. solani* than the Apron fungicide seed treatment.

## Chapter 5: Brassica plant biofumigation and mustard seed coating treatment

### 5.1 Introduction

Control of soil-borne pests and diseases in high-value agriculture and horticulture can be carried out by soil fumigation, using toxic, volatile compounds applied to the soil. The most widely used compound has been methyl bromide (MeBr) which is highly effective (Martin, 2003; Matthiessen & Kirkegaard, 2006). Other compounds such as chloropicrin, 1,3-dichloropropene and methyl isothiocyanate are used, but are less effective (Matthiessen & Kirkegaard, 2006). Soil fumigation, especially in glasshouses, remains the standard practice to manage soilborne pathogens such as plant – parasitic nematodes, bacteria, and fungi, especially in high-value crops. However, increasing regulatory pressure due to the inherent and broad-spectrum toxicity and negative effect on overall soil health, and increasing demand for organic produce, has created a growing interest in biological fumigants. Many plants and microorganisms emit volatile compounds, which can potentially be used as bio-fumigants (Bui & Desaeger, 2021). There is growing interest in the use of natural, plant-derived compounds for pest control because these are considered to be more environmentally friendly than synthetic compounds (Brennan et al., 2020; Morri et al., 2020). Bio fumigation is an example of such an approach.

Bio fumigation refers to the suppression of soil-borne pests by the decomposition of buried chopped plant material with high levels of glucosinolates (GSL) (Angus et al., 1994; Brown & Morra, 1997; Kirkegaard et al., 1993; Kirkegaard & Matthiessen, 2004).

Glucosinolates are secondary plant metabolites that occur naturally in Brassicaceae plants. Plants that are high in GSLs, such as mustard or arugula, have long been known for their unique flavour and scent (Bellostas et al., 2007). Glucosinolates are hydrolysed by the enzyme myrosinase when plant tissue is disrupted in the presence of water, which releases biological active products such as isothiocyanates, cyanides and thiocyanates (Gimsing & Kirkegaard, 2009). Glucosinolates can be divided into three groups; aliphatic, aromatic, and indole glucosinolates depending on the amino acid side chain (Mithen, 2001b; Velasco et al., 2008; Wittstock & Halkier, 2002). Aliphatic glucosinolates are reported to be most toxic to *Rhizoctonia* species (Li et al., 2013).

Biofumigation can be achieved by incorporating fresh plant material (green manure), frozen or dried plant material into the soil, using cover crops in rotation cropping systems, or as intercrops using seed meals or by using extracted pure bioactive compounds (Kirkegaard and Matthiessen 2004; Lazzeri et al. 2004; Morra and Kirkegaard 2002)

For this experiment, brassica crop residues and ground mustard seeds were formulated and tested for their biocontrol ability against *R. solani* on variety Green Ace F1 in the laboratory and glasshouse.

## **5.2 Materials and methods**

### **5.2.1 Brassica plant biofumigant treatment**

#### **Frozen plant material**

Four brassica species (white mustard, radish, cauliflower, and Pak Choi) were evaluated for their ability to suppress *R. solani*. Seeds from each species was sown in 80-cell seedling trays at Lincoln University's Horticulture Research Glasshouse. After two weeks in the glasshouse, they were transplanted into an outside plot. At two months old the plants were harvested. The leaves and stem were cut into small pieces (1-2 cm) with a knife and mixed thoroughly before the chopped tissues were macerated in a food blender. After that, they were put into Ziplock bags and stored at -20 °C until use (Abdallah et al., 2020; Kushad et al., 1999; Morra & Kirkegaard, 2002; Thanh, 2018). Morra and Kirkegaard (2002) reported that freezing caused extensive cell membrane disruption and permitted greater contact between glucosinolates and the enzyme myrosinase releasing isothiocyanates which are toxic to soil-borne pathogens.

#### **Fresh plant material**

In the Lincoln University Horticulture Research Glasshouse, four seeds each of five brassica species cauliflower, brown mustard, white mustard, Pak Choi, and radish were grown in 1.5-L pots containing potting mix (Appendix 2) in a controlled environment with daily watering. At 30 DAS four leaves from each species were sampled for measurement of glucosinolates levels (Section 5.2.2). After seven weeks, the plants were harvested and washed under running tap water. The leaves, stems and roots were cut into small pieces (1-2 cm) with a knife and mixed thoroughly before the chopped tissues were macerated in a food blender (Figure 5.1) and added to soil (see Section 5.3.2) within 1hour after maceration.



A.

B.

C.

Figure 5.1 Chopped Pak Choi tissue (A) Pak Choi tissue after maceration in food blender (B) and frozen macerated tissues in a Ziplock bag (C).

## 5.2.2 Measurement of glucosinolates level

### Fresh tissue disruption

The level of glucosinolates in leaves of cauliflower, brown mustard, white mustard, radish, and Pak Choi grown in the glasshouse and a garden plot (Section 5.2.1), were determined in the laboratory. Eppendorf tubes (2mL) were used to punch out leaf discs from one leaf. Two from the base of the leaf and two from the top of the leaf (Soth et al., 2022). The Eppendorf tubes containing the four leaf discs were then weighed and this weight subtracted from that of an empty Eppendorf tube to provide leaf disc weight (Doheny-Adams et al., 2017). The tubes containing the leaf discs were dipped immediately into liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  until the next day for further processing.

### Frozen tissue disruption

Aluminium foil was used to cover the bench to reduce contamination. A sterile mortar, pestle and spatula were placed on the bench and liquid nitrogen was poured over them to freeze. Frozen leaves (section 5.2.1) were dropped in the mortar and ground into powder with further liquid nitrogen additions when necessary. The ground powder (minimum weight of 0.1g was required) was transferred into new sterile 2ml tubes, immediately dropped into a container containing liquid nitrogen and then removed and stored at  $-80^{\circ}\text{C}$  for 24 hours.

### Glucosinolate extraction

Glucosinolates were extracted using the cold methanol method (Doheny-Adams et al., 2017). The methanol (80%) was prepared by mixing 800 ml of absolute methanol into 200 ml of sterile water precooled at  $-20^{\circ}\text{C}$ . The Brassica leaf samples species were removed from the  $-80^{\circ}\text{C}$  freezer, and 1.755 ml of 80% methanol was added to each tube. The tubes were placed in a microtube rack and then filled with liquid nitrogen. The leaf tissues were disrupted by grinding them into powder using sterile plastic pestles. The tubes were vortexed for 5 minutes and left at room temperature for 30 minutes. After that, the tubes were shaken using a “Thermo Fisher Scientific Orbital Shaker” for 30 minutes at 70 revolution per second (rev/s) then removed and centrifuged (Centrifuge 5415D Eppendorf) at 4000 rpm for 10 minutes. The supernatant for each sample was poured carefully into new 2ml (Thermo Scientific™ Abgene™ 2D Barcoded) Screw Cap tubes and stored at  $-20^{\circ}\text{C}$  for glucosinolates measurement the next day. The sinigrin stock solution (Appendix 42) and sodium tetrachloropalladate solution (Appendix 43) were prepared and the glucosinolates contents were determined by spectrophotometry.

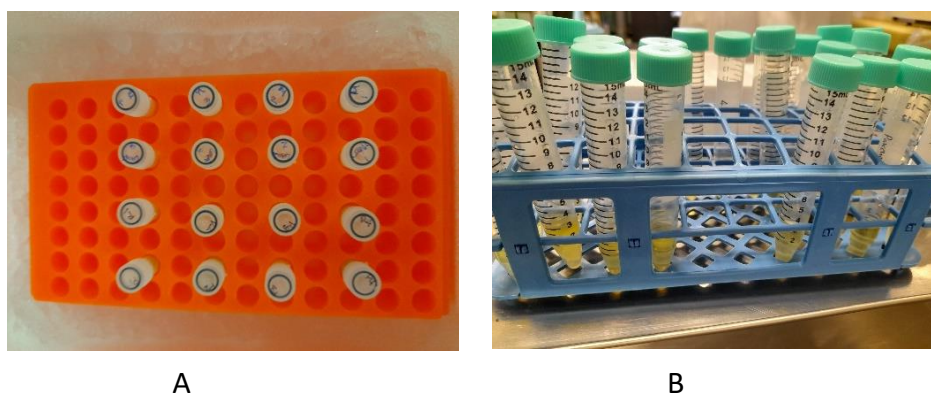


Figure 5.2 Supernatant of Brassica species in 2ml tubes stored in ice (A). 100μL of each Brassica species supernatant samples (stored in  $-20^{\circ}\text{C}$ ) was poured into sodium tetrachloropalladate solution in each 15ml falcon tubes (B).

### Spectrophotometric estimation of glucosinolate levels

100μL of each Brassica species supernatant (Figure 5.2 A) samples was extracted and poured into 15ml falcon tubes. After that, 3ml of 2mM sodium tetrachloropalladate solution (Appendix 43) and 0.3ml of sterile water (Figure 5.3 A) were added into the 100μL of the Brassica species supernatant (Figure 5.2 B) and the sinigrin solution (Appendix 42) (Figure 5.3 B). The tubes were covered with aluminium foil to avoid light sensitivity and incubated at room temperature for one hour. Sodium tetrachloropalladate was used to develop colour as

it is known to interact with glucosinolates causing an hyperchromic shift in the visible region (Mawlong et al., 2017).

After incubation, absorbance was measured at 425nm using a spectrophotometer (GENESYS 10uv Scanning, Thermo Fisher). 1ml of the solution for each species (Figure 5.2B) was extracted and poured into the spectrophotometer cuvettes which were then placed into the rack. "Measure blank" was selected for number 0 (zero) and continued at 0.05,0.1,0.15,0.2 and 0.25 mg/ml. Then the standard curve was plotted based on absorbance at a 425 nm and the glucosinolates level was analysed using linear regression (Hu et al., 2010) (Appendix 44). An overview of the spectrophotometric protocol is shown in Appendix 45.

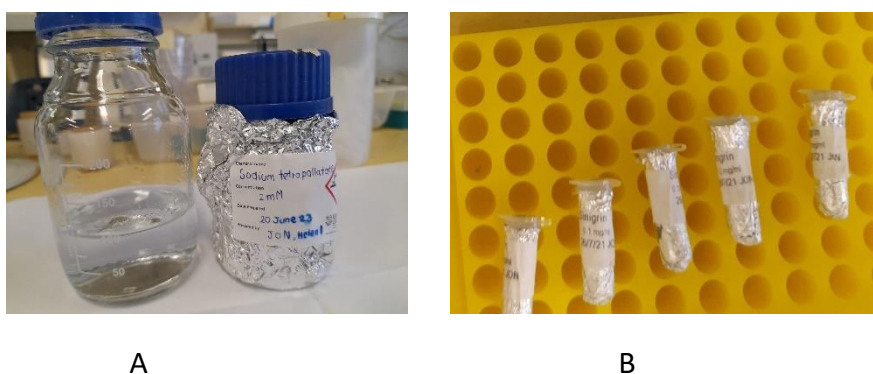


Figure 5.3 Sterile water and sodium tetra palladate wrapped with aluminium foil (A). Sinigrin standard solutions prepared and wrapped with aluminium foil for calibration on the spectrophotometer (B).

### 5.2.3 Inhibition of *R. solani* in the glasshouse

The decision as to which of the brassica species should be used for this glasshouse experiment was dependent on the glucosinolates content measured in Section 5.2.2.

Cauliflower and Pak Choi were selected because the former had the greatest glucosinolates levels in fresh tissue while the latter had the greatest in frozen tissue (Table 5.1 and 5.2).

A mixture of sieved soil and pumice (3:1) was placed into 1.9L pots and the macerated frozen and fresh plant tissues were added at 2% by weight and thoroughly mixed. The pots were watered to 70% water holding capacity (Abdallah et al., 2020). Each pot was enclosed in a black plastic bag for two weeks to limit the escape of volatiles from the bio fumigation treatments. After two weeks the bags were removed (Thanh, 2018).



Figure 5.4 Biofumigation treated pots enclosed in black plastic bags at Lincoln University's Horticulture Research Glasshouse.

### **Inoculating incubated biofumigant media with *R. solani***

*R. solani* isolate (043-4) was grown in wheat bran and incubated at 25 °C for two weeks (Section 3.2.2) prior to use in the glasshouse. For each pot a weight of 386g of incubated media was mixed with 0.25g of *R. solani* inoculated wheat bran. The control (+ and - pathogen) pots had the same mixture apart from no bio fumigation material. Seven seeds of variety Green Ace F1 were sown at a depth of 1cm, and the pots were watered as required. Variety Green Ace F1 was chosen because of its susceptibility to *R. solani*. There was a total of 48 pots (6 treatments x 8 replicates). The pots were arranged in a randomized complete block design (RCBD).

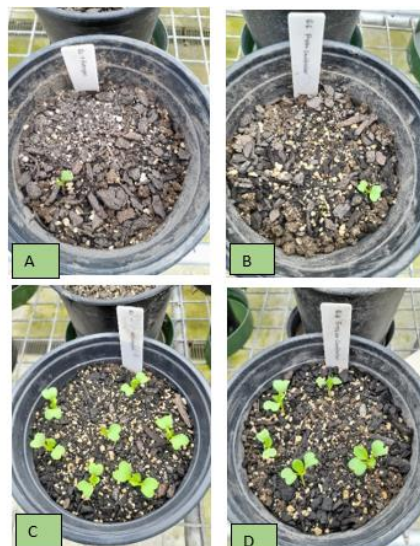


Figure 5.5 Variety Green Ace F1 seedlings at 14 DAS at Lincoln University's Horticulture Research Glasshouse for the following treatments: Control (no biofumigation) in the presence (A) and the absence (C) of the pathogen and fresh (B) and frozen (D) cauliflower in the presence of *R. solani*

#### 5.2.4 Assessment at 30 DAS after sowing

Seedling emergence was assessed daily until 30 DAS. Seedlings which died post emergence, the number of diseased seedlings, the number of healthy seedlings and the number of surviving seedlings were assessed at 30 DAS. The seedlings were then carefully removed from the pots, washed under tap water and the disease score was assessed using the 0 to 5 disease scale of Rittmo et al. (2008). Seedlings were assessed for the following parameters: shoot length, hypocotyl diameter, number of leaves leaf surface area, root and shoot length and plant dry weight as described in Section 3.2.2 and Section 4.4.3. However, in this experiment the root- length, surface area and volume were measured using a root scanner (winRHIZO™. Tron).

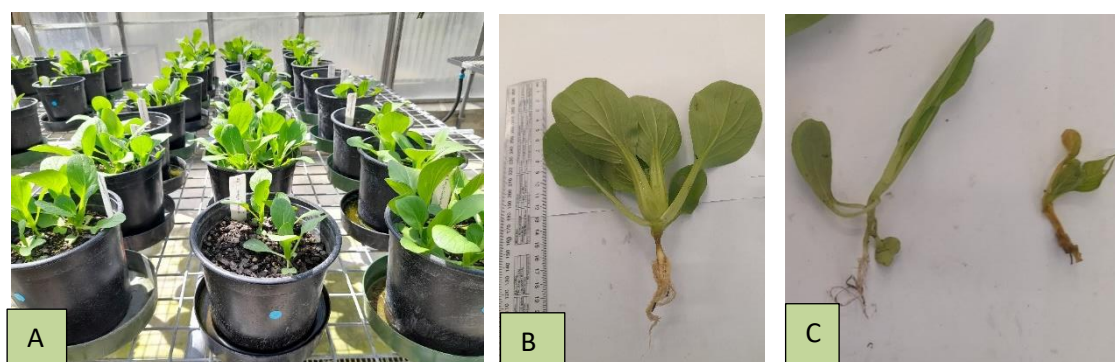


Figure 5.6 Variety Green Ace F1 seedlings at 30 DAS at Lincoln University's Horticulture Research Glasshouse (A). Seedlings grown in pots with frozen cauliflower added in the presence of *R. solani* (B) Seedling grown in pots with fresh cauliflower added in the presence of *R. solani* (C).

#### 5.2.5 Brassica Seed Meal treatment

##### Ground mustard seed spread plate technique for inhibition of *R. solani*

Using the method of Chung et al. (2002) fresh seeds of *B. juncea* (brown mustard) were ground using a sterile mortar and pestle and 0.1g ,0.25g and 0.5g of ground seed meal was mixed with sterile distilled water at a rate of 1:2 (w/v) in petri dishes. For the control, ground mustard was not added. There were six replicates of each treatment. Petri dishes containing PDA amended with 150 ppm streptomycin sulphate were placed upside down. The lid of each plate was inoculated with *R. solani* by placing an agar block with mycelia (0.5cm in diameter) obtained from 3-day old PDA cultures on the fresh agar. The ground seed mustard for each of the three rate treatments was poured into the base of each dish, and a sterile hockey stick was used to the spread it evenly over the dish. The petri dishes were then sealed with two layers of parafilm and incubated at 28°C for 4 days (Figure 5.7).

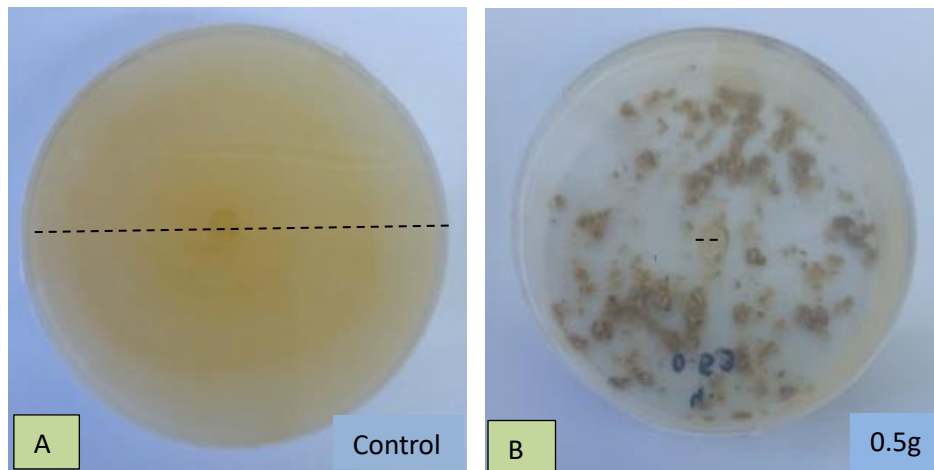


Figure 5.7 *R. solani* hyphal growth at day 4 in the control (A). Hyphal growth of *R. solani* at day 4 with 0.5g ground mustard in the media (B). The dotted lines show the diameter of the colonies.

#### **Ground mustard seed coating with polymer for inhibition of *R. solani***

A second method was the application of ground mustard via a seed coating which included a polymer (see Section 4.2.3). There were three rates of ground mustard (25 ,50 and 75 % (w/w) plus 0.28  $\mu$ l polymer. Twenty-five Pak Choi seeds were mixed with 0.175g ,0.35g or 0.525g ground mustard plus polymer while the control had polymer only. The PDA media containing streptomycin was prepared and the *R. solani* was added according to the protocol used for the spread plate technique. Twenty-five coated Pak Choi seeds were placed on each plate; for each treatment there were 10 replicate plates.

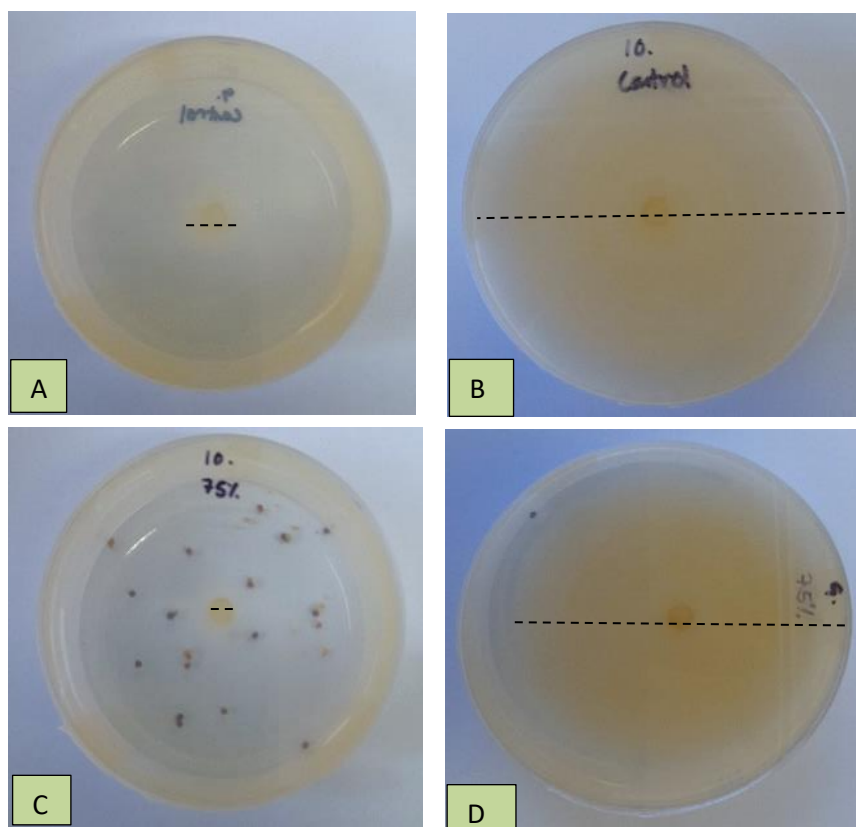


Figure 5.8 *R. solani* hyphal growth at Day 1 (A) and Day 4 (B) for the control (polymer only) and for 75% w/w of ground mustard seed coating at day 1 (C) and Day 4 (D). The dotted lines show the diameter of the colony.

## 5.3 Results

### 5.3.1 Glucosinolates level for frozen and fresh leaves.

Frozen Pak Choi had a significantly higher glucosinolates level than the frozen cauliflower and white mustard but did not differ from the frozen radish (Table 5.1).

Table 5.1 Level of glucosinolates in seven week old frozen leaves of Brassicaceae species.

Species	Glucosinolates level (mg/ml)
Cauliflower	0.09 b
White mustard	0.05 b
Radish	0.11 ab
Pak Choi	0.19 a
LSD 5%	0.07
Significance	P<0.05

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

The glucosinolates level differed among the fresh leaves of Brassicaceae species (Table 5.2). Cauliflower had a higher glucosinolates level than radish and brown mustard but did not differ from white mustard and Pak Choi (Table 5.2).

Table 5.2 Level of glucosinolates in seven week old fresh leaves of Brassicaceae species.

Species	Glucosinolates level (mg/ml)
Cauliflower	0.21 a
White mustard	0.15 ab
Radish	0.11 bc
Pak Choi	0.12 abc
Brown mustard	0.03 c
LSD 5%	0.10
Significance	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 5.3.2 Glasshouse assessment at 30 DAS

There was a significant difference ( $P<0.001$ ) among the macerated biofumigant treatments for maximum seedling emergence. The no pathogen control had the highest number of seedlings emerged and the pathogen control the lowest. In the presence of the pathogen the macerated frozen cauliflower treatment had a significantly higher maximum seedling emergence than the other biofumigant treatments. Seedling emergence for the macerated fresh Pak Choi treatment did not differ from the pathogen control (Table 5.3).

The number of seedlings which died post emergence did not differ among the treatments. However, there was a significant difference ( $P<0.001$ ) among the treatments for the number of surviving seedlings (Table 5.3). The control (- pathogen) had the highest number of surviving seedlings, followed by the frozen cauliflower which had more surviving seedlings, than all the remaining treatments. The pathogen had the lowest number of surviving seedlings (Table 5.3). There were no significant differences among the treatments for the number of diseased seedlings but there was a significant difference ( $P<0.001$ ) for the number of healthy seedlings; the no pathogen control had more healthy seedlings than all the other treatments, while the pathogen control had the lowest number of healthy seedlings but did not differ from both fresh biofumigation treatments. The frozen cauliflower and Pak Choi had more healthy seedlings than all the other treatments except for the no pathogen control and fresh cauliflower. The disease score was very low for all the

treatments with only the pathogen control differing from the no pathogen control (Table 5.3).

Table 5.3 Effect of macerated biofumigation treatments on maximum seedling emergence, seedlings which died post emergence, surviving seedlings and diseased and healthy seedlings per pot at 30 DAS for variety Green Ace F1 in the presence of *R. solani*.

Treatments	Maximum seedling emergence	Seedlings which died post emergence	Surviving seedlings	Diseased seedlings	Healthy Seedlings	Disease score
No pathogen (Control)	7.0 a	0.0 a	7.0 a	0.0 a	7.0 a	0.0 b
Pathogen (Control)	1.6 d	0.2 a	1.4 d	0.8 a	0.6 d	1.0 a
Fresh Pak Choi	2.6 cd	0.1 a	2.5 c	0.8 a	1.7 d	0.5 ab
Fresh cauliflower	2.8 c	0.2 a	2.6 c	0.6 a	2.0 cd	0.4 ab
Frozen Pak Choi	3.1 c	0.1 a	3.0 c	0.4 a	2.6 c	0.3 b
Frozen cauliflower	4.3 b	0.3 a	4.0 b	0.1 a	3.9 bc	0.1 b
LSD 5%	1.03	0.4	1.1	0.8	1.3	0.74
Significance	P<0.001	NS	P<0.001	NS	P<0.001	P<0.05

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 5.3.3 Assessment of above ground seedling parameters at 30 DAS

Shoot length was reduced for the pathogen and fresh Pak Choi treatments but the other biofumigant treatments did not differ from the no pathogen control (Table 5.4). The hypocotyl diameter was reduced by the pathogen control in the two fresh biofumigant treatments, but the frozen treatments did not differ from the control (Table 5.4).

The number of leaves per pot differed significantly (P<0.001) among the treatments, with the no pathogen control having more leaves than all other treatments. The frozen cauliflower treatment had the second highest number of leaves per pot, but this did not differ from the frozen Pak choi treatment. The pathogen control had the smallest number of leaves per pot (Table 5.4).

There was a significant difference (P<0.001) in the leaf surface area per pot among the treatments with the no pathogen control having a greater leaf surface area per pot than the other treatments. The frozen treatments had the second highest leaf surface area per pot, but the two fresh treatments did not differ from the pathogen control (Table 5.4).

Table 5.4 Effect of macerated biofumigation treatments on the shoot length (cm), hypocotyl diameter (mm), number of leaves per pot, and leaf surface area per pot at 30 DAS for variety Green Ace F1 in the presence of *R. solani*.

Treatments	Shoot length (cm)	Hypocotyl diameter (mm)	Number of leaves per pot	Leaf surface area (cm <sup>2</sup> ) per pot
No pathogen (Control)	13.0 a	0.11 a	38.8 a	684.9 a
Pathogen (Control)	10.1 b	0.08 b	7.1 d	119.1 d
Fresh Pak Choi	10.2 b	0.07 b	13.9 c	254.1 cd
Fresh cauliflower	11.9 ab	0.07 b	14.6 c	272.7 cd
Frozen Pak Choi	12.2 ab	0.09 ab	16.9 bc	308.6 b
Frozen cauliflower	12.2 ab	0.10 a	22.6 b	422.8 b
LSD 5%	2.5	0.02	6.0	138.1
Significance	P<0.05	P<0.05	P<0.001	P<0.001

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 5.3.4 Assessment of seedling root parameters at 30 DAS

There was a significant difference ( $P<0.001$ ) among the treatments for root length and root surface area. Root length and root surface area for the no pathogen control did not differ from that of the two frozen biofumigant treatments while the root length and surface area for the fresh Pak Choi treatment did not differ from that of the pathogen control (Table 5.5).

There was also a significant difference ( $P<0.05$ ) in root volume among the treatments. The no pathogen control treatment had the highest root volume but did not differ from the two frozen biofumigant treatments and the fresh cauliflower. However, the root volume for the fresh macerated cauliflower did not differ from that of the frozen cauliflower, fresh Pak Choi, or the pathogen control (Table 5.5).

Table 5.5 Effect of macerated biofumigation treatments on the root length (cm), root surface area(cm<sup>2</sup>) and root volume(cm<sup>3</sup>) at 30 DAS for variety Green Ace F1 in the presence of *R. solani*.

Treatments	Root length (cm)	Root surface area (cm <sup>2</sup> )	Root volume (cm <sup>3</sup> )
No pathogen (Control)	120.9 a	20.6 a	0.28 a
Pathogen (Control)	54.6 cd	9.9 b	0.15 b
Fresh Pak Choi	53.2 c	9.8 b	0.14 b
Fresh cauliflower	85.1 b	15.6 ab	0.24 ab
Frozen Pak Choi	92.0 ab	17.0 a	0.27 a
Frozen cauliflower	111.7 ab	18.6 a	0.25 ab
LSD 5%	30.7	6.0	0.12
Significance	P<0.001	P<0.001	P<0.05

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

### 5.3.5 Assessment of seedling fresh and dry weight at 30 DAS

Seedling fresh weight per pot differed significantly ( $P<0.001$ ) among the treatments at 30 DAS. The no pathogen control had a higher fresh weight than all the other treatments. The macerated frozen cauliflower had the second highest fresh weight which differed from that of other three macerated biofumigant treatments. The pathogen control had the lowest fresh weight per pot, but this did not differ from the two fresh biofumigant treatments (Table 5.6).

There was a significant difference ( $P<0.001$ ) among the treatments for total dry weight of seedlings per pot. The no pathogen control had the highest dry weight per pot. The dry weight did not differ among the four biofumigant treatments, but only the frozen cauliflower biofumigant treatment had a dry weight greater than that of the pathogen control (Table 5.6).

Table 5.6 Effect of macerated biofumigation treatment on the fresh and dry weight of seedlings per pot at 30 DAS for variety Green Ace F1 in the presence of *R. solani*

Treatments	Total fresh weight (g)	Total dry weight (g)
No pathogen (Control)	58.8 a	2.6 a
Pathogen (Control)	11.9 d	0.4 c
Fresh Pak Choi	17.7 cd	0.9 bc
Fresh cauliflower	18.1 cd	0.9 bc
Frozen Pak Choi	25.00 c	1.3 bc
Frozen cauliflower	32.7 b	1.5 b
LSD 5%	11.4	0.5
Significance	P<0.001	P<0.001

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 5.3.6 Assessment of the ground mustard seed spread plate technique for inhibition of *R. solani* at 4DAS.

There was a significant difference (P<0.001) among the treatments for hyphal growth of *R. solani* at 4 DAS. The control had the greatest hyphal growth, the 0.10g rate reduced hyphal growth by almost 50%, and the 0.25g and 0.50g rates prevented growth of hyphae at 4 DAS (Table 5.7).

Table 5.7 Effect of ground mustard seed spread plate technique on inhibition of *R. solani*

Treatments	Hyphal growth of <i>R. solani</i> (cm)
0.00g (control)	8.36 a
0.10g	4.00 b
0.25g	0.00 c
0.50g	0.00 c
LSD 5%	1.07
Significance	P<0.001

Letters were assigned to a Fisher 's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 5.3.7 Assessment of the ground mustard seed coating with polymer on inhibition of *R. solani* at 4DAS.

The hyphal growth of *R. solani* differed significantly ( $P < 0.001$ ) among the treatments, being reduced by all three application rates. Hyphal growth was slower as application rate increased (Table 5.8).

Table 5.8 Effect of ground mustard seed coating with polymer on inhibition of *R. solani*

Treatments	Hyphal growth of <i>R. solani</i> (cm)
0.00g (control)	8.12 a
0.18g	7.62 b
0.35g	7.54 bc
0.53g	7.42 c
LSD 5%	0.11
Significance	$P < 0.001$

Letters were assigned to a Fisher's 5% level unprotected LSD procedure. Means with the same letter are not significantly different at  $P = 0.05$ .

## 5.4 Discussion

There is an increasing interest in the biofumigant properties of toxic glucosinolate hydrolysis products as a method to control agricultural pests (Gimsing & Kirkegaard, 2009). Evaluating biofumigation potential requires rapid and accurate quantification of glucosinolates, but the methods of extraction prior to analysis is time consuming, expensive and hazardous (Doheny-Adams et al., 2017).

The most common analytical techniques utilized for the determination and quantification of these compounds are chromatographic and spectrometry techniques (Theunis et al., 2022; Wu et al., 2021; Zeng et al., 2021). These analytical techniques provide direct and accurate quantification of the different phytochemicals in the sample matrix after extraction and dilution (Hooshmand & Fomsgaard, 2021). However, in this experiment a simple spectrophotometric method was used, similar to that of Mawlong et al. (2017), and a sinigrin standard was used to determine the glucosinolate level (GSL) of each Brassica species. Aliphatic GSLs are the most abundant and can be classified according to their sulphur containing side chains. Li et al. (2024), suggested that five glucosinolates (sinigrin, gluconapin, glucobrassicin, gluconasturtiin and progoitrin) could be used as standards for total glucosinolate content. Sinigrin has been widely studied (Wu et al., 2023). It is

accessible and affordable and has a wide linear range when used for total glucosinolate content (TGC) determination (Li et al., 2024). GSL structure and their compounds make GSLs analysis very challenging (Mithen et al., 2000). For this reason, standard curves using sinigrin alone may underestimate the TGC. Researchers have tried to improve the accuracy of TGC determination by using regression curves with a variety of individual GSLs as a mixed standard (Li et al., 2024). Blaževića et al. (2020) reported that there were approximately 200 GSLs identified by modern spectroscopy methods (such as Nuclear Magnetic Resonance and Mass Spectrum) as of 2018, and more GSLs are estimated to be identified in the future.

In this laboratory experiment, frozen and fresh leaf tissues of four Brassicaceae species were assessed for sinigrin (GSLs) concentrations. For the frozen leaf tissue, the highest sinigrin concentration was recorded in Pak Choi but did not differ from radish. For the fresh leaf tissue, cauliflower had the highest sinigrin concentration but did not differ from white mustard and Pak Choi. Bhandari et al. (2015) reported that the compositions and concentrations of individual GSLs varied among crops, tissues, and growth stages. Bhandari et al. (2015) reported that sinigrin was a major GSL in cauliflower, Pak Choi and kale shoots. Mustard leaf tissue had the highest concentration of GSLs sinigrin among nine Brassica species assessed (Rao & Viswanath, 2023). Soth et al. (2022) measured the total glucosinolates content using the same method of extraction as used in this experiment, and showed that radish leaves contained a higher content of sinigrin glucosinolates than cauliflower, cabbage and broccoli. Bhandari et al. (2015) found the lowest GSL concentrations in radish across all tissues examined.

Yeo et al. (2021) grew three types of Pak Choi (white, green, and pale green) harvested at 85 days, froze samples immediately in liquid nitrogen, and subsequently stored them at  $-80^{\circ}\text{C}$  until they were analysed for their glucosinolate content using high-performance liquid chromatography (HPLC). The HPLC analysis revealed the presence of 13 glucosinolate compounds, with the green Pak Choi having the highest individual and total glucosinolate contents among the three cultivars.

Therefore, the concentration and distribution of the GSL in plants of the Brassicaceae family vary according to a number of factors, namely species (Bellostas et al., 2004), variety (Choi et al., 2014), plant organ (Bellostas et al., 2004; Brown et al., 2003) or plant age (Brown et al., 2003; Fahey et al., 1997) and developmental stage (Rhee et al., 2020). Moreover,

environmental conditions such as season (Cartea & Velasco, 2007) , biotic (Verkerk et al., 2009) or abiotic stress factors are known to play a role in the production and content of these compounds (Khan et al., 2011; Martínez-Ballesta et al., 2015).

GSLs are not bioactive themselves but rely on an activating enzymes (e.g. myrosinase) to produce the bioactive agents (Plaszko et al., 2021). Of these the most potent antifungal GSL decomposition products are isothiocyanates (Abdallah et al., 2020). One of the factors that influences the amount of isothiocyanates (ITC) released into the soil, is whether the tissue is fresh or frozen before incorporation (Gimsing & Kirkegaard, 2009; Matthiessen & Kirkegaard, 2006). In the present glasshouse experiment, in the presence of the pathogen the macerated frozen cauliflower treatment allowed a significantly higher maximum seedling emergence, survival and healthy seedlings than the other biofumigant treatments while seedling emergence for the macerated fresh Pak Choi treatment did not differ from the pathogen control.

ITC release is partly dependent on tissue maceration, effective incorporation, and a reduction of volatilization losses from the soil by using a plastic cover (Tagele et al., 2021). Price et al. (2005) reported that cover plastic sheets also raise the soil temperature. Increasing the soil temperature using solarization has been found to enhance biofumigant efficacy (Rubayet et al., 2018). Tissue disruption at a cellular level caused by freezing, resulted in extensive cell membrane disruption and permitted greater contact between glucosinolates and myrosinase that catalyses glucosinolate to release ITC into the soil (Morra & Kirkegaard, 2002). Plant tissues that are incorporated into the soil without first being broken down will release lower levels of ITCs, sometimes less than 1% (Gimsing & Kirkegaard, 2009). Freezing and thawing increases the effectiveness of ITCs in inhibiting soil borne pathogens (Hanschen et al., 2017; Price et al., 2005). According to Brennan et al. (2020), speed is also an important consideration when incorporating biofumigant materials into the soil as fast incorporation traps volatilising compounds in the soil. In this experiment the fresh macerated tissues were incorporated 1 hr after tissue maceration. The highest GSLs levels in the soil have been recorded within the first 30 minutes of their incorporation in the soil (Gimsing & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009). Effectiveness of biofumigation declines slowly as exposure time increases (Morris et al., 2020).

However, a recent study has identified positive effects of incorporation of fresh tissues. Fresh chopped leaves of six Brassica species (mustard, broccoli, cabbage, cauliflower, Knol Khol and, radish) were incorporated into soil and assessed for their ability to reduce the impact of the wilt-causing pathogen (*Fusarium oxysporum f.sp. melongenae*) on egg plant in a glasshouse study. All the treatments improved emergence from 51% in the untreated control, to 61 to 93% (Rao & Viswanath, 2023). Brassica biofumigation is now considered to an increasingly feasible method of managing soil-borne plant pathogens and pests (Curto et al., 2016; Dewitte et al., 2019).

In this experiment the above ground parameters including the shoot length, hypocotyl diameter, number of leaves, and leaf surface area were higher in the control without pathogen and the frozen cauliflower treatment. Isothiocyanates (ITCs) and other defensive volatile organic constituents (VOCs) upon brassica tissue disruption, protect the host against the pathogen attack (Plaszko et al., 2021). Lazzeri et al. (2004) used fresh and frozen *B. juncea* macerated tissues incorporated in pots infested with *Pythium*. After 15 days pots with frozen *B. juncea* decreased the number of colony forming units of *Pythium* (CFUs) by 95%, while the fresh *B. juncea* pots had 84% less CFUs. Lazzeri et al. (2004) found that in fresh brassica tissues, after 3 minutes of grinding, around 75% of the GSLs contained in *B. juncea* were not recovered, probably due to their disappearance after hydrolysis. But the frozen tissue of *B. juncea* maintained its GSLs level for more than three hours, being sufficient time for residue incorporation before the start of GSLs hydrolysis. Frozen tissues release GSL degradation compounds directly into soil during thawing (Price et al., 2005; Tagele et al., 2021) and resulted in maximum ITC concentrations from 40 to 75nmol ITC g<sup>-1</sup> soil, thus increasing ITC release efficiencies to 14 to 26% (Morra & Kirkegaard, 2002). Morra and Kirkegaard (2002) concluded that most of the ITC will be released within the first 4 days after tissue incorporation. The exact timing of this release will vary based on soil chemical and physical characteristics, temperature and moisture (Tagele et al., 2021). Complete hydrolysis is necessary to achieve the highest level of ITC release, so thorough maceration of plant tissue followed by rapid soil incorporation and addition of water is ideal (Matthiessen & Kirkegaard, 2006).

A large number of studies have investigated the antifungal activity of different ITCs against diverse fungi (Sharma et al., 2023), including the control of *R. solani* (Taylor, 2013). Chung et al. (2002) and Yulianti et al. (2006) found that *R. solani* radial growth in culture was

decreased when exposed to allyl isothiocyanates (AITC) and 2-phenylethyl (PEITC) including benzyl (BITC) and methyl (MITC) (Taylor, 2013). ITCs interfere with the production of the pathogen virulence factors, including biofilm production, hyphal morphogenesis, filamentations (Tagele et al., 2021) and antioxidant production which is one of the main targets (Plaszko et al., 2021). ITCs inhibit oxygen uptake through the uncoupling of oxidative phosphorylation in mitochondria which results in a reduction in oxygen consumption and mitochondrial depolarization (Sharma et al., 2023), leading to poorer survival of the fungal pathogen (Tagele et al., 2021).

In this glasshouse study, the below ground parameters including the root length, root surface area, and root volume were higher in the control without pathogen and the two frozen biofumigant treatments. They were lower in the two fresh biofumigant treatments and the control with pathogen *R. solani*. Shavnam and Raj (2024) reported the effect of soil solarization and biofumigation using fresh cauliflower leaves on the viability of four pathogens (*P. ultimum*, *F. oxysporum*, *R. solani*, and *S. rolfisii*). They buried 5 to 10g of the pathogens in nylon mesh bags at 5 and 10 cm depths in the soil and investigated the influence of the cauliflower leaves on pathogen impacts. They found that the incidence of damping-off was reduced in tomato, chilli, and capsicum. Fresh cauliflower and cabbage leaves have also been reported to reduce the effect of plant parasitic nematodes and improve plant growth parameters (shoot and root length, fresh and dry weight) of okra (Das & Behera, 2019).

In this experiment the presence of the pathogens inhibited root development. *R. solani* is a necrotrophic pathogen that obtains nutrients by killing its host plant cells before colonization (Ajayi & Bradley, 2018) which is associated with toxic cellular enzymes and pectinolytic and cellulolytic enzymes (Bateman, 1970). Plants with affected roots may exhibit poor root growth, leading to less vigorous plants with reduced water and nutrient uptake (Ajayi & Bradley, 2018; Mayo et al., 2015).

Brassica biofumigants show considerable inhibitory activity against plant fungal pathogens (Wang et al., 2014). Baysal-Gurel et al. (2020) used brassica based biofumigation for control of *R. solani* and *P. nicotianae* in hydrangea and viburnum woody plants. The cuttings were grown in biofumigated and non biofumigated control pots, and root rot disease severity was evaluated for 4 weeks. The viburnum and hydrangea cuttings grown in green mustard,

arugula and turnip incorporated soil had significantly higher root fresh weights than the inoculated, non biofumigated control plants. The decline of soil-borne pathogens in biofumigated soil results in a decrease in disease incidence (Ascencion et al., 2015; Handiseni et al., 2016; Hansen & Keinath, 2013; Plaszkó et al., 2021) and increased plant vegetative growth and yield (Cheah et al., 2006; Handiseni et al., 2016; Oka et al., 2007; Wang & Mazzola, 2019) due to release of allelochemicals (Meghvansi & Varma, 2015; Zeist et al., 2019). Biofumigation can also enhance soil fertility by improving the soil structure and other soil physicochemical properties and microbial activity (Tagele et al., 2021). Using plant material in biofumigation can have long-lasting beneficial effects on the soil microbiome, even though ITCs are usually only present/active in the short-term.

In this experiment, the pathogen control had the lowest fresh and dry weights per pot and the frozen cauliflower treatment had a fresh and dry weight per pot significantly greater than that of the pathogen control, but significantly lower than that of the no pathogen control. However, on a per plant basis, the fresh and dry weights of the frozen cauliflower treatment (8.2g and 0.37g) did not differ from the no pathogen control (8.4g and 0.37g), and therefore there was no growth promotion; the differences on a per pot basis were because of differences in surviving plant number caused mostly by pathogen induced post-emergence seedling death. In New Zealand, the cultivation and incorporation of *B. rapa* and *B. napus* reduced clubroot disease caused by the protozoan *Plasmodiophora brassicae* in Chinese cabbage, cauliflower and broccoli and increased crop yield (Cheah et al., 2001).

ITC are compounds very similar to the chemical fumigant metam sodium. ITC released from mustard was shown to be suppressive to *R. solani* in a controlled laboratory study (Charron & Sams, 1999). In the present laboratory experiment, the two higher concentrations of ground mustard (0.25g and 0.50g) had completely inhibited hyphal growth of *R. solani* after four days in the spread plate experiment. The ground mustard seed coating with polymer results indicated that *R. solani* hyphal growth decreased as application rate increased, a result similar to that reported by Sarhan et al. (2020). Fayzalla et al. (2009) showed that the bioactive compounds in mustard were more effective at higher concentrations. Sarhan et al. (2020), used mustard (*Brassica juncea*) and canola (*Brassica napus*) seed meals to control damping-off and root-rot diseases of chickpea. Mustard seed meal at the highest concentration (2.5g/pot) reduced *R. solani* damping off to 20 % and increased plant survival to 80% in green house study. In the laboratory, a high rate of mustard seed meal (0.025g)

slowed down the mycelium growth of *R. solani* by 88% compared to *R. solani* grown without mustard seed meal.

Laboratory and greenhouse experiments were also conducted by Abdallah et al. (2020) to evaluate the biofumigation effect of *B. juncea*, used as dry plants, seed meal, seed powder, methanol extract, and fresh plants (at the vegetative and flowering stages), against *R. solani*. Their results showed that hexane defatted seed meal was the most efficient, inhibiting the fungal growth by 61.5%.

Earlier reports (Charron & Sams, 1999; Mayton et al., 1996; Smolinska et al., 2003) stated that the volatile substances released from ground mustard seed in in-vitro assays showed a strong fungicidal effect, towards soil-borne pathogens. Lord et al. (2011) assessed the biocidal activity of the glucosinolates released from *B. juncea* on *Globodera pallida* on potato in polyethylene covered soil and found that *B. juncea* caused over 95 % mortality in encysted *G. pallida* eggs. *B. juncea* was found to be rich in ITCs and is well-known in bioassay screenings of Brassicaceae species as a very effective biofumigant ( Hanschen & Winkelmann, 2020).

Morris et al. (2020) concluded that the different Brassica incorporation methods (direct incorporation of fresh tissue, freezing before incorporation, drying before incorporation, freeze drying before incorporation, or crushing of seeds) did not differ in their effectiveness. Different incorporation methods preserve glucosinolates and ITCs to varying degrees. The lack of tissue damage should have prevented the mixing of glucosinolates and myrosinase needed to produce toxic ITC (Gimsing & Kirkegaard, 2009).

## Chapter 6: Field experiment

### 6.1 Introduction

Pak choy (*Brassica campestris* ssp. *chinensis*) is an important and widely cultivated leafy vegetable known for its nutritional value and culinary versatility (Kim et al., 2024). In China, Pak Choi accounts for 30% to 40% of the country's total annual vegetable consumption (Ding et al., 2022). However, soil-borne plant pathogens, including *R. solani*, are widely distributed and attack Pak Choi (Grosch et al., 2012). *R. solani* causes damping-off and root rot, leading to significant yield losses (Sturrock et al., 2015; Williamson-Benavides & Dhingra, 2021).

Fungicides are commonly used to control pathogens, but they can harm the environment and may pose health risks to humans and other non-target species (Grosch et al., 2012). Increasingly, farming practices are being developed based on biocontrol methods, which, while sometimes not providing the same level of control as fungicides, have fewer negative effects (Jaiswa et al., 2022). The demand for seeds treated with microbial biological control agent, prior to planting is increasing (Cortés-Rojas et al., 2021). Plant beneficial microbes (PBMs) applied via seed coating promote crop growth, and yield, and can provide protection against pathogens (Rocha et al., 2019). *Trichoderma* species are endophytic plant symbionts that have been used as seed treatments to control pathogens such as *R. solani* in radish, *Fusarium oxysporum* in tomato, *Sclerotinia sclerotiorum* in lettuce and *Fusarium culmorum* in durum wheat, and to enhance plant growth and yield (Cortés-Rojas et al., 2021; Kthiri et al., 2020; Lee, 2018). Their ability to reduce the impact of *R. solani* on Pak Choi was also demonstrated (see Chapter 4).

Ground seeds from Brassica species are high in glucosinolates and can release significant amount of toxic volatile compounds, offering potential for control of soil-borne pathogens (Smolinska et al., 2003). Ground seeds from mustard (*B. juncea*) were used as a seed coating to improve plant growth and development and most importantly, control pathogens such as *R. solani* on cabbage in a greenhouse (Chung et al., 2002) and *Fusarium culmorum* on wheat in both green house and field conditions (Kowalska et al., 2021). Ground seeds from mustard (*B. juncea*) used as seed coating inhibited the growth of *R. solani* in petri plates (See Chapter 5).

Seed coating allows precise application of small amount of inoculants and is therefore cost effective (Oliveira et al., 2016a; Rocha et al., 2019; Roupael et al., 2017). After confirming that *Trichoderma* LU785 and ground brown mustard applied as seed treatment provided some protection against *R. solani* in Pak Choi under controlled environment conditions (See Chapters 4 and 5), these two treatments, as well as a commercial fungicide seed coat product (Apron) were tested for their effectiveness in a field trial with soil known to be infested with *R. solani* (Kandula et al., 2015).

## 6.2 Materials and methods

### 6.2.1 Site

A site at Lincoln University Research Farm known to contain *R. solani* was used for the field experiment. The soil type was a Templeton silt loam. Resident weeds were sprayed with the herbicide Roundup (Ravensdown Glyphosate G360, containing 360g/L glyphosate as the isopropylamine salt in the form of a soluble concentrate) applied at 2.0 L per hectare using fan nozzles with a pressure of 240 kPa (Ravensdown Ltd, n.d). The trial area was then cultivated by rotary hoeing to produce a seed bed. On September 21<sup>st</sup>, 2023, 3kg of granular Crop Mustard fertilizer was applied to the field using a "Scotts" Hand-Held Broadcast spreader (Figure 6. 1), prior to the transplantation of seedlings.



Figure 6.1 " Scotts" Hand-Held Spreader used to apply Crop Mustard fertilizer to the field before transplanting of seedlings.

### 6.2.2 Seed coating

Pak Choi seeds, variety Green Ace F1, were used for the field experiment because this variety was more susceptible to *R. solani* (section 3.4.3 and 4.4.3). There were five treatments: *Trichoderma* LU785, *Trichoderma* LU785 plus Apron, Apron, ground mustard and bare seed (control). Polymer (Seedworx™ – Biofriendly -1) was applied to all treatments

(Section 4.2.3) apart from the Apron and the control. The treatments were applied to 2.4g of seeds. Trichoderma LU785 was used for seed coating due to its effectiveness in controlling *R. solani* infection (Section 4.4.2 and 4.4.3) in the greenhouse experiment while brown mustard was used for its ability to inhibit *R. solani* hyphal growth (Sections 5.3.6 and 5.3.7) in laboratory plate tests.

Six petri dishes of Trichoderma LU78 were cultured (Section 4.2.1) and the spores were harvested after seven days of colony growth (Section 4.2.2). For the Trichoderma with polymer treatment, seeds were placed in a 15ml falcon tube, with 0.036g of Trichoderma spores, and vortexed for 3 minutes. Then polymer (0.0936ml) was added to the seeds and the tube was vortexed for another 3 minutes.

For the Trichoderma plus Apron with polymer treatment a stock solution of Apron was prepared (see Section 4.2.5). From this stock solution 0.061ml was added to the seeds and Trichoderma spores in a 15ml falcon tube which was vortexed for 3 minutes. 0.0936ml of polymer stock solution was then added and the tube vortexed for another 3 minutes.

For the Apron without polymer treatment, the fungicide was applied to the seeds as described in Section 4.2.5. For the seed meal with polymer treatment 0.525g ground mustard seed (75% w/w) was used. The seeds and ground mustard seed were placed into a 15ml falcon tube and 0.0936ml of polymer solution was added. After vortexing, seeds from all the treatments were tipped into petri plates in a laminar flow cabinet and stirred periodically as the coat dried over two hours.

The plates for all five treatments were then sealed with parafilm, placed in a sterilized plastic container, and stored at 4°C until sowing in the greenhouse.

### **6.2.3 Raising transplants in the greenhouse**

A soil medium was prepared in a ratio of 2:1:1, consisting of 10 litres of sieved black soil, 5 litres of pumice, and 5 litres of peat (Figure 6.2). Fifteen seedling trays (6 cm x 10 cm x 5cm) were filled with the media for each treatment (Figure 6.2b). Seed sowing was done on September 12<sup>th</sup>, 2023. One seed was sown in each cell (45ml volume), and the trays were watered as needed.



Figure 6.2 A mixture of growing media made from sieved black soil, peat, and pumice (a). Seedling trays placed in the Lincoln University Horticulture Greenhouse after seeds were sown (b).

#### 6.2.4 Drenching of *Trichoderma* spores

*Trichoderma* spore concentration per seed was assessed on 8<sup>th</sup> September by using the method outlined in (section 4.2.4) and Appendix 20. The results were only 2.80E+06 spores per seed for *Trichoderma* with polymer treatment and 2.75E+06 spores per seed for *Trichoderma* plus Apron with polymer treatment, less than the 5.00E+06 required. To correct this further *Trichoderma* spores were applied to the soil for both original *Trichoderma* seed treatments by drenching on September 11<sup>th</sup> (3DAS) using a watering can. A stock spore suspension was made by harvesting spores (Section 4.2.2) from six plates using 20 ml of water per plate and putting them into a 250 ml Schott bottle. The procedure is outlined in Appendix 46. A concentration of 3.00E+06 spores per cell was applied Appendix 46.

#### 6.2.5 Field soil *Trichoderma* colony forming units

Twenty-six days before transplanting seedlings into the field, a 7.5mm diameter soil corer was used to obtain 10-15g soil samples from a depth of 5-7 cm to determine resident *Trichoderma* levels. Thirty samples were collected in the field in a zig-zag pattern. The soil samples were composited into a bag, thoroughly mixed by hand, and sieved using a 4mm sieve. The sample was placed in plastic zip-lock bag, labelled, and stored at 4 °C. The colony-forming units were then determined using the method described by Lee (2018). Four 10g subsamples were taken from the zip-lock bag, placed in a 250 ml flask, and diluted by adding 90 ml of water, creating a 10<sup>-1</sup> dilution. Flasks were labelled and the contents mixed using a “Thermo Fisher Scientific Orbital Shaker” at 200 rpm for 10 minutes. The flasks were then carefully removed from the shaker without disturbing the soil material.

1 ml of the suspension was extracted using a pipette and transferred into a 15 ml falcon tube containing 9 ml of water ( $10^{-2}$  dilution) and vortexed for 10 seconds. 1 ml of the diluted suspension was extracted from the  $10^{-2}$  tube and transferred into a 15 ml tube containing 9 ml of water ( $10^{-3}$  dilution) and vortexed again for 10 seconds. Next 1 ml of diluted suspension from the  $10^{-3}$  tube was transferred into another 15ml tube containing 9ml of water ( $10^{-4}$  dilution). After each dilution, 0.1 ml (100  $\mu$ l) was extracted from each using a pipette (Figure 6.3) and poured into separate TSM plates ( $4 \times 4 \times 3 = 48$  plates) starting from the lowest concentration ( $10^{-4}$ ) to the highest concentration ( $10^{-1}$ ). A hockey stick was used to evenly spread the suspension on the plates. Three replicate plates were prepared for each dilution.

Lids were placed on the plates which were then labelled, sealed, put on sterilized trays and covered with black plastic bags. They were stored at room temperature (25 °C) in the dark within growth cabinet for seven days. After seven days, the Trichoderma colonies were counted by eye (Figure 6.4).

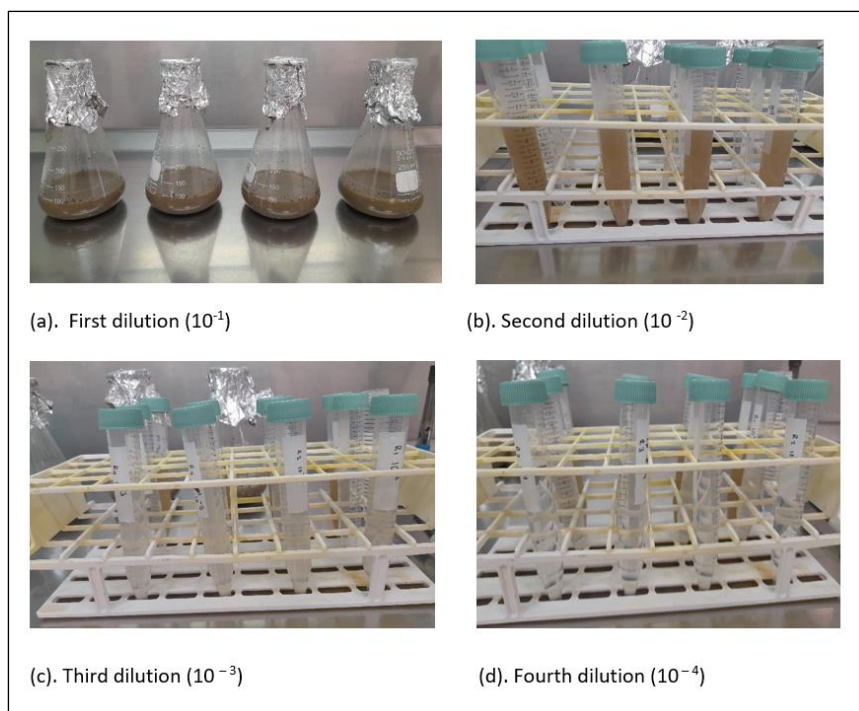


Figure 6.3 Soil sample dilutions for drenching Trichoderma level in the field soil. The first dilution was in four 250ml flasks. The colour was quite dark, reflected the original colour of the soil, but slightly lighter due to the water added (a). Second dilution was in 15 ml falcon tubes. Colour noticeably lighter than the first dilution as the soil particles were more dispersed in the water (b). Third dilution appeared much lighter or tan (c). Fourth dilution colour was very faint, almost transparent.

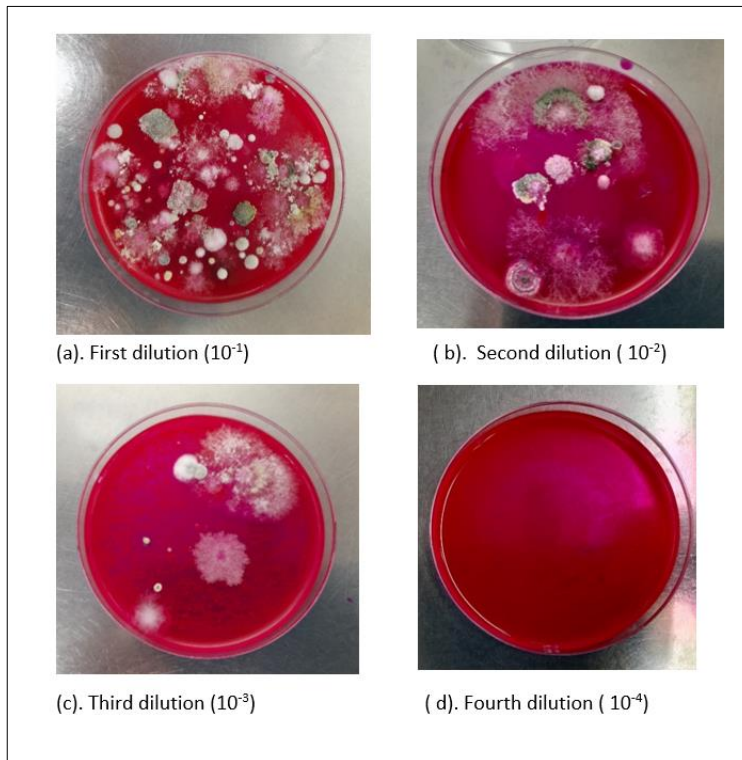


Figure 6.4 The growth of colonies after 7 days of incubation. The first dilution had a high number of colonies, densely packed and often overlapping (a). In the second dilution, the number of colonies decreased, and the colonies were spaced out (b). The third dilution showed a reduced number of colonies with greater separation between individual colonies (c). The fourth dilution yielded no colonies (d).

### 6.2.6 Soil moisture content

Five 10g field soil samples were placed in metal containers (Figure 6.5), dried in a drying “MATEST” oven at 105 °C for 24 hours, and then weighed using a “Thermo Fisher Scientific” scale.

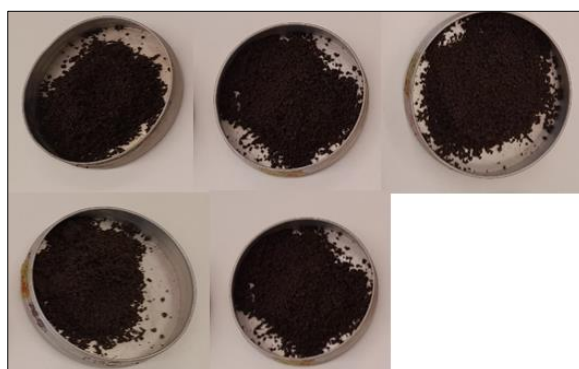


Figure 6.5 Five 10g samples of moist soil were placed on metal containers for drying.

After drying, the samples were weighed, and dry weight recorded. The moisture content was then calculated using the formular in Appendix 48. The soil moisture content was around 25% (Table 6.1).

Table 6.1 Moisture content of soil samples from Lincoln University Research Farm collected on September 15, 2023.

Soil Samples	Wet weight (g)	Dry weight (g)	Moisture content (%)
S1	10	7.98	25.31
S2	10	8.03	24.53
S3	10	8.01	24.84
S4	10	7.99	25.15
S5	10	7.97	25.47

### 6.2.7 Colony forming units (CFUs) per gram of dry soil

The CFUs (Section 6.2.5) were converted to CFUs per gram of dry soil for each of the soil dilution using the formular in Appendix 49. Each dilution had different CFUs (Appendix 49). Each dilution had different CFUs (Appendix 49). The optimum dilution for an accurate CFU value was considered to be from the 1/100<sup>th</sup> dilution due to no crowding of colonies (Appendix 49) was used to calculate the original concentration of CFU within the three samples (Appendix 49). The 1/100<sup>th</sup> dilution provided a sufficient number of colonies to measure the original CFU (Appendix 49). Using the CFU per ml and the dry weight of soil (Table 6.1), the CFU per gram of dry soil was calculated for the field trial (Appendix 49).

### 6.2.8 SeedlingTrichoderma root colonization prior to field transplantation

Four weeks after sowing in the greenhouse, fifteen seedlings (Figure 6.8) were randomly selected from all trays within each treatment to assess Trichoderma root colonization. The roots and TSM media were prepared as described in Section 4.3.3 and Appendix 21. After allowing a seven-day incubation period at 25 °C, Trichoderma colonies were counted on the TSM plates. There were 5 treatments with 8 replicate plates for each treatment.

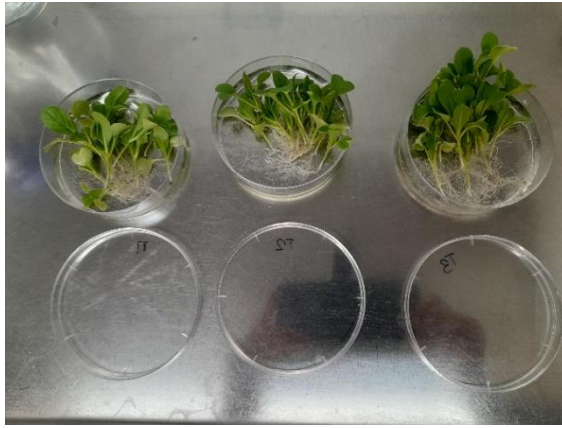


Figure 6.6 4-week-old seedlings were harvested and prepared to determine Trichoderma root colonization.

### 6.2.9 Field trial experimental design

The design was a completely randomised block design (RCBD), with 8 blocks and 5 treatments per block. The plot size was 1.4m x 1.8 m, with rows spaced 15 cm apart, and each row was one meter long (Figure 6.7). The total area of the trial was 117.42m<sup>2</sup> (0.011742ha).

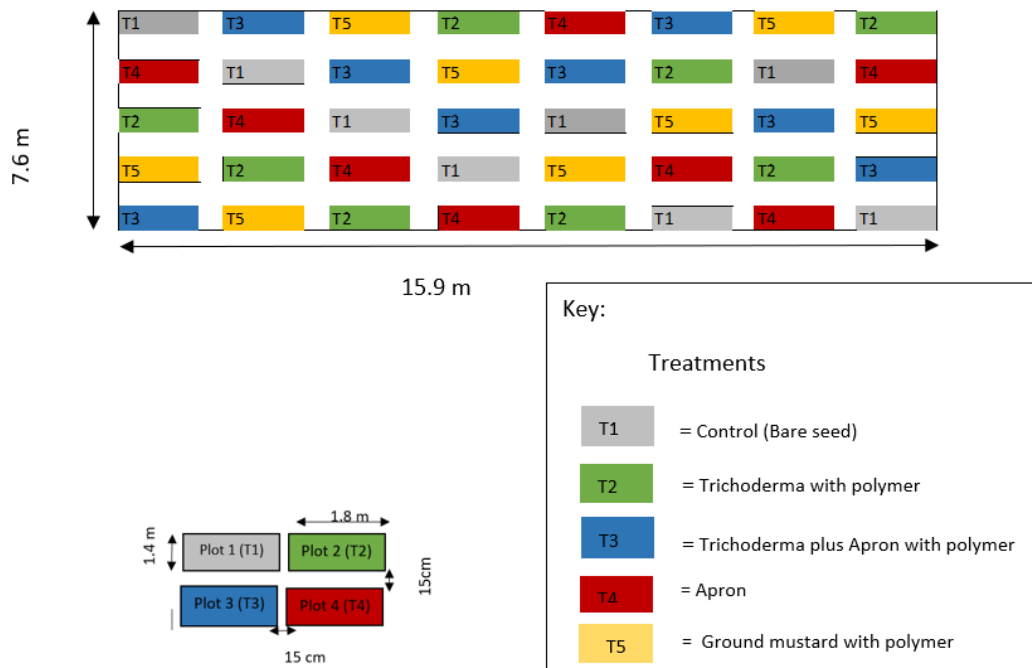


Figure 6.7 An example of the experimental design.

### 6.2.10 Transplanting of seedlings

On October 18th, 2023, the 37-day-old largest and healthiest seedlings were manually transplanted (Figure 6.8a) at a depth of 2 cm to 3 cm. The seedlings were transplanted into 5 rows with a spacing of 15cm between plants within each row. There were 12 plants sown in each row, with a total of 60 plants per plot. After transplanting, each plot was marked with wooden sharp sticks. Netting was used to protect the seedlings from birds. Irrigation was applied manually via a hose as needed (Figure 6.8b) and no insecticide was applied.

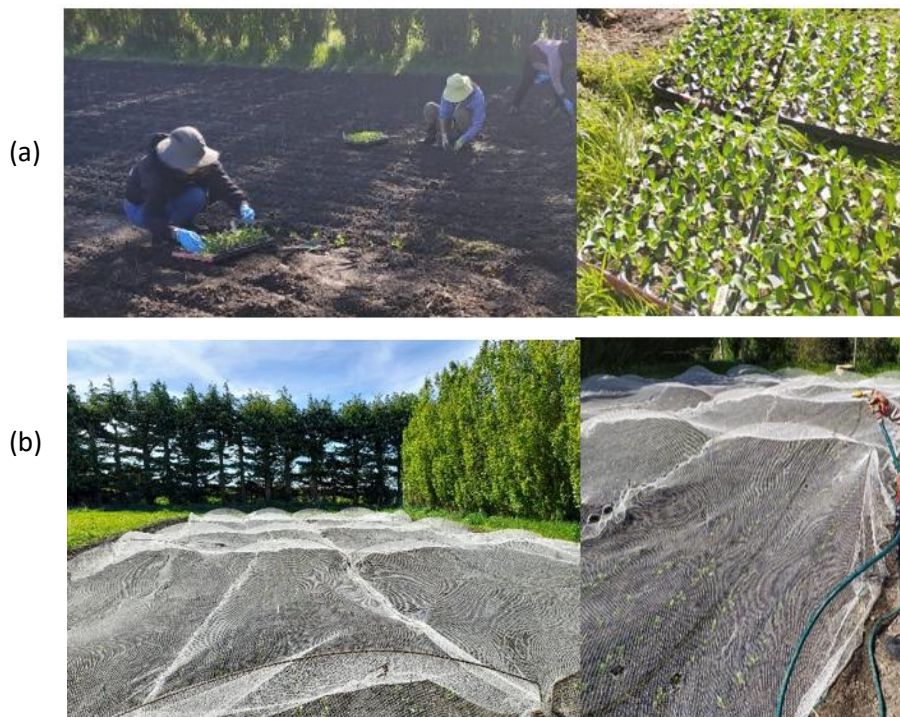


Figure 6.8 37 -day old seedlings transplanted manually in the field (a). Seedlings covered by netting and a hose used for hand-watering the seedlings (b).

### 6.2.11 Data collection at 20 days after sowing (DAS)

At 20 DAS in the glasshouse, the number of seedlings: Healthy (green circle) have vibrant green leaves and stems, strong roots, and shoots. They grow uniformly with firm, upright stems. Unhealthy (yellow circle) show stunted or irregular growth, and their stems are usually soft and weak, and absent (red circle) for each cell per treatment were counted (Figure 6.9).



Figure 6.9 Assessment of seedling numbers at 20 DAS in the greenhouse.

### 6.2.12 Data collection at 27 days after transplanting (DAT)

At 27DAT, all eight replicates of each treatment were assessed to record plant growth development. Leaf-miner damage was at low levels and was not evaluated.



Figure 6.10 Netted plants (red) were harvested 27 days after transplanting.

Four plants were carefully extracted from each of three inner rows, placed in plastic bags (size 25 x 30 cm) and stored in a chiller overnight. The next day, the plants were washed using a water-spray gun to remove soil from the root and leaf surfaces. After washing, the plants were ambient air dried on a bench. Out of 12 plants harvested per plot, 3 plants were subsampled to measure leaf surface area (Section 3.3.2), leaf area index and below ground parameters (Section 5.2.4). The shoots and roots were cut to separates using secateurs and dried in the oven (Section 4.3.2). The remaining 9 plants were also dried in the oven. The total plant dry weight at harvest was the sum of the two samples (9 and 3 plants). The area

sampled was 30 x 45cm, and data were converted to per m<sup>2</sup> (Appendix 50). After assessment, data were analysed (see Section 3.3.2).

### 6.2.13 Data collection at 41 DAT

At 41 DAT, four plants were harvested from each plot. One plant from each treatment was used to assess Trichoderma root colonization. To assess root colonization, 6 root pieces were placed on TSM plate, with 6 replicate plates, resulting in a total of 240 plates (5 treatments x 8 replicate plots x 6 replicate plates) using the method described in Section 4.3.3 and Section 6.2.8. The remaining three plants from each plot were prepared and assessed for below-ground parameters, following the same procedures used for the first sampling at 27 DAT.



Figure 6.11 Roots from each of the five treatments were assessed for root length, root surface area and root volume at 41 DAT.

## 6.3 Results

### 6.3.1 Soil Trichoderma levels

The soil Trichoderma levels for the field trial was considered from 1/100<sup>th</sup> dilution and converted to CFUs per gram of dry soil. The CFU counts from 1/10<sup>th</sup> to 1/1000<sup>th</sup> and the formula for working out the CFU in original sample and CFU per gram of dry soil (g) is given in (Appendix 49). The 1/100<sup>th</sup> dilution from the four subsamples were replicated three times and the CFU counts were shown in the red rectangle (Table 6.2). Sample one yielded CFU

counts of 10, 7, and 13. Sample 2 had the CFU counts of 11, 8, and 4. Sample 3, produced CFU counts of 9, 21, and 27. Sample 4 had CFU counts of 15, 13, and 14. The average CFU per gram of dry soil for all four samples was 158 and this was the soil Trichoderma level for the field trial.

Table 6.2 The soil Trichoderma level from 1/100<sup>th</sup> dilution, combined from the four subsamples and converted to CFU per gram of dry soil (g).

		Sample number				Mean
		Sample 1	Sample 2	Sample 3	Sample 4	
		1/100th	1/100th	1/100th	1/100th	
		0.01	0.01	0.01	0.01	
Replicate Number	1	10	11	9	15	
	2	7	8	21	13	
	3	13	4	27	14	
	Average	10.00	7.67	19.00	14	
Wet weight		10	10	10	10	
CFU in original sample		1000	767	1900	1400	
Dry weight of sample		7.98	8.03	8.01	7.99	
<b>CFU per gram of dry soil (g)</b>		<b>125</b>	<b>95</b>	<b>237</b>	<b>175</b>	<b>158</b>

### 6.3.2 Assessment at 20 days after sowing in the greenhouse.

Seedling emergence did not differ among the treatments (Table 6.3) averaging 97 %.

Therefore, there were no differences among the seedlings which failed to emerge. The Trichoderma plus Apron plus polymer and the ground mustard plus polymer treatments had a significantly greater number of healthy seedlings (and fewer unhealthy seedlings) than the control and other treatments (Table 6.3).

Table 6.3 Effect of treatment on seedling emergence, and the number of healthy and unhealthy seedlings out of 60 cells per tray at 20 DAS for variety Green Ace F1 in green house.

Seed treatments	Emerged seedlings	No emerged seedlings	Healthy seedlings growth	Unhealthy seedling growth
Control (bare seed)	58.1 ab	1.9 ab	52.0 b	6.1 a
Trichoderma + polymer	57.7 b	2.3 a	51.8 b	5.9 a
Trichoderma and Apron + polymer	59.3 a	0.7 b	56.2 a	3.1 b
Apron (no polymer)	57.8 b	2.2 a	53.3 b	4.5 ab
Ground mustard + polymer	59.2 a	0.8 b	56.6 a	2.6 b
LSD 5%	1.3	1.3	2.7	1.9
Significance	P<0.05	P<0.05	P<0.001	P<0.05

Letters were assigned to a Fisher's 5% unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

### 6.3.3 Trichoderma root colonization before transplanting into the field.

There was a highly significant difference among the treatments in the mean number of Trichoderma colonies isolated from the Pak Choi roots. The treatments containing Trichoderma, had a higher mean number of colonies and root colonization percentages, (41% and 47%) than the treatments without Trichoderma and the control (from 6% to 8%, Table 6.4).

### 6.3.4 Assessment at 27 days after transplanting (DAT) into the field

There was no significant difference in leaf area, leaf area index, root surface area, and root volume among the treatments (Table 6.5). However, the root length differed, with the ground mustard with polymer treatment having a greater root length than the control and Trichoderma plus polymer treatment. However, this was not significantly different from the Trichoderma and Apron with polymer and the Apron treatments (Table 6.5).

Table 6.4: Number of Trichoderma colonies isolated from Pak Choi roots and the percentage of roots with colonies at 28 DAS.

Treatments	Mean number of colonies isolated per TSM plate	Root colonization (%)
Control	0.3 a	5.6
Trichoderma with polymer	2.5 b	41.7
Trichoderma and Apron with polymer	2.8 b	47.2
Apron without polymer	0.5 a	8.3
Ground mustard with polymer	0.3 a	5.6
LSD 5%	0.92	
Significance	P < 0.001	

Letters were assigned to a Fisher's 5% unprotectd LSD procedure. Means with the same letter are not significantly different at P= 0.05

Table 6.5: Effect of seed treatment on leaf surface area, leaf area index, root length, root surface area, and root volume (m<sup>3</sup>) per plant for the variety Green Ace F1, 27 days after transplanting into the field.

Seed treatments	Leaf surface area (cm <sup>2</sup> )	Leaf area index (m <sup>2</sup> )	Root length (cm)	Root surface area (cm <sup>2</sup> )	Root volume (cm <sup>3</sup> )
Control (bare seed)	472.9 a	2.10 a	17.3 b	11.1 a	0.60 a
Trichoderma + polymer	506.1 a	2.25 a	18.0 b	11.2 a	0.60 a
Trichoderma and apron + polymer	554.7 a	2.47 a	22.8 ab	12.8 a	0.62 a
Apron (no polymer)	508.7 a	2.26 a	20.9 ab	12.5 a	0.62 a
Ground mustard + polymer	481.7 a	2.14 a	24.6 a	13.4 a	0.62 a
LSD 5%	86.10	0.38	5.9	2.4	0.13
Significance	NS	NS	P<0.05	NS	NS

Letters were assigned to a Fisher's 5% unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

There was no significant difference among the five treatments in terms of shoot, root, and total dry weight of the plant (Table 6.6).

Table 6.6 Effect of seed treatment on shoot, root, and total dry weight per plant for the variety Green Ace F1, 27 days after transplanting into the field.

Seed treatments	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)
Control (bare seed)	144.7 a	17.1 a	161.8 a
Trichoderma + polymer	147.6 a	16.5 a	164.1 a
Trichoderma and apron + polymer	161.2 a	18.2 a	179.4 a
Apron (no polymer)	147.6 a	18.0 a	165.6 a
Ground mustard + polymer	144.4 a	18.1 a	162.5 a
LSD 5%	32.6	4.6	36.8
Significance	NS	NS	NS

Letters were assigned to a Fisher's 5% unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05

### 6.3.5 Assessment at 41 (DAT)

Root length, root surface area and root volume did not differ among the treatments at 41 DAT (Table 6.6). However, there was a significant difference (P<0.05) in root dry weight.

Trichoderma plus Apron with polymer had a higher root dry weight than the control but did not differ significantly from the other three treatments (Table 6.7).

Table 6.7 Effect of seed treatment on plant root length, root surface area, root volume and root dry weight for variety Green Ace F1 41 days after transplanting into the field.

Seed Treatments	Root length (cm)	Root surface area (cm <sup>2</sup> )	Root volume (cm <sup>3</sup> )	Root dry weight (g)
Control (bare seed)	93.8 a	28.7 a	9.6 a	33.2 b
Trichoderma + polymer	97.6 a	30.3 a	10.1 a	38.7 ab
Trichoderma and apron + polymer	103.5 a	31.5 a	10.5 a	41.3 a
Apron (no polymer)	95.4 a	28.8 a	9.6 a	35.0 ab
Ground mustard + polymer	91.3 a	27.8 a	9.3 a	34.7 ab
LSD 5 %	22.3	8.1	2.7	7.5
Significance	NS	NS	NS	P<0.05

Letters were assigned to a Fisher's 5% unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

### 6.3.6 Trichoderma root colonization after transplanting into the field

There was a highly significant difference (P<0.001) in Trichoderma root colonization among the treatments. The ground mustard plus polymer treatment and Trichoderma plus Apron with polymer had the highest root colonization, with ground mustard having the highest mean colony count of 2.4 (40.0 % colonization) (Table 6.8). However, the Trichoderma plus Apron with polymer treatment did not significantly differ from the Trichoderma treatment with polymer, with mean colony numbers of 1.6 and 1.9 (26.7% and 31.7% colonization), respectively. The control and Apron treatments had the lowest root colonization (Table 6.8).

Table 6.8 Number of Trichoderma colonies isolated from Pak Choi roots and the percentage of roots with colonies at 41 DAT.

Treatments	Mean number of colonies isolated	Root colonization (%)
Control	0.2 b	3.3
Trichoderma with polymer	1.6 a	26.7
Trichoderma and Apron with polymer	1.9 a	31.7
Apron without polymer	0.1 b	1.7
Ground mustard with polymer	2.4 a	40.0
LSD 5%	0.9	
Significance	P< 0.001	

Letters were assigned to a Fisher's 5% unprotected LSD procedure. Means with the same letter are not significantly different at P= 0.05.

## 6.4 Discussion

In New Zealand *R. solani* is an economically important soil-borne pathogen of potatoes, causing black scurf on tubers, shoots and stolon canker on young plants (Das et al., 2014). This pathogen also affects Brassica crops, causing damping off and root rot (Kandula et al., 2011; Lee et al., 2023). Fungicide seed treatments such as Apron (Holden, 2022) can effectively increase seedling emergence (Silva et al., 2013), enhance growth and development of plants (Javed et al., 2022) and control soil-borne pathogens such as *R. solani* (Hoose et al., 2022; Lee, 2018; Silva et al., 2013). In agriculture, fungicides may be commonly applied to seeds using a film coating (Accinelli et al., 2018; Pedrini et al., 2020; Pedrini et al., 2018). This method uniformly distributes the treatment directly onto the seeds. Only small amounts of fungicide are required to produce a treatment effect, which reduces the potential exposure of active substances to non-target organisms and increases the economic efficiency of the treatments (Munkvold, 2009; Nuyttens et al., 2013). However, the use of chemical fungicides can result in environmental damage (Usta, 2013). Alternative control methods such as the use of beneficial microorganisms (Lahlali et al., 2022) and biofumigation with Brassicaceae plant volatiles (Hanschen, 2020) are a possible way to ensure minimal disruptive influences on the environment (Lahlali et al., 2022). The polymer acts as a protective cover for the biological seed coating treatments, which are an efficient mechanism for placement of microbial inoculum into soil where they can subsequently colonize the seedling roots and protect against soil-borne pathogens (Sujatha et al., 2023).

*Trichoderma* spp. have been identified worldwide as having the ability to promote plant growth and induce plant resistance against pathogens (Halifu et al., 2019; Yao et al., 2023b). In New Zealand a study demonstrated that coating of radish seeds with selected isolates of *Trichoderma* significantly increased bulb yield in soil naturally infested with *R. solani* (Lee et al., 2023). Biofumigation using Brassica spp as seed meal, as a green manure amendment, or in the crop rotation was considered to be effective in controlling multiple soil-borne pathogens when the glucosinolates in Brassica spp are hydrolysed into the soil (Matthiessen & Kirkegaard, 2006). A study in Egypt indicated that biofumigation using mustard (*B. juncea*) protected chickpea plants against *R. solani* and increased the number of nodules per plant (Sarhan et al., 2020).

In this study, *T. hamatum* LU785, *T. hamatum* LU785 combined with Apron (both with polymer), Apron alone, and ground mustard with polymer were formulated into a Pak Choi seed coating with bare seed as the control. These treatments were tested in a Templeton silt loam soil naturally infested with *R. solani* to examine their biocontrol ability. The hypotheses were that; (i) Trichoderma seed coating would increase seedling emergence and ameliorate plant growth (e.g., plant growth parameters and weight); (ii) Trichoderma would provide control of *R. solani* as effectively as Apron; and (iii) *R. solani* would reduce seedling emergence and plant growth by causing root rot and damping-off.

#### **6.4.1 In the greenhouse**

Cortés-Rojas et al. (2021) and Coninck et al. (2020) have reported that Trichoderma seed treatments can have positive effects on germination and seedling emergence. Lee (2018) however found that in the field, seedling emergence for Trichoderma treated radish seeds did not differ from that of the control. However, in the present experiment the seedlings for transplanting were raised in the greenhouse and there was no significant difference among the treatments in the number of emerged seedlings.

However, there were significant differences in the number of emerged seedlings which were healthy (no symptoms of disease). The Trichoderma plus Apron treatment and ground mustard had a slightly higher number of healthy seedlings. Healthy seedlings had no wire stem symptoms (Sujatha et al., 2023). The combination of Trichoderma with Apron fungicide increased the number of healthy seedlings whereas Trichoderma alone and Apron alone did not.

This synergistic response has been previously reported. A combination of Trichoderma spp with thiophanate-methyl fungicide seed treatment reduced damping-off caused by *Fusarium* spp. in beans under both greenhouse and field conditions (Abd-El-Khair et al., 2019). The synergetic effect of biocontrol agents such as Trichoderma spp. combined with reduced levels of fungicides can suppress disease equally to a fungicide application at full strength Howell (1991) suggested that this synergetic effect of biocontrol agents such as Trichoderma spp. combined with fungicides might allow a reduced level of fungicide application to achieve similar disease suppression to the fungicide applied at full strength.

Apron XL seed treatment has been shown to be highly effective against many oomycete pathogens including *Pythium* and *Phytophthora* spp (Margot, 1983). It is not labelled for

controlling *R. solani* (Syngenta-seedcare, n.d). Hwang et al. (2006) reported that Apron XL when combined with other fungicides can significantly reduce the impact of *R. solani* on seedling establishment. However, Apron XL does not provide protection against Aphanomyces root rot (Margot, 1983; Vincelli, 1992).

Brassica spp. seed meal or green manure can suppress soil borne pathogens (Sarwar et al., 1998) and enhance plant growth and yield (Morris et al., 2020) by released volatile compounds, mainly isothiocyanates (ITCs) as a result of hydrolysis of brassica tissue in the soil (Brown & Morra, 1997). Brassicas, particularly mustard, can be used as an alternative method to control root rot disease where methyl bromide is banned (Abdallah et al., 2020; Rokunuzzaman et al., 2016). Lord et al. (2011) considered that the biocidal activity of the glucosinolates from *B. juncea* is comparable with the efficacy of chemical pesticides and antibiotics. Mustard seeds contain higher glucosinolates content than other plant parts (Lazzeri et al., 2004). The fungicidal effects of mustard and canola seed meals were tested against three pathogenic fungi (*Fusarium. oxysporum*, *Sclerotinia. sclerotiorum* and *R. solani*) on chickpea under both greenhouse and field conditions. Both seed meals significantly reduced damping-off. Mustard seed meal had the highest plant survival rate ( 61- 65%) and the lowest damping-off rate ( 34-38 %) among the three pathogenic fungi (Sarhan et al., 2020). Shaban et al. (2011) also found that mustard seed meal was the most effective treatment for reducing root rot and wilt disease incidence on lupin plants (by 88 and 88 % respectively). A more recent study showed that using seed meal alone or in combination with other techniques had promising results for controlling plant diseases (Hanschen and Winkelmann, 2020).

In this study, Trichoderma root colonization was assessed among the treatments before the seedlings were transplanted into the field. Not surprisingly the two Trichoderma treatments had 41 and 47% Trichoderma root colonization at 60 DAS. Ferreira and Musumeci (2021) have stated that Trichoderma will colonize the roots independently of the inoculation method. For example, *T. harzianum* T -22 colonized the entire root surface irrespective of when it was applied as a seed treatment, on soil surface or in granules incorporated into the potting mix (Harman, 2000). Trichoderma spp. are versatile plant symbionts attracted by chemical signals emitted by plant roots (Brotman et al., 2013). The initial steps of symbiosis establishment involve hyphal attachment, penetration, and colonization within the apoplast of plant roots (Halifu et al., 2019; Risoli et al., 2022; Yedidia et al., 1999).

#### 6.4.2 In the field

In the field trial only healthy (no wire stem) seedlings were transplanted, with the assumption that *R. solani* inoculum already present in the field soil would have an impact on the transplants. Unfortunately, as a result of a communication error, disease incidence was not recorded during the final harvest at 41 DAT. The symptoms of naturally occurring *R. solani* infection in field conditions, can resemble those caused by other diseases or abiotic stress, making accurate identification challenging without laboratory tests (Aydin, 2022). Though the disease incidence data were not recorded, other studies have reported that as plants mature (reaching or at the flowering stage) (Figure 6.14) they develop stronger and more robust tissues which can provide resistance to *R. solani* infection (Acharya et al., 1984). Kang et al. (2015) reported that the plant's natural defence mechanisms involve protective chemicals like Salicylic (SA) acid (Lee et al., 2006) and an upregulation of pathogenesis-related protein (PR) genes which enhance the plant's resistance to pathogen attack.



Figure 6.14 Pak Choi plants at 27 DAT

Many studies have shown that *Trichoderma* spp. can promote plant growth and alleviate abiotic and biotic stress in the field (Hirst et al., 2024). However, at 27 DAT leaf surface area, leaf area index, root surface area and the plant dry weight did not differ among the treatments. Only the root length differed among the treatments and the ground mustard treatment had a greater root length than the control and *Trichoderma* treatment. Meyer et al. (2015) reported that mustard seed meal has been effective in reducing root-knot nematode populations, leading to healthier root systems in the field. The glucosinolates in mustard seed meal breaks down into bioactive compounds that suppress harmful soil organisms, thereby promoting healthier root development and plant growth (Meyer et al., 2015). But the ground mustard root length did not differ from that of the *Trichoderma* and Apron with polymer and Apron treatment. *Trichoderma* can effectively manage root rot up to 60 days after sowing and enhance plant growth under field conditions (Sain et al., 2023). Apron

fungicide reduced the number of diseased plants in soil inoculated with *F. graminearum* and increased maize plant biomass (Aveling et al., 2012).

During the second harvest at 41 DAT only below ground parameters were recorded. There was no significant difference in root length, surface area or volume among the treatments. However, the Trichoderma plus Apron treatment significantly increased root dry weight whereas the Trichoderma alone and Apron alone did not differ from the control. Increases in root weight following Trichoderma treatment have been reported in many species (Abdelkhalek et al., 2022; Lee, 2018; Velasco et al., 2008), but in the present trial the synergistic response recorded for increased root length at 27 DAT presumably explains the increased root weight at 41DAT for this treatment.

At 41 DAT root colonization by Trichoderma was 27% and 32% for the Trichoderma and Trichoderma plus Apron treatments, but surprisingly it was 40% for the ground mustard treatment.

Field conditions often provide more diverse dynamic environment compared to the controlled conditions of a greenhouse (Carro-Huerga et al., 2021; Rivera-Méndez, 2020). Topolovec-Pintarić (2018) pointed out that for Trichoderma to be effective, it must first be isolated, studied, and formulated for soil application. However, reintroducing it to the soil is challenging due to competition with existing rhizosphere microbes for colonization. This competition may explain the slight decrease in the number of Trichoderma root colonies in both Trichoderma treatments in the field compared to greenhouse.

The Trichoderma colonizing the roots of the plants grown from the ground mustard seed treatment must have been already present in the field soil as no Trichoderma was added for this treatment. In the glasshouse the control, Apron and ground mustard treatments all had around 6-8% root colonization, indicating a Trichoderma presence initially. Mustard biofumigation can effectively reduce the population of soil borne pathogens but not inhibit beneficial soil microbes. For example Saranya et al. (2024) conducted experiments on red rot (sugar cane disease) caused by the fungal pathogen *Colletotrichum falcatum*, using mustard biofumigation under in vitro and in vivo conditions and examining its effect on other soil microbiota such as the biocontrol agent *Trichoderma harzianum*. In vitro the volatiles from mustard seed meal gave 100% inhibition of *C. falcatum* at 0.5% concentration (w/v), whereas *T. harzianum* was not inhibited, even by up to a 1% concentration.

Another study by Saranya et al. (2024) using mustard seed extract in poisoned food technique. A 57% inhibition of mycelial growth of *C. falcatum* was found at a 2.5% concentration of mustard seed, while beneficial organisms like *T. harzianum*, *Metarhizium anisopliae* and *Nomuraea rileyi* were not affected. The native isolate *Trichoderma* sp. *T-Nam*, a potential biocontrol agent of *S. rolfsii*, showed high degree of tolerance to biofumigation (Goswami & Pariyar, 2023). Therefore, selective inhibition of microbes by biofumigation may help maintain a healthy soil microbiome allowing *Trichoderma* spp at the rhizosphere to colonize the plant roots. This may explain the high *Trichoderma* root colonization recorded for the ground mustard treatment.

Recent studies have demonstrated that biopolymer seed coatings can attract naturally occurring soil microbes, including beneficial *Trichoderma* species (Usmanova et al., 2024). These biopolymers play a protective role for plants against pathogenic fungi through various mechanisms (Othman et al., 2022). Additionally, they serve as carriers for active ingredients, allowing for controlled release (Korbecka-Glinka et al., 2022). Whether this occurred in this trial could not be determined as there was no polymer only treatment.

## Chapter 7: General Discussion

*Rhizoctonia solani* is a soil-borne fungal pathogen that negatively affects a wide range of economically important crops, including grains, vegetables, and forage (Senapati et al., 2022). Symptoms such as damping-off, root rot, and stem cankers can cause economic losses in crops, and in Brassica crops, yield losses can range from 20 % to 60% (Akber & Fang, 2024). Pak Choi is a significant vegetable in Asia, particularly in countries like China (Ding et al., 2022), Japan (Klein, 2021), and Malaysia (Henny & Gobilik, 2022). Pak Choi has also become increasingly available and popular in supermarkets in Australia and New Zealand (Thomson, 2005). The cultivation and sale of Pak Choi contribute to New Zealand's local economy. While there is no available information on the production value, Pak Choi production supports New Zealand's emphasis on sustainable and diverse agricultural practices (Vegetables.co.nz, 2021).

This research was conducted to determine (i) the effects of the pathogen on Pak Choi growth, and (ii) to assess the effect of different biological control methods on the pathogen.

Fungicides are still the most widespread method for disease control and those available to control *R. solani* include azoxystrobin, flutolanil, and pencycuron (Campion et al., 2003; Djébalí & Belhassen, 2010), which can be applied as seed or soil treatments. Chemical application can be efficient in reducing pathogen damage in the short term. However, the regular application of chemical fungicides increases the risk of pathogen resistance, with consequent environmental damage (Chaudhary et al., 2024). Alternative control methods, such as biological controls, are considered environmentally friendly and essential for sustainable farming (Abbas et al., 2022).

*Trichoderma* spp. have been proven to be beneficial biocontrol agents worldwide in agriculture and horticulture (Adhikari, 2023; Yao et al., 2023b), with more than 60 % of the registered bio-fungicides globally being *Trichoderma* (Abbas et al., 2017). They are commonly found in soils, roots, and leaves, and are abundant in various types of soils and dead plant tissues (Enshasy et al., 2020; Natsiopoulou et al., 2024). Studies have confirmed that *Trichoderma* spp. are effective biocontrol agents against plant pathogens, using mechanisms such as antibiosis, mycoparasitism, and competition for nutrients to reduce the

negative impact of pathogens on plant growth and productivity (Alizadeh et al., 2024; Natsiopoulou et al., 2024; Rodrigue et al., 2023).

The control of soil-borne pests and diseases in commercial agriculture and horticulture systems has proved to be difficult. In glasshouses or small production areas, soil fumigation, using toxic, volatile compounds applied to the soil was formerly a common practice (Gimsing & Kirkegaard, 2009). This involved the application of methyl bromide, a synthetic compound known for its high efficacy in pest suppression (Gareau, 2010; Martin, 2003). However, the use of methyl bromide has been banned due to its negative impact on the environment, including contributing to depletion of the ozone layer (Gareau, 2010).

Biofumigation was an alternative method used to suppress pests and diseases through the hydrolysis products of glucosinolates, particularly the isothiocyanates, which are released from the damage of Brassicaceae plant tissues (Gimsing & Kirkegaard, 2009). Some of the best brassica species used for biofumigation are *B. juncea* (Sencenbaugh et al., 2024), *B. napus*, *Raphanus sativus* (Reddy, 2012) and *Eruca sativa* (Aydinli & Mennan, 2018). They are incorporated into the soil as green manure or cover crops, where they decompose and release bioactive compounds that help manage soil health and reduce pathogen loads (Santos et al., 2021). They are used in various regions around the world, including North and South America, Europe, Asia, South Africa, Brazil, Australia (Santos et al., 2021), and New Zealand (Falloon et al., 2007).

As already stated, the research reported in this thesis explored the impacts of *R. solani* on two Asian cabbage varieties (Mei Qing Choi and Green Ace F1) and the ability of differing biocontrol methods to reduce the impact of the pathogen. Two New Zealand strains of *R. solani* (043-4) kindly supplied by the New Zealand Plant and Food Research Institute were assessed in a preliminary glasshouse study for their ability to infect the host plants and the amount of inoculum required to reduce the plant population by 50%. Strain 043-4, at an inoculum rate of 0.25g per pot resulted in close to 50% survival of seedlings in variety Green Ace F1, and this was the rate selected and used in Experiment 2. Strain 043-4 was more virulent than 043-1, and had previously been identified as the strain most commonly associated with the disease black scurf in potato production in New Zealand (Das et al., 2014).

The second experiment involved the use of four New Zealand Trichoderma isolates from the Lincoln University “trichobank” collection (*T. atroviride* LU32, *T. hamatum* LU785, *T. harzianum* LU1347 and *T. polysporum* LU1358) which Lee (2018) had previously used for control of *R. solani* and the promotion of plant growth in radish. They were applied as a seed coating to evaluate their effectiveness in controlling *R. solani* strain 043-4 and in influencing plant growth parameters for the two Pak Choi varieties under glasshouse conditions.

None of the treatments completely prevented the failure of some seedlings to emerge or post-emergence seedling death. However, *T. hamatum* LU785 significantly improved seedling emergence and survival rates for both Pak Choi varieties and had higher root colonization percentages, indicating strong biocontrol and growth promoting properties. The beneficial activities of *T. hamatum* include antibacterial, antifungal, antiviral, herbicidal, insecticidal/pesticidal, antioxidant, and plant growth promotion activities (Lodi et al., 2023). In addition, *T. atroviride* LU32 increased plant fresh and dry weights for variety Green Ace F1 in the presence of *R. solani* and showed the second highest root colonization percentages. *T. atroviride* LU32 also increased leaf chlorophyll concentration, leaf number, and leaf area compared to the control in the presence of *R. solani*, and all the Trichoderma treatments resulted in longer root and shoot lengths. These results are consistent with previous reports which show that Trichoderma spp. can better support plant growth under pathogen stress (Poveda et al., 2020; Sood et al., 2020; Yao et al., 2023b). Studies have also reported that *T. atroviride* had positive impacts on plant growth and weight (Liu et al., 2023; Rao et al., 2022). This isolate is commercially available in New Zealand as Tenet® and Sentinel® for the control of onion white rot in Allium species and *Botrytis cinerea* in grapes and glasshouse tomatoes (agrimm.nz, n.d). From this glasshouse experiment *T. hamatum* LU785 was effective for reducing the pathogen impacts on seedling emergence and early growth while *T. atroviride* LU32 provided broader benefits for plant development. However, *T. hamatum* LU785 was chosen to be used in the field, because it significantly improved seedling emergence and survival rates for both Pak Choi varieties, which is crucial for establishing a strong crop stand in the field.

Overall, Trichoderma isolates demonstrated good performance compared to both the control and Apron fungicide in enhancing Pak Choi growth and controlling *R. solani*. While Apron fungicide has its uses for control of some, pathogens including oomycetes causing damping off of seedlings (syngenta-seedcare, n.d) it does not have a label claim for control

*R. solani*. Trichoderma inoculation is a promising tool for sustainable agriculture (Sharma et al., 2023), offering multiple benefits from pathogen control to enhance plant growth and soil health (Wang et al., 2024).

In experiment three, the biofumigant properties of glucosinolates (GSLs) were first evaluated in the laboratory by assessing the sinigrin concentrations in both frozen and fresh leaf tissues of four Brassicaceae species. Based on their glucosinolate levels, cauliflower and Pak Choi were selected and used for biofumigation in the glasshouse experiment.

In the presence of the pathogen, the frozen cauliflower treatment resulted in significantly higher seedling emergence, survival, and healthy seedlings compared to the other biofumigant treatments. Root and shoot parameters, including root length, root surface area, root volume, shoot length, hypocotyl diameter, number of leaves, and leaf surface area, were higher in the frozen cauliflower treatment and these results in some cases did not differ from the non-pathogen control. These findings suggest that the method of tissue preparation and incorporation (Brennan et al., 2020) plays a crucial role in the effectiveness of biofumigation, with frozen tissues showing enhanced biofumigant activity due to better ITC release (Morra & Kirkegaard, 2002). Studies have shown that frozen tissues release higher quantities of ITC compared to fresh tissues because, freezing causes ice crystals to form with plant cells leading to cell rupture (Angelino et al., 2015). When the plant tissue is disrupted, the enzyme myrosinase comes into contact with glucosinolates and in the presence of water, hydrolysis reaction occurs and releases the ITCs (Brown & Morra, 1996).

This study contributes to the understanding of how different Brassica tissue treatments can be optimized for improved disease and pest control, as well as plant health, in agricultural systems. Morra and Kirkegaard (2002) suggested that thorough tissue disruption at a cellular level by freezing the tissues increased release efficiencies to 14 and 26%. The resulting maximum ITC concentrations of 40–75 nmol ITC g<sup>-1</sup> soil are much nearer to those used in commercial pest control, making it likely that these levels will effectively suppress pest and pathogens. A study done by Rodríguez et al. (2023) confirmed that the application of freeze-dried kale tissues to six week old pepper seedlings in pot trials significantly reduced pathogen (*R. solani*) damage. Ashiq et al. (2022) conducted two experiments (Petri dish and closed jar) to assess the biofumigation potential of frozen leaves from Brassicaceae species (*Brassica juncea*, *Raphanus sativus*, *Eruca sativa*) against *Fusarium graminearum*, a major

cereal pathogen. In the Petri dish experiment, mycelial plugs of *F. graminearum* were exposed to frozen leaves of the Brassica species. The results showed that frozen *B. juncea* significantly suppressed the mycelial growth of *F. graminearum* by 87-100%. In the closed jar experiment, soil was amended with chopped frozen Brassica tissues, and the results indicated that each frozen Brassica species tissue suppressed *F. graminearum* by 41-55%. Kirkegaard et al. (1996) reported up to 50% suppression of *F. graminearum* mycelial growth by ground freeze-dried tissue of the *B. juncea* shoots 10–500 mg per Petri dish.

In the laboratory petri dish experiment, the two higher concentrations of ground mustard incorporated into the medium (0.25g and 0.50g per petri dish) completely inhibited the hyphal growth of *R. solani* after four days. Additionally, results from ground mustard seed coating experiment indicated that *R. solani* hyphal growth decreased as the application rate increased. This finding is consistent with previous research, which showed that bioactive compounds in mustard seed are more effective at higher concentrations of 2% w/w (Abdallah et al., 2020), and 25mg (Fayzalla et al., 2009; Sarhan et al., 2020).

In experiment four, Trichoderma LU785 and ground mustard seed applied as seed treatments, were able to reduce the impacts of *R. solani* in Pak Choi under controlled environmental conditions. These treatments were chosen for field trials to see if they could also support early plant growth and provide some resistance to diseases, along with a commercial fungicide (Apron XL). The field trial site had soil known to be infested with *R. solani* (Kandula et al., 2015).

Seedlings for transplanting into the field were raised in a greenhouse. Seedling emergence did not differ between any of the treatments and the control, but seed treatment with Trichoderma plus Apron incorporated in a polymer, and ground mustard in the polymer had more healthy seedlings.

At 27 days after transplanting into the field there were no differences in plant growth parameters except for root length, which the ground mustard with polymer treatment had increased. By 41 days after transplanting, plants grown from the Trichoderma plus Apron with polymer treatment, had a significantly greater root dry weight which was greater than for either the Trichoderma or Apron fungicide treatments alone. The possibility of generating a synergistic effect with the application of fungicides and biological antagonists, such as Trichoderma spp. has been reported by several authors. In a study conducted by

Wang et al. (2005), the combination of Trichoderma and fludioxonil fungicide was found to provide greater control of root rot and significantly increase the survival rate of coneflower seedlings (*Echinacea* spp.) when used against *Fusarium* sp. in greenhouses. Lezama et al. (2023) evaluated the in vitro compatibility of four species of Trichoderma (*T. hamatum*, *T. asperellum*, *T. konigiopsis*, and *T. harzianum*) with three broad-spectrum fungicides (Captan, Mancozeb and Chlorothalonil) through potential antagonism against the MA-FC120 strain of *F. solani*. Results showed that *Trichoderma asperellum* presented high compatibility with the fungicides Captan and Mancozeb in concentrations of 450, 900, and 1350 mg L<sup>-1</sup>. The use of antagonistic strains together with the fungicide Chlorothalonil in its three concentrations showed a negative effect on the growth of Trichoderma species, which caused low and null compatibility against the MA-FC120 strain of *F. solani*. There are reports of many factors causing the tolerance of Trichoderma strains to pesticides, such as the change in function of oxidoreductase genes and ABC transporter genes resulting in the tolerance of Trichoderma spp. to dichlorvos, Mancozeb, thiram, tebuconazole, and carbendazim (Hirpara & Gajera, 2018; Sun et al., 2019).

However, by 41 days after transplanting into the field the root colonization for the Trichoderma treatments was lower than that recorded in the glasshouse, while for the ground mustard, in polymer seed treatment root colonization was greater than for the Trichoderma treatments. It is possible that the selective inhibition by mustard biofumigation reduced antagonist in the microbiome thus allowing a greater proliferation of soil resident Trichoderma which then invaded the plant roots. Galletti et al. (2008) reported that in *in vitro* tests, Trichoderma isolates were generally less sensitive to the toxic volatiles from *Brassica carinata* seed meal compared to pathogen assayed such as *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum*. In addition, Garain et al. (2021) experimented on the effectiveness of biofumigation using Indian mustard (*Brassica juncea*) against a fungus called *Sclerotium rolfsii*, which causes collar rot disease in betelvine plants. The beneficial fungus Trichoderma sp. T-Nam was also tested to see how it responded to the biofumigation. Results indicated that the beneficial fungus, Trichoderma, was more tolerant to the biofumigation. Garain et al. (2021) also found that when biofumigation was combined with Trichoderma in the soil, it completely stopped *S. rolfsii* from colonizing the soil and reduced the incidence of collar rot disease in betelvine by 95%. However, whether this is what happened requires further investigation. Whether the presence of the polymer

attracted beneficial microbes as suggested by Usmanova et al. (2024) also requires investigation, but this is unlikely as an explanation as other treatments, which also included the polymer did not have an increase in root colonization in the field.

This research indicates that both *Trichoderma* species and biofumigation show potential as biocontrol methods for Asian cabbage. *Trichoderma* spp. are beneficial microorganisms that provide protection against *R. solani* and enhance plant growth (Abbas et al.,2022). They suppress plant pathogens by producing and releasing cell wall-degrading enzymes and antibiotics. Biofumigation suppresses soilborne pathogens by incorporating glucosinolate containing Brassica plant materials as green manure or seed meals. This process releases volatiles, mainly isothiocyanates, which are toxic to various pathogens (Matthiessen & Kirkegaard, 2006), and also enhances plant growth and development. However, further studies are required before commercial application. These include:

- (1) The necessity of extensive field trials to confirm the efficacy and safety of these methods under various environmental conditions. This will help in understanding their practical applicability and any potential limitations.
- (2) Research should focus on optimizing the application methods for both *Trichoderma* spp. and biofumigation. This includes determining the best formulations, dosages, and timing for application to maximize their effectiveness.
- (3) Long-term studies are required to assess the sustainability and environmental impact of these biocontrol methods. This includes monitoring soil health, non-target effects, and potential resistance development in pathogens.
- (4) Conducting a cost-benefit analysis to evaluate the economic feasibility of these methods for farmers. This includes comparing the costs of these biocontrol methods against those of chemical controls and assessing their market potential.
- (5) Research should also explore how these biocontrol methods can be integrated with existing agricultural practices and pest management strategies to provide an integrated pest management approach to crop protection.

## APPENDICIES

### Appendix 1: Potato Dextrose Agar (PDA)

- Mix 39 g of PDA and 1 litre of distilled water in a 1 litre Schott bottle using a magnetic mixer.
- Autoclave the medium for 15 minutes at 121 °C
- Pour 18ml of medium by hand into sterile petri plates on a flat surface in a laminar flow cabinet

### Appendix 2: Lincoln University Nursery potting mix

Ingredients;

- 3-4 month mix, volume – 500L.
- Media - 400 L bark ,100 L pumice.
- Fertilizers- Osmocote exact ;16- 3.9 -10g N:P: K (3-4 month) 1500g.
- Horticulture lime 500g.
- Hydrflo 500g

### Appendix 3: Lincoln University seed raising mix

Ingredients;

- 3-4 month mix, volume – 200L.
- Media – 120 L peat and 80 L pumice
- Fertilizers -Osmocote exact mini;15-3.9-9.1g N:P:K (3-4 month) 400g
- Dolomite lime 500g
- Hydroflo 200g

TWO-WAY FACTOR ANALYSES AT 20 DAYS ASSESSEMENT FOR EXPERIMENT ONE

**Appendix 4: Effect of *R.solani* isolate 043-1 and 043-4 inoculum rates on surviving seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
	043-1	043-4
<b>Main effect means</b>		
<b><i>R. solani</i> rates</b>		
0g	10 a	10.0 a
0.1g	9.8 a	8.0 a
0.25g	9.6 a	6.5 a
0.5g	8.5 b	4.5 b
1g	6.5 c	3.0 c
LSD (5%)	1.04	1.79
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	9.28 a	6.60 a
Green Ace F1	8.48 b	6.20 a
LSD (5%)	0.65	1.13
Significance	P<0.05	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 10.0 a		
043-1: 8.6 b		
043-4: 5.5 c		
LSD (5%): 1.37		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	10.0	10.0
0.1g	9.8	8.0
0.25g	9.6	7.0
0.5g	9.0	5.0
1g	8.0	3.0
<b>Green Ace F1</b>		
0g	10.0	10.0
0.1g	9.8	8.0
0.25g	9.6	6.0
0.5g	8.0	4.0
1g	5.0	3.0
LSD (5%)	0.71	2.53
Significance	P<0.05	NS

**Appendix 5: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the number of seedlings per pot which died post emergence at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	0.0 a	0.0 a
0.1g	0.0 a	1.1 a
0.25g	0.0 a	2.3 b
0.5g	0.6 a	3.1 b
1g	2.3 b	1.1 a
LSD (5%)	0.95	1.15
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	0.20 a	1.28 a
Green Ace F1	0.96 b	1.78 a
LSD (5%)	0.60	0.73
Significance	P<0.05	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 0.00 a		
043-1: 0.72 b		
043-4: 1.91 c		
LSD (5%): 0.66		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	0.0	0.0
0.1g	0.0	1.0
0.25g	0.0	2.0
0.5g	0.0	2.2
1g	1.0	1.2
<b>Green Ace F1</b>		
0g	0.0	0.0
0.1g	0.0	1.2
0.25g	0.0	2.7
0.5g	1.2	4.0
1g	3.6	1.0
LSD (5%)	1.35	1.63
Significance	P<0.05	NS

**Appendix 6: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the number of healthy seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
	043-1	043-4
<b>Main effect means</b>		
<b><i>R. solani</i> rates</b>		
0g	10.0 a	10.0 a
0.1g	9.8 a	5.8 b
0.25g	8.9 a	3.1 c
0.5g	7.4 b	0.8 d
1g	4.8 c	1.5 d
LSD (5%)	1.31	1.02
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	9.28 a	4.62 a
Green Ace F1	7.12 b	3.92 b
LSD (5%)	0.83	0.64
Significance	P<0.001	P<0.05
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 10.0 c		
043-1: 7.75 b		
043-4: 2.83 a		
LSD (5%) :1.71		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	10.0	10.0
0.1g	9.8	5.8
0.25g	9.6	4.6
0.5g	9.0	1.7
1g	8.0	1.0
<b>Green Ace F1</b>		
0g	10.0	10.0
0.1g	9.8	5.9
0.25g	8.3	1.7
0.5g	5.9	0.0
1g	1.6	2.0
LSD (5%)	1.85	1.45
Significance	P<0.001	P<0.05

**Appendix 7: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the number of diseased seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means <i>R. solani</i> rates</b>	<b>043-1</b>	<b>043-4</b>
0g	0.0 a	0.0 a
0.1g	0.0 a	2.1 bc
0.25g	0.65 b	3.3 bc
0.5g	1.05 b	3.6 c
1g	1.70 c	1.5 ab
LSD (5%)	0.4	1.98
Significance	P<0.001	P<0.05
<b>Variety</b>		
Mei Qing Choi	0.0 a	1.98 a
Green Ace F1	1.3 b	2.28 a
LSD (5%)	0.23	1.25
Significance	P<0.001	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 0.00 a		
043-1: 0.85 a		
043-4: 2.66 b		
LSD (5%) :1.44		
Significance :P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	0.0	0.0
0.1g	0.0	2.2
0.25g	0.0	2.4
0.5g	0.0	3.3
1g	0.0	2.0
<b>Green Ace F1</b>		
0g	0.0	0.0
0.1g	0.0	2.1
0.25g	1.3	4.3
0.5g	2.1	4.0
1g	3.4	1.0
LSD (5%)	0.52	1.37
Significance	P<0.001	NS

**Appendix 8: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the disease score of seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means <i>R. solani</i> rates</b>	<b>043-1</b>	<b>043-4</b>
0g	0.0 a	0.0 a
0.1g	0.0 a	1.6 b
0.25g	0.3 ab	2.0 b
0.5g	0.6 b	2.3 b
1g	1.7 c	3.7 c
LSD (5%)	0.39	0.93
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	0.0 a	1.85 a
Green Ace F1	1.1 b	2.05 a
LSD (5%)	0.24	0.59
Significance	P<0.001	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 0.00 a		
043-1: 0.68 b		
043-4: 2.43 c		
LSD (5%): 0.88		
Significance :P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	0.0	0.0
0.1g	0.0	1.5
0.25g	0.0	2.0
0.5g	0.0	2.2
1g	0.0	3.5
<b>Green Ace F1</b>		
0g	0.0	0.0
0.1g	0.0	1.75
0.25g	0.7	2.0
0.5g	1.2	2.5
1g	3.5	4.0
LSD (5%)	0.55	0.64
Significance	P<0.001	P<0.001

**Appendix 9: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the root length of seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
	043-1	043-4
<b>Main effect means.</b>		
<b><i>R. solani</i> rates</b>		
0g	9.4 a	9.4 a
0.1g	8.5 a	7.6 a
0.25g	8.3 a	8.2 a
0.5g	8.4 a	7.9 a
1g	8.3 a	7.8 a
LSD (5%)	1.38	1.85
Significance	NS	NS
<b>Variety</b>		
Mei Qing Choi	8.84 a	8.22 a
Green Ace F1	8.44 a	8.26 a
LSD (5%)	0.87	0.57
Significance	NS	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 9.4 b		
043-1: 8.44 a		
043-4: 7.94 a		
LSD (5%): 0.87		
Significance: P<0.05		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	9.5	9.5
0.1g	7.8	7.5
0.25g	9.6	8.0
0.5g	8.8	8.0
1g	8.2	7.9
<b>Green Ace F1</b>		
0g	9.2	9.2
0.1g	9.2	7.8
0.25g	7.0	8.3
0.5g	8.1	7.9
1g	8.5	7.8
LSD (5%)	1.95	2.62
Significance	NS	NS

**Appendix 10: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the shoot length of seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means.</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	9.0 ab	9.0 a
0.1g	9.3 a	8.5 a
0.25g	9.1 ab	8.6 a
0.5g	8.6 bc	7.9 a
1g	8.3 c	7.8 a
LSD (5%)	0.63	1.39
Significance	P<0.05	NS
<b>Variety</b>		
Mei Qing Choi	10.3 a	9.57 a
Green Ace F1	7.4 b	7.26 b
LSD (5%)	0.19	0.88
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 9.0 a		
043-1: 8.8 a		
043-4: 8.2 a		
LSD (5%): 1.70		
Significance: NS		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	10.4	10.4
0.1g	10.6	9.2
0.25g	10.7	9.1
0.5g	9.9	9.5
1g	9.9	9.4
<b>Green Ace F1</b>		
0g	7.7	7.7
0.1g	7.9	7.8
0.25g	7.5	8.1
0.5g	7.3	6.3
1g	6.7	6.2
LSD (5%)	0.90	1.97
Significance	NS	NS

**Appendix 11: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the fresh weight of seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means <i>R. solani</i> rates</b>	<b>043-1</b>	<b>043-4</b>
0g	17.2 a	17.2 a
0.1g	16.7 a	14.1 a
0.25g	16.9 a	13.0 b
0.5g	15.1 a	10.5 b
1g	12.2 b	5.1 c
LSD (5%)	2.25	4.05
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	20.5 a	15.5 a
Green Ace F1	10.8 b	8.6 b
LSD (5%)	1.42	2.56
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 17.2 b		
043-1: 15.2 b		
043-4: 10.7 a		
LSD (5%): 5.39		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	22.6	22.6
0.1g	20.5	16.7
0.25g	22.2	18.3
0.5g	19.4	13.8
1g	17.4	5.7
<b>Green Ace F1</b>		
0g	11.8	11.8
0.1g	12.8	11.5
0.25g	11.6	7.8
0.5g	10.7	7.1
1g	7.0	4.5
LSD (5%)	3.19	5.73
Significance	NS	NS

**Appendix 12: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the dry weight of seedlings per pot at 20 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	2.8 b	2.8 c
0.1g	2.8 b	2.2 b
0.25g	2.6 ab	2.1 b
0.5g	2.4 ab	1.8 b
1g	2.1 a	1.1 a
LSD (5%)	0.60	0.61
Significance	P<0.05	P<0.001
<b>Variety</b>		
Mei Qing Choi	3.0 a	2.4 a
Green Ace F1	2.1 b	1.6 b
<b><i>R. solani</i> isolates &amp; control</b>		
LSD (5%)	0.38	0.39
Significance	P<0.001	P<0.001
0g : 2.8 b		
043-1: 2.5 b		
043-4: 1.8 a		
LSD (5%): 0.70		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	3.4	3.4
0.1g	3.1	2.4
0.25g	3.4	2.8
0.5g	2.7	2.1
1g	2.3	1.2
<b>Green Ace F1</b>		
0g	2.3	2.3
0.1g	2.5	1.9
0.25g	1.9	1.4
0.5g	2.0	1.5
1g	1.9	1.0
LSD (5%)	0.87	0.87
Significance	NS	NS

TWO-WAY FACTOR ANALYSES AT 33 DAYS ASSESSEMENT FOR EXPERIMENT ONE

**Appendix 13: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the number of healthy plants per pot at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	9.8 a	9.7 a
0.1g	9.6 ab	6.2 b
0.25g	8.8 bc	4.2 bc
0.5g	8.3 cd	3.5 c
1g	7.5 d	2.0 d
LSD (5%)	0.93	1.38
Significance	P<0.001	P<0.001
<b>Variety</b>		
Mei Qing Choi	8.84 a	6.40 a
Green Ace F1	8.85 a	3.90 b
LSD (5%)	0.59	0.87
Significance	NS	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 9.8 c		
043-1: 8.5 b		
043-4: 4.0 a		
LSD (5%): 1.05		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	9.75	10.0
0.1g	9.5	8.5
0.25g	9.0	5.75
0.5g	8.2	5.0
1g	7.7	2.75
<b>Green Ace F1</b>		
0g	10.0	9.5
0.1g	9.75	4.0
0.25g	8.75	2.75
0.5g	8.50	2.0
1g	7.25	1.25
LSD (5%)	1.32	1.92
Significance	NS	NS

**Appendix 14: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the shoot length of plants per pot at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	13.2 a	13.20 a
0.1g	13.6 a	12.61 ab
0.25g	13.4 a	11.93 b
0.5g	12.7 a	12.02 b
1g	13.1 a	12.58 ab
LSD (5%)	1.05	1.11
Significance	NS	NS
<b>Variety</b>		
Mei Qing Choi	14.88 a	14.23 a
Green Ace F1	11.60 b	10.71 b
LSD (5%)	0.66	0.70
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 13.2		
043-1: 13.2		
043-4: 12.2		
LSD (5%) :1.17		
Significance: NS		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	15.0	15.00
0.1g	15.15	14.11
0.25g	14.81	13.81
0.5g	14.55	13.97
1g	14.90	14.29
<b>Green Ace F1</b>		
0g	11.41	11.41
0.1g	12.61	11.11
0.25g	12.17	10.06
0.5g	10.96	10.08
1g	11.32	10.88
LSD (5%)	1.49	1.57
Significance	NS	NS

**Appendix 15: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the root length of plants per pot at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means <i>R. solani</i> rates</b>	<b>043-1</b>	<b>043-4</b>
0g	9.7 a	9.7 a
0.1g	10.6 a	9.7 a
0.25g	9.3 a	9.1 a
0.5g	9.2 a	9.1 a
1g	10.5 a	9.2 a
LSD (5%)	1.56	2.47
Significance	NS	NS
<b>Variety</b>		
Mei Qing Choi	10.38 a	9.29
Green Ace F1	9.48 a	9.51
LSD (5%)	0.98	1.56
Significance	NS	NS
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 9.7 a		
043-1: 9.9 a		
043-4: 9.3 a		
<b>LSD (5%) :1.15</b>		
<b>Significance: NS</b>		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	10.4	10.4
0.1g	10.6	10.1
0.25g	9.75	7.6
0.5g	10.2	8.8
1g	10.9	9.3
<b>Green Ace F1</b>		
0g	9.8	9.0
0.1g	10.7	9.2
0.25g	9.0	10.6
0.5g	8.3	9.5
1g	10.19	9.0
LSD (5%)	2.21	3.50
Significance	NS	NS

**Appendix 16: Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the root length of plants per at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	930.4 a	930.4 a
0.1g	1033.0 a	902.9 a
0.25g	1017.6 a	641.7 b
0.5g	934.0 a	602.9 b
1g	968.9 a	318.1 c
LSD (5%)	162.3	205.1
Significance	NS	P<0.001
<b>Variety</b>		
Mei Qing Choi	1140 a	874 a
Green Ace F1	813 b	484 a
LSD (5%)	102.7	129.7
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 930.4 b		
043-1: 988.4 a		
043-4: 616.4 c		
LSD (5%): 162.7		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	1147	1147
0.1g	1156	1124
0.25g	1139	890
0.5g	1119	782
1g	1140	429
<b>Green Ace F1</b>		
0g	714	714
0.1g	910	682
0.25g	896	393
0.5g	749	424
1g	798	207
LSD (5%)	229.6	290.0
Significance	NS	NS

**Appendix 17 : Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the fresh weight of plants per pot at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means <i>R. solani</i> rates</b>	<b>043-1</b>	<b>043-4</b>
0g	63.7 a	63.9 c
0.1g	76.5 b	59.6 c
0.25g	71.7 ab	36.1 b
0.5g	60.5 a	36.7 b
1g	66.0 ab	20.0 a
LSD (5%)	12.4	10.5
Significance	NS	P<0.001
<b>Variety</b>		
Mei Qing Choi	82.3 a	57.4 a
Green Ace F1	53.2 b	29.2 b
LSD (5%)	7.8	6.6
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g: 63.7 b		
043-1: 68.7 b		
043-4: 38.1 a		
LSD (5%): 11.9		
Significance: P<0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	78.5	78.5
0.1g	90.3	73.1
0.25g	85.5	56.8
0.5g	72.1	49.5
1g	85.3	29.0
<b>Green Ace F1</b>		
0g	49.1	49.1
0.1g	62.8	46.3
0.25g	58.0	15.4
0.5g	49.0	24.0
1g	46.7	11.0
LSD (5%)	17.6	14.9
Significance	NS	NS

**Appendix 18 : Effect of *R. solani* isolates 043-1 and 043-4 inoculum rates on the dry weight of plants per pot at 33 DAS for varieties Mei Qing Choi and Green Ace F1**

Treatment	<i>R. solani</i> isolate	
<b>Main effect means</b>	<b>043-1</b>	<b>043-4</b>
<b><i>R. solani</i> rates</b>		
0g	3.1 a	3.1 a
0.1g	3.7 a	2.8 a
0.25g	3.4 a	1.9 b
0.5g	2.9 a	1.8 b
1g	3.3 a	1.0 c
LSD (5%)	0.74	0.57
Significance	NS	P<0.001
<b>Variety</b>		
Mei Qing Choi	4.07	2.79
Green Ace F1	2.58	1.50
LSD (5%)	0.47	0.36
Significance	P<0.001	P<0.001
<b><i>R. solani</i> isolates &amp; control</b>		
0g : 3.1 a		
043-1: 3.3 a		
043-4: 1.9 b		
LSD (5%): 0.6		
Significance: P< 0.001		
<b><i>R. solani</i> rate x variety interaction means</b>		
<b>Mei Qing Choi</b>		
0g	3.9	3.9
0.1g	4.4	3.3
0.25g	4.1	2.8
0.5g	3.6	2.3
1g	4.1	1.4
<b>Green Ace F1</b>		
0g	2.3	2.3
0.1g	3.0	2.2
0.25g	2.7	1.0
0.5g	2.3	1.2
1g	2.4	0.6
LSD (5%)	1.05	0.81
Significance	NS	NS

## Appendix 19: Malt Yeast Extract Agar

### Recipes

- Malt extract 10g (6g)
- Yeast extract 1.0 g (0.6g)
- Agar 16.7 g (10g)
- Distilled water 1000 ml (600ml)

## Appendix 20: Determining Trichoderma spore numbers using a haemocytometer.

- Place a microscope glass cover slip (size 26 x 76 mm & 0.1 mm thick) on the haemocytometer.
- Pipette 10 $\mu$ L of sample from a cap tube and place it under the slide.
- Using the 'Olympus bx51' microscope on lens 'UPLSAPO 4 x 0.16', count the number of spores in the centre grid and four corner grids of the haemocytometer.
- Calculate the average spore number: Average the grids and multiply by 25 (as there are 25 squares in the haemocytometer).
- Calculate the number of spores per mL (volume of slide) by multiplying  $1 \times 10^4$  eg; the number of spore counts (mean)  $\times$  25 grids  $\times 10^4 =$  spores mL $^{-1}$ .
- number of spore counts (mean)  $\times$  25 grids  $\times 10^4 =$  spores mL $^{-1}$ .

## Appendix 21: Root TSM media

### TSM recipes

- Malt extract: 6.0g
- Yeast extract: 0.6g
- Terrachlor (quintozene): 0.12g
- Rose Bengal: 1.8ml
- Micro Agar: 10g
- Distilled water: 600ml
- Antibiotic: 0.6ml chloramphenicol stock solution (100mg/ml)

TWO-WAY FACTOR ANALYSES AT 30 DAYS ASSESSMENT FOR EXPERIMENT TWO

**Appendix 22: Effect of seed treatment and *R. solani* on maximum seedling emergence per pot at 30 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	4.4 c	4.0 b
LU132	5.3 ab	4.9 ab
LU785	5.9 a	5.3 a
LU1347	4.9 bc	4.8 ab
LU1358	5.3 ab	4.5 ab
Apron	5.2 b	4.1 b
LSD (5%)	0.7	0.9
Significance	P<0.05	P<0.05
<b>Pathogen</b>		
0.25g	3.4	2.2
0.0 g	6.9	6.9
LSD (5%)	0.4	0.5
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	1.8	1.0
LU132	3.7	2.8
LU785	4.8	3.6
LU1347	2.7	2.8
LU1358	3.8	2.0
Apron	3.3	1.2
<b>No pathogen</b>		
Control	7.0	7.0
LU132	6.8	7.0
LU785	7.0	7.0
LU1347	7.0	6.7
LU1358	6.8	7.0
Apron	7.0	7.0
LSD (5%)	0.9	1.3
Significance	P<0.001	P<0.05

**Appendix 23: Effect of seed treatment and *R. solani* on the number of seedlings per pot which died post emergence at 30 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	0.3 b	0.2 a
LU132	0.5 ab	0.1 a
LU785	1.0 a	0.5 a
LU1347	0.5 ab	0.5 a
LU1358	0.5 ab	0.4 a
Apron	0.4 b	0.3 a
LSD (5%)	0.6	0.5
Significance	P<0.05	NS
<b>Pathogen</b>		
0.25g	1.0	0.6
0.0 g	0.0	0.0
LSD (5%)	0.4	0.3
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.6	0.3 a
LU132	1.0	0.2 a
LU785	2.0	1.0 a
LU1347	1.0	1.0 a
LU1358	1.0	0.7 a
Apron	0.7	0.5 a
<b>No pathogen</b>		
Control	0.0	0.0
LU132	0.0	0.0
LU785	0.0	0.0
LU1347	0.0	0.0
LU1358	0.0	0.0
Apron	0.0	0.0
LSD (5%)	0.8	0.7
Significance	NS	NS

**Appendix 24: Effect of seed treatment and *R. solani* on the number of seedlings per pot which survived at 30 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatments</b>		
Control	4.1 b	3.9 b
LU132	4.8 a	4.8 a
LU785	4.9 a	4.8 a
LU1347	4.4 ab	4.3 ab
LU1358	4.8 a	4.2 b
Apron	4.8 a	3.9 b
LSD (5%)	0.6	0.6
Significance	P<0.05	P<0.05
<b>Pathogen</b>		
0.25g	2.3	1.6
0.0 g	6.9	6.9
LSD (5%)	0.4	0.4
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	1.2	0.7
LU132	2.7	2.6
LU785	2.8	2.6
LU1347	1.7	1.8
LU1358	2.8	1.3
Apron	2.6	0.7
<b>No pathogen</b>		
Control	7.0	7.0
LU132	6.8	7.0
LU785	7.0	7.0
LU1347	7.0	6.7
LU1358	6.8	7.0
Apron	7.0	7.0
LSD (5%)	0.9	0.9
Significance	P<0.05	P<0.05

**Appendix 25: Effect of seed treatment and *R. solani* on the number of diseased seedlings per pot at 30 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	0.4 a	0.3 a
LU132	0.7 a	0.3 a
LU785	0.5 a	0.4 a
LU1347	0.4 a	0.4 a
LU1358	0.4 a	0.0 a
Apron	0.5 a	0.1 a
LSD (5%)	0.4	0.4
Significance	NS	NS
<b>Pathogen</b>		
0.25g	0.9	0.5
0.0 g	0.0	0.0
LSD (5%)	0.2	0.2
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.7	0.5
LU132	1.4	0.6
LU785	1.0	0.8
LU1347	0.7	0.8
LU1358	0.8	0.0
Apron	1.0	0.2
<b>No pathogen</b>		
Control	0.0	0.0
LU132	0.0	0.0
LU785	0.0	0.0
LU1347	0.0	0.0
LU1358	0.0	0.0
Apron	0.0	0.0
LSD (5%)	0.3	0.6
Significance	NS	NS

**Appendix 26: Effect of seed treatment and *R. solani* on the number of healthy seedlings per pot at 30 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatments</b>		
Control	3.8 a	3.6 c
LU132	4.1 a	4.5 a
LU785	4.4 a	4.4 a
LU1347	4.0 a	3.9 b
LU1358	4.4 a	4.2 ab
Apron	4.3 a	3.8 bc
LSD (5%)	0.7	0.6
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	1.4	1.1
0.0 g	6.9	6.9
LSD (5%)	0.4	0.3
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.5	0.2
LU132	1.3	2.0
LU785	1.8	1.8
LU1347	1.0	1.0
LU1358	2.0	1.3
Apron	1.6	0.5
<b>No pathogen</b>		
Control	7.0	7.0
LU132	6.8	7.0
LU785	7.0	7.0
LU1347	7.0	6.7
LU1358	6.8	7.0
Apron	7.0	7.0
LSD (5%)	0.5	0.8
Significance	NS	P<0.05

**Appendix 27: Effect of seed treatment and *R. solani* on the SPAD value (leaf chlorophyll concentration) of plants at 20 DAS for varieties Mei Qing Choi and Green Ace F1.**

Main effect means	Varieties	
	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	20.2 a	22.7 b
LU132	22.7 a	30.5 a
LU785	23.8 a	27.7 ab
LU1347	23.9 a	28.5 ab
LU1358	22.2 a	23.2 ab
Apron	22.4 a	24.9 ab
LSD (5%)	5.0	7.4
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	20.82	20.9
0.0 g	24.22	30.2
LSD (5%)	2.9	4.3
Significance	P<0.05	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	16.2	14.9
LU132	21.1	30.5
LU785	23.7	25.1
LU1347	23.1	29.6
LU1358	20.4	15.5
Apron	20.4	19.8
<b>No pathogen</b>		
Control	24.2	30.4
LU132	24.3	30.4
LU785	23.9	30.3
LU1347	24.7	27.4
LU1358	23.9	30.9
Apron	24.3	29.9
LSD (5%)	7.1	10.60
Significance	NS	P<0.05

TWO-WAY FACTOR ANALYSES AT 60 DAYS ASSESSEMENT FOR EXPERIMENT TWO

**Appendix 28: Effect of seed treatment and *R. solani* on the number of plants per pot that survived at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	4.1 b	3.9 b
LU132	4.3 ab	4.7 a
LU785	4.9 a	4.5 a
LU1347	4.2 b	3.9 b
LU1358	4.7 ab	4.2 ab
Apron	4.7 ab	3.8 b
LSD (5%)	0.7	0.6
Significance	P<0.05	P<0.05
<b>Pathogen</b>		
0.25g	2.0	1.3
0.0 g	6.9	6.9
LSD (5%)	0.4	0.4
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	1.2	0.7
LU132	1.8	2.3
LU785	2.8	2.0
LU1347	1.3	1.0
LU1358	2.5	1.3
Apron	2.3	0.5
<b>No Pathogen</b>		
Control	7.0	7.0
LU132	6.8	7.0
LU785	7.0	7.0
LU1347	7.0	6.7
LU1358	6.8	7.0
Apron	7.0	7.0
LSD (5%)	1.0	0.9
Significance	P<0.05	P<0.05

**Appendix 29: Effect of seed treatment and *R. solani* on the number of dead plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	0.0 b	0.0 b
LU132	0.5 a	0.2 ab
LU785	0.0 b	0.3 ab
LU1347	0.2 ab	0.4 a
LU1358	0.2 ab	0.0 b
Apron	0.2 ab	0.0 b
LSD (5%)	0.4	0.4
Significance	P<0.05	P<0.05
<b>Pathogen</b>		
0.25g	0.3	0.3
0.0 g	0.0	0.0
LSD (5%)	0.2	0.2
Significance	P<0.05	P<0.05
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.0	0.0
LU132	0.9	0.3
LU785	0.0	0.6
LU1347	0.4	0.8
LU1358	0.3	0.0
Apron	0.3	0.0
<b>No pathogen</b>		
Control	0.0	0.0
LU132	0.0	0.0
LU785	0.0	0.0
LU1347	0.0	0.0
LU1358	0.0	0.0
Apron	0.0	0.0
LSD (5%)	0.5	0.5
Significance	NS	P<0.05

**Appendix 30: Effect of seed treatment and *R. solani* on the number of diseased plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatments</b>		
Control	0.4 a	0.3 a
LU132	0.3 a	0.2 a
LU785	0.5 a	0.1 a
LU1347	0.2 a	0.0 a
LU1358	0.3 a	0.2 a
Apron	0.4 a	0.0 a
LSD (5%)	0.5	0.3
Significance	NS	NS
<b>Pathogen</b>		
0.25g	0.6	0.2
0.0 g	0.0	0.0
LSD (5%)	0.3	0.2
Significance	P<0.001	P<0.05
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.7	0.5
LU132	0.5	0.3
LU785	1.0	0.2
LU1347	0.3	0.0
LU1358	0.5	0.3
Apron	0.7	0.0
<b>No pathogen</b>		
Control	0.0	0.0
LU132	0.0	0.0
LU785	0.0	0.0
LU1347	0.0	0.0
LU1358	0.0	0.0
Apron	0.0	0.0
LSD (5%)	0.7	0.5
Significance	NS	NS

**Appendix 31: Effect of seed treatment and *R. solani* on the number of healthy plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatments</b>		
Control	3.8 b	3.6 c
LU132	4.1 ab	4.5 a
LU785	4.4 a	4.4 a
LU1347	4.0 ab	3.9 b
LU1347	4.4 a	4.0 ab
Apron	4.3 ab	3.8 bc
LSD (5%)	0.6	0.7
Significance	P<0.05	P<0.05
<b>Pathogen</b>		
0.25g	1.4	1.1
0.0 g	6.9	6.9
LSD (5%)	0.3	0.4
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	0.5	0.2
LU132	1.3	2.0
LU785	1.8	1.8
LU1347	1.0	1.0
LU1358	2.0	1.0
Apron	1.6	0.5
<b>No pathogen</b>		
Control	7.0	7.0
LU132	6.8	7.0
LU785	7.0	7.0
LU1347	7.0	6.7
LU1358	6.8	7.0
Apron	7.0	7.0
LSD (5%)	0.4	1.0
Significance	P<0.05	P<0.05

**Appendix 32: Effect of seed treatment and *R. solani* on plant disease score at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	1.0 a	1.0 a
LU132	1.0 a	0.5 ab
LU785	1.0 a	0.5 ab
LU1347	0.5 a	0.0 b
LU1358	0.5 a	0.4 ab
Apron	1.0 a	0.0 b
LSD (5%)	0.9	0.8
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	1.7	0.8
0.0 g	0.0	0.0
LSD (5%)	0.6	0.5
Significance	P<0.001	P<0.05
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	2.0	2.0
LU132	2.0	1.0
LU785	2.0	1.0
LU1347	1.0	0.0
LU1358	1.0	0.7
Apron	2.0	0.0
<b>No pathogen</b>		
Control	0.0	0.0
LU132	0.0	0.0
LU785	0.0	0.0
LU1347	0.0	0.0
LU1358	0.0	0.0
Apron	0.0	0.0
LSD (5%)	1.2	1.2
Significance	NS	NS

**Appendix 33: Effect of seed treatment and *R. solani* on the SPAD value (leaf chlorophyll concentration) of plants at 50 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	28.1 a	26.8 b
LU132	30.3 a	36.6 a
LU785	33.3 a	32.7 ab
LU1347	33.3 a	26.4 b
LU1358	30.3 a	27.2 ab
Apron	29.9 a	29.2 ab
LSD (5%)	6.7	9.8
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	27.8	23.4
0.0 g	33.9	36.2
LSD (5%)	3.9	5.7
Significance	P<0.05	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	21.8	18.0
LU132	27.4	35.7
LU785	32.4	29.9
LU1347	31.9	17.2
LU1358	27.0	17.9
Apron	26.4	21.9
<b>No pathogen</b>		
Control	34.3	35.5
LU132	33.2	37.5
LU785	34.1	35.5
LU1347	34.7	35.5
LU1358	33.6	36.5
Apron	33.4	36.5
LSD (5%)	9.4	6.9
Significance	NS	NS

**Appendix 34: Effect of seed treatment and *R. solani* on the shoot length (cm) of plants at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatment</b>		
Control	14.1 a	11.9 a
LU132	14.6 a	14.9 a
LU785	15.8 a	12.9 a
LU1347	15.6 a	12.5 a
LU1358	14.6 a	12.8 a
Apron	13.5 a	11.8 a
LSD (5%)	4.1	4.6
Significance	NS	NS
<b>Pathogen</b>		
0.25g	13.1	10.7
0.0 g	16.3	14.9
LSD (5%)	2.3	2.7
Significance	P<0.05	P<0.05
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	11.4	8.3
LU132	12.5	14.6
LU785	14.9	11.5
LU1347	15.4	10.4
LU1358	12.8	10.5
Apron	11.3	8.6
<b>No pathogen</b>		
Control	16.7	15.4
LU132	16.7	15.2
LU785	16.6	14.2
LU1347	15.6	14.6
LU1358	16.4	15.0
Apron	15.6	14.9
LSD (5%)	5.7	6.5
Significance	NS	NS

**Appendix 35: Effect of seed treatment and *R. solani* on the root length (cm) of plants at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	11.9 a	11.3 a
LU132	13.1 a	15.9 a
LU785	12.2 a	13.6 a
LU1347	14.8 a	13.1 a
LU1358	12.9 a	12.2 a
Apron	12.5 a	11.2 a
LSD (5%)	3.9	5.2
Significance	NS	NS
<b>Pathogen</b>		
0.25g	12.4	12.3
0.0 g	13.4	13.5
LSD (5%)	2.2	3.0
Significance	NS	NS
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	11.2	9.0
LU132	12.3	18.5
LU785	10.9	13.9
LU1347	16.4	13.3
LU1358	11.8	9.8
Apron	11.6	9.0
<b>No pathogen</b>		
Control	12.6	13.5
LU132	13.8	13.3
LU785	13.4	13.2
LU1347	13.1	12.9
LU1358	13.9	14.5
Apron	13.3	13.3
LSD (5%)	5.5	7.4
Significance	NS	NS

**Appendix 36: Effect of seed treatment and *R. solani* on the hypocotyl diameter (mm) of plants at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatments</b>		
Control	2.7 a	2.8 a
LU132	2.8 a	3.6 a
LU785	2.8 a	3.4 a
LU1347	3.3 a	3.3 a
LU1358	2.8 a	3.1 a
Apron	2.7 a	2.5 a
LSD (5%)	0.8	1.3
Significance	NS	NS
<b>Pathogen</b>		
0.25g	2.7	2.9
0.0 g	3.0	3.3
LSD (5%)	0.5	0.7
Significance	NS	NS
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	2.2	2.1
LU132	2.6	4.0
LU785	2.8	3.2
LU1347	3.6	3.1
LU1358	2.6	2.8
Apron	2.2	2.0
<b>No pathogen</b>		
Control	3.1	3.4
LU132	3.0	3.2
LU785	2.7	3.5
LU1347	3.0	3.4
LU1358	2.9	3.4
Apron	3.2	2.9
LSD (5%)	1.2	1.8
Significance	NS	NS

**Appendix 37: Effect of seed treatment and *R. solani* on the number of leaves per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	28.1 ab	32.6 ab
LU132	29.1 ab	39.0 a
LU785	32.3 a	34.9 ab
LU1347	29.3 ab	30.6 a
LU1358	26.0 b	35.4 a
Apron	30.1 ab	31.5 a
LSD (5%)	5.9	9.7
Significance	P<0.05	NS
<b>Pathogen</b>		
0.25g	12.8	11.5
0.0 g	45.5	56.5
LSD (5%)	3.4	4.2
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	9.7	7.5
LU132	10.5	20.5
LU785	17.0	15.0
LU1347	10.0	8.8
LU1358	16.3	11.5
Apron	13.3	5.7
<b>No pathogen</b>		
Control	46.5	57.7
LU132	47.7	57.5
LU785	47.5	54.8
LU1347	48.6	52.3
LU1358	35.7	59.3
Apron	46.8	57.3
LSD (5%)	8.4	10.4
Significance	P<0.05	NS

**Appendix 38: Effect of seed treatment and *R. solani* on the leaf surface area of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

Main effect means	Varieties	
	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	656.6 a	826.3 ab
LU132	1624.1 a	1069.8 a
LU785	1760.8 a	851.4 ab
LU1347	1898.1 a	787.4 b
LU1358	1764.4 a	1077.2 a
Apron	1871.3 a	712 b
LSD (5%)	1308.3	265
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	522	393
0.0 g	2669	1382
LSD (5%)	755.3	153
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	410.2	281.5
LU132	460.2	770.5
LU785	628.5	359.8
LU1347	443.1	347.7
LU1358	592.8	386.3
Apron	599.5	213.0
<b>No pathogen</b>		
Control	903	1371
LU132	2788	1369
LU785	2893	1343
LU1347	3353	1227
LU1358	2956	1768
Apron	3143	1211
LSD (5%)	1850.2	374
Significance	NS	NS

**Appendix 39: Effect of seed treatment and *R. solani* on the fresh weight (g) of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	111.2 a	118.1 a
LU132	95.2 a	127.8 a
LU785	113.3 a	117.0 a
LU1347	106.6 a	105.9a
LU1358	123.2 a	120.6 a
Apron	111.2 a	101.2 a
LSD (5%)	29.09	50.61
Significance	NS	NS
<b>Pathogen</b>		
0.25g	54.2	47.2
0.0 g	165.9	183.0
LSD (5%)	17.3	18.5
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	44.1	32.4
LU132	44.0	92.2
LU785	66.7	43.6
LU1347	35.9	44.1
LU1358	85.4	46.1
Apron	49.2	24.9
<b>No pathogen</b>		
Control	178.2	203.8
LU132	146.3	163.4
LU785	159.8	190.4
LU1347	177.2	167.6
LU1358	160.9	195.1
Apron	173.1	177.5
LSD (5%)	36.7	45.2
Significance	NS	P<0.05

**Appendix 40: Effect of seed treatment and *R. solani* on the dry weight (g) of plants per pot at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	Varieties	
Main effect means	Mei Qing Choi	Green Ace F1
<b>Seed treatments</b>		
Control	14.0 a	15.5 ab
LU132	14.7 a	20.3 a
LU785	15.3 a	17.8 ab
LU1347	16.6 a	17.1 ab
LU1358	16.3 a	16.7 ab
Apron	15.0 a	14.2 b
LSD (5%)	3.3	5.9
Significance	NS	P<0.05
<b>Pathogen</b>		
0.25g	11.9	9.1
0.0 g	18.9	24.8
LSD (5%)	1.9	3.4
Significance	P<0.001	P<0.001
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	9.8	6.2
LU132	10.8	14.0
LU785	13.3	10.4
LU1347	12.2	9.3
LU1358	12.9	8.6
Apron	11.6	5.8
<b>No pathogen</b>		
Control	18.2	24.7
LU132	18.6	26.6
LU785	17.2	25.1
LU1347	20.9	24.8
LU1358	19.7	24.8
Apron	18.4	22.6
LSD (5%)	4.7	9.8
Significance	NS	P<0.05

**Appendix 41: Number of Trichoderma colonies at 60 DAS for varieties Mei Qing Choi and Green Ace F1.**

	<b>Varieties</b>	
<b>Main effect means</b>	<b>Mei Qing Choi</b>	<b>Green Ace F1</b>
<b>Seed treatment</b>		
Control	1.4 c	1.2 c
LU132	4.2 a	3.9 ab
LU785	4.6 a	4.4 a
LU1347	4.3 a	4.0 ab
LU1358	2.8 b	3.2 b
LSD (5%)	0.9	0.9
Significance	P<0.001	P<0.001
<b>Pathogen</b>		
0.25g	2.80	2.92
0.0 g	4.12	3.76
LSD (5%)	0.6	0.56
Significance	P<0.001	P<0.05
<b>Seed treatments x pathogen interaction</b>		
<b>Pathogen</b>		
Control	1.4	1.2
LU132	3.2	3.0
LU785	3.6	3.8
LU1347	3.2	3.4
LU1358	2.6	3.2
<b>No pathogen</b>		
Control	1.4	1.2
LU132	5.2	4.8
LU785	5.6	5.0
LU1347	5.4	4.6
LU1358	3.0	3.2
LSD (5%)	1.2	1.2
Significance	P<0.05	NS

#### Appendix 42: Preparation and calculations on sinigrin stock solution

1. Sinigrin (Sigma Aldrich) powder (10mg) was weighed (Thermo Fisher Scientific scale) and transferred to a 50ml falcon tube.
2. 10ml of sterile water was added and mixed with a vortex for 5minutes.
3. Solution was filtered using a 0.2  $\mu\text{m}$  filter syringe and the standard solution was prepared using the formular;  $C_1 V_1 = C_2 V_2$  for 1mg/ml stock solution.

Solution concentration	0.05	0.1	0.15	0.2	0.25	mg/ml
Stock volume	100	200	300	400	500	Final volume
MQ water	1.9	1.8	1.7	1.6	1.5	2mL

For example:  $C_1 V_1 = C_2 V_2$

$$V_1 = \frac{0.05 \times 2}{1}$$

$$V_1 = 0.1 \text{ ml stock in } 1.9 \text{ (2mL - 0.1 = 1.9ml water)}$$

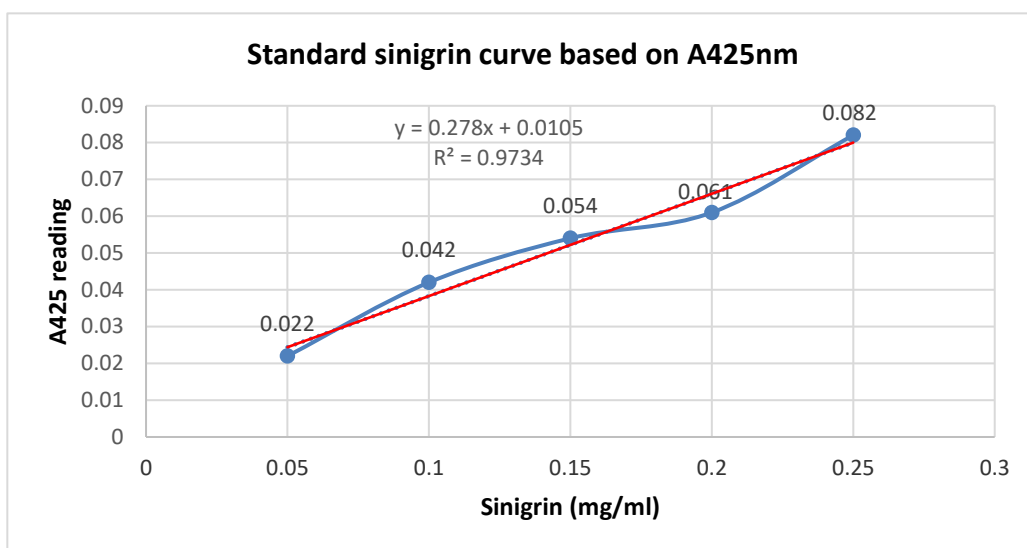
4. All the tubes were wrapped with aluminium foil and stored at  $-20^\circ\text{C}$ .

#### Appendix 43: Preparation of Sodium tetrachloropalladate solution

1. Sodium tetrachloropalladate (Sigma Aldrich,58.8mg) was weighed (Thermo Fisher Scientific scale) and transferred to a 15ml falcon tube.
2. Concentrated HCL (absolute,170  $\mu\text{l}$ ) was added and mixed until the sodium tetrachloropalladate was dissolved and the solution was poured into 100ml of sterile water in a Schott bottle (Mawlong et al., 2017).
3. The Schott bottle was covered with aluminium foil to avoid light sensitivity.

**Appendix 44: Linear regression calculations showing the glucosinolates level.**

Sinigrin standard (mg/ml)	A425 reading
0.05	0.022
0.1	0.042
0.15	0.054
0.2	0.061
0.25	0.082



Example: A 425 reading = 0.031

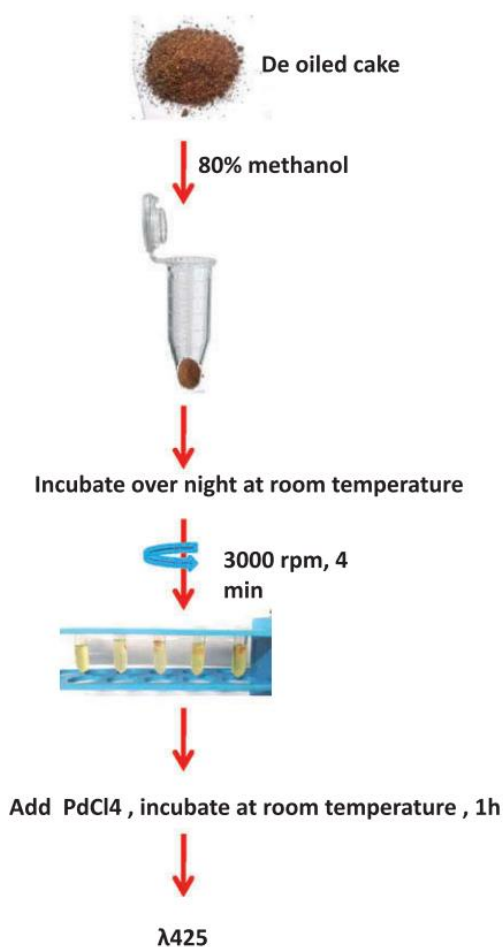
Formular = (A425 – slope/x )

slope = 0.0105

x = 0.278

Glucosinolates (mg/ml) = 0.074

## Appendix 45: Spectrophotometric method of analysis



Source: "A simple spectrophotometric method for estimating total glucosinolates in de-oiled cake by Mawlong et al, 2017, International Journal of Food Properties, 20 p 3276. Copyright 2017 Taylor & Francis Group, LLC

## Appendix 46: Trichoderma spore drenching procedure

1. The total number of cells per treatment was 800, plus 100 spare cells, being 900 tray was 900 cells.
2. The total volume of spore suspension for each treatment was 4500ml (900 cells x 5 ml volume per cell).
3. Trichoderma LU785 spores were harvested (Section 4.2.2) and the number of spores per ml was calculated using the haemocytometer.

Treatments	Haemocytometer counts spores/ml	Spore concentration (ml <sup>-1</sup> ) per cell
Trichoderma with polymer	12.8	3.00E + 06
Trichoderma and Apron with polymer	12.8	3.00E + 06

4. Formular:  $C_1V_1 = C_2V_2$  was used to work out how much stock spores suspension was needed to make the spore suspension for application.

$C_1$  = Concentration of the stock spore suspension (320,000,000)

$V_1$  = Volume of the stock spore suspension needed (?)

$C_2$  = Desired concentration of the spore suspension for application (3000 000)

$V_2$  = Total volume of the spore suspension for application (5000 ml).

Rearrange the formular:

$$\frac{C_2 \times V_2}{C_1}$$

$$V_1 = \frac{300,000 \times 5000}{320}$$

$$V_1 = 46.875$$

Therefore, 46. 875 ml stock spores' suspension was needed to make the spore suspension for application.

5. In the laboratory, two 250 ml Scotch bottles were filled with 4.875 ml of stock spores' suspension (Figure 6.2) and were taken to the green house.
6. In the greenhouse the two Trichoderma treatment trays were separated from the other three treatments.
7. Spore suspensions were poured into a 5000 ml (5L) watering can filled with water, mixed thoroughly with a bamboo stick, and applied evenly over each cell, one at a time, for each of the Trichoderma treatments.

#### **Appendix 47: Trichoderma selective media for measuring Trichoderma in soil samples.**

##### 1. Ingredients:

- Malt yeast Extract 6.0g
- Yeast extract 0.6g
- Terrachlor (quintozene) 0.12g
- Rose Bengal (50mg/ml) 1.8 ml
- Micro agar 10g
- Distilled water 600 ml
- Sterile Streptomycin sulphate + Chlortetracycline HCL solution 6ml (antibiotics)

The streptomycin was not autoclaved because it becomes inactive by 50% within minutes at 100 °C

##### 2. Procedure

- The ingredients were weighed and dissolved in 1 Litre of distilled water in a Schott bottle, resulting in a 600 ml agar solution.
- The medium was wet-cycled and autoclaved at 121 °C and 15 Psi for 15 minutes.
- Approximately 16 ml of the medium was poured into sterile petri dishes in a lamina flow cabinet.
- The lids were placed onto the plates, then the plates were put into a black bag and stored in the chiller.

#### **Appendix 48: Formular for calculating soil moisture content.**

Formular used:

$$\text{Moisture content (\%)} = \frac{(\text{Wet weight} - \text{Dry weight}) \times 100}{\text{Dry weight}}$$

## Appendix 49: Microbial analysis for soil samples for CFU per gram of dry soil

Formular:

1. 
$$\text{CFU/ml} = \frac{\text{Number of colonies counted} \times \text{Dilution factor}}{\text{Volume plated in ml}}$$
  
2. 
$$\text{CFU/gram of dry soil} = \frac{\text{CFU/ml of the original suspension}}{\text{Weight of dry soil in grams}}$$

Soil Trichoderma level				
Samples	Replicate	Dilution factors and the colony counts per plate		
		1/10 <sup>th</sup>	1/100 <sup>th</sup>	1/1000 <sup>th</sup>
One	1	10	8	2
	2	7	4	2
	3	13	5	2
Two	1	11	2	0
	2	8	5	2
	3	4	5	0
Three	1	9	3	0
	2	21	5	1
	3	27	5	3
Four	1	13	2	2
	2	14	5	1
	3	15	5	2
Average		12.7	4.5	1.4

## Appendix 50: Area sampled.

Data per meter of three seedling rows were converted to square meter (m<sup>2</sup>) as follows;

1. At 27 days after transplanting (12 plants)
  - The area sampled was (30 x 45cm) to m<sup>2</sup>
  - Data value in 0.135m<sup>2</sup> = x in m<sup>2</sup>
  - Data value x 1 ÷ 0.135 = Data value/m<sup>2</sup>

2. At 41 days after transplanting (3 plants)

- The area sampled was ( 15cm x 15 cm = 225 cm<sup>2</sup>)
- 225cm<sup>2</sup> x 3 plants = (675cm<sup>2</sup>) to m<sup>2</sup>
- Data value in 0.0675 m<sup>2</sup> =  $x$  in m<sup>2</sup>
- Data value x 1 ÷ 0.0675 = Data value/m<sup>2</sup>

3. Results were converted to square meter (m<sup>2</sup>) per plant

- Area sampled was 15cm x 15 cm = 225 cm<sup>2</sup>
- Data value in 0.0225 m<sup>2</sup> =  $x$  in m<sup>2</sup>
- Data value x 1 ÷ 0.0675 = Data value/m<sup>2</sup>

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