Comparative efficacy of *Trichoderma harzianum* application methods for controlling *Rhizoctonia solani* in peas (*Pisum sativum* L.)

By

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DECLARATION

I hereby declare that this dissertation has been the result of my own original efforts and investigations, and such work has not been presented elsewhere for any degree. All additional sources of information have been acknowledged by means of references.

Masuka Motion ……………………… …………………………..

Reg No. R125894R Signature Date

I, the undersigned, certify that Masuka Motion, a candidate for the Bachelor of Science Agronomy Honours Degree has presented this dissertation with the title:

Comparative efficacy of *T. harzianum harzianum* application methods for controlling *R. solani* in peas (*Pisum sativum* L.)

That the dissertation is acceptable in form and content, that satisfactory knowledge of the field covered by the dissertation was demonstrated by the candidate through oral examination held on 13/05/16.

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I declare and certify that I have supervised this thesis and it is now ready for evaluation.

ABSTRACT

The use of *Trichoderma* has been widely used to control *R. solani* but however there is need to evaluate the different mechanisms used to administer the Biological Control Agent into the plant environment. A greenhouse experiment to compare the efficacy of three *T. harzianum* application methods i.e. seed bio-priming, seed coating and soil treatment to control *R. solani* in peas was carried at Kutsaga Research Station. The main objective of this study was to evaluate the efficacy of *T. harzianum* application methods for controlling root rot and damping off caused by *R. solani* in peas. Seeds were sown in artificially infested soil in 22.5 cm wide pots. Data on pre emergence, post emergence, root rot severity and *T. harzianum* CFU concentration in the root rhizosphere were recorded and analysed using GenStat. There were significant differences between all application methods on all measured parameters. *T. harzianum* seed bio-priming have significantly recorded the lowest pre and post emergence damping off disease incidence of 8 % and 11 % respectively. *T. harzianum* soil treatment have significantly suppressed root rot severity and recorded the lowest severity score of 1.5. In determining the *T. harzianum* CFU concentration in the root rhizosphere *T. harzianum* soil treatment significantly recorded the highest population increase rate at 6 WAS. Seed biopriming have greatly reduced both pre emergence and post emergence damping off and also resulted in low disease severity cause by *R. solani*. *T. harzianum* applied as soil treatment have greatly suppressed root rot severity and recorded a significant increase in CFU in the root rhizosphere.

DEDICATION

I would like to dedicate this project to my brothers Prof. A. J. Masuka, T. Masuka, Dr G. Masuka and all my family members. You have inspired me to pursue my goals. I owe you my gratitude. You are the best.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
BCA	Biological Control Agent
CFU	Colony Forming Unit
LSD	Least Significance Differences
WAS	Weeks after Sowing

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CHAPTER ONE

1.0 INTRODUCTION AND JUSTIFICATION

1.1 Study background

Increase in European market price and demand of peas have led to an increase in area under pea production in many countries including Zimbabwe (Goodwin, 2009). Despite the introduction of new high yielding cultivars and increase in area pea production the average yield obtained per unit area is still very low (Khan *et al*., 2013). These low yields can be attributed to several biotic and abiotic factors affecting pea production. Biotic factors such as weeds, pests and diseases are reported to cause substantial yield losses in pea production if not managed well (Goodwin, 2009). Among these biotic factors, plant diseases are reported to be the major yield-limiting in pea production when conditions are favourable (Xue, 2002). Pea is affected by a wide range of diseases caused by plant pathogenic bacteria, fungi, nematodes and viruses however fungi is reported to pose serious threat to pea production (Kikkert *et al*., 2011).

Seedling damping off and root rot are reported among the most economically important and devastating seedling stage diseases (Xue 2002). More than twenty fungal pathogens were reported to cause seedling damping off and root rot in peas (Gaurilčikienė, 2012) but however, *Rhizoctonia solani* was reported to be highly destructive pathogen (Hamid *et al*., 2012). *R. solani* can cause plant diseases over a wide range of soil temperature, pH, type, fertility levels and moisture levels (Bost, 2006). Xue (2002) reported that under favourable conditions, root rot and damping off can account for more than 70% yield loss. The pathogen can attack any part of the root system up to a short distance above the soil surface any time between germination and maturity. The pathogen can cause poor seed emergence and infection of the root system inhibit root growth due to poor nutrient and water uptake and can ultimately result in stunted growth and the infected plants can die (Oyarzun, 1993). Due to its wide host range and longer overwintering ability in the soil, *R. solani* is difficult to control by cultural methods (Grosch *et al*., 2003).

Disease management is a pre requisite to ensure high quality produce and higher yield (Kumar *et al*., 2014). Fungicides and fumigants have been widely used in *R. solani* damping off and root rot control in peas. However, the continuous use of these chemicals may not always be desirable. The toxicological environmental concerns arising from use of these chemicals have compelled researchers to look for ecofriendly strategies for disease management (Arcury, 2003). More so, the efficacy of these chemicals are further reduced by development and emergence of resistant pathogen strains (Radheshyam *et al*., 2012). Biological control is a viable and alternative eco- friendly way of disease management (Sharma *et al*., 2012). Disease bio control is the use of antagonist microorganisms to suppress plant disease causing pathogens (Gveroska, 2011).

Several Biological Control Agents (BCAs) such as *T. harzianum* spp., *Bacillus* spp. and *Pseudomonas* spp. have been widely used in many crops controlling a wide range of plant pathogens (Moller *et al.*, 2003). Among these BCAs, *T. harzianum* spps are extensively studied and recommended for use in management of many fungal diseases (Kumar *et al*., 2012). *T. harzianum* is reported to employ several mechanisms such as; mycoparasitism, antibiosis and induction of plant resistance in plant disease control (Mancini, 2013). Although *T. harzianum* have been widely used in plant disease control there is still need to identify the best application method used to administer the pathogen into the plant environment (Kumar *et al*., 2014). The application method of *T. harzianum* is very important for successful plant disease control because it determines the establishment of *T. harzianum* in the plant environment. Mancini, (2013) reported that these application methods can determine the mode of action employed by *T. harzianum* in controlling the disease causing pathogens. Seed treatment, seed biopriming, seedling dip and soil application are among the commonly used application methods in controlling soil borne fungal pathogens (Kumar *et al*., 2014).

Since the application method used to administer *T. harzianum* as a BCA into the plant environment is very important for successful disease control. It is important to evaluate different *T. harzianum* application methods in controlling root rot and damping off in peas since there is little work conducted on their efficacies. Therefore the thrust of this study was laid to compare the efficacy of *T. harzianum* application methods i.e. seed biopriming, seed coating and soil treatment for controlling root rot and damping off caused by *R. solani* in peas.

1.2 Objectives

1.2.1 Main objectives

- To evaluate the efficacy of different *T. harzianum* application methods for controlling damping off and root rot in peas caused by *R. solani*.

1.2.2 Specific objectives

- To evaluate the effect of *T. harzianum* application methods on pre emergence and post emergence damping off
- To evaluate the effectiveness of *T. harzianum* application method on *R. solani* root rot severity.
- To determine *T. harzianum* colony forming units (CFU) concentration in the root rhizosphere at 6 weeks after sowing (WAS).

1.3 Hypotheses

- There are significant differences between *T. harzianum* application methods on pre emergence and post emergence damping off caused by *R. solani*
- There are significant differences *T. harzianum* application methods on *R. solani* root rot severity
- There is significant differences in *T. harzianum* CFU concentration in the root rhizosphere at 6 WAS.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Challenges faced in pea production

In their growing environment, plants are subjected to several disease causing pathogenic microorganisms, such as bacteria, fungi, viruses, nematodes and oomycetes. Among these disease causing pathogens, plant pathogenic fungi and oomycete poses serious threats to crop production by causing most of the world's important plant diseases, which ultimately threaten global food security (Blandón-Díaz 2011).

Peas (*Pisum sativum* L) is susceptible to a wide range of diseases causing pathogens (Kikkert *et al*., 2011) however plant pathogenic fungi is reported to pose a serious threat in pea production when severe (Xue, 2002). Fungal diseases can be classified according to time of occurrence during the crop's growing season. Seedling stage diseases occur between sowing and early establishment of seedlings while foliar stage diseases occur during the later stages of the plant growth stages (Kikkert *et al*., 2011). Seedling stage fungal diseases are of economic importance and pose devastating consequences to pea production if not managed well (Seed Treatment and Environment Committee 1999). Seedling damping off and root rot are among the most economically important seedling stage diseases of peas.

More than twenty fungal pathogens have been reported to cause seedling damping off, seed and root rot of peas (Xue, 2002). *R. solani* seedling damping off and root rot are the major yield-limiting seedling stage diseases in peas (El-Mohamedy, 2008). *R. solani* can attack any part of the root system up to a short distance above the soil surface mainly during germination and early seedling establishment. Seed infection can result in failure to germinate; die soon after germination before reaching the soil surface or the seedling may die after emergence. The effects stated above can result in poor seed emergence and stand establishment and ultimately results in severe yield losses (Kikkert *et al*., 2011). Seedlings which escape damping off can also be affected by root rot disease.

2.1.1 *R. solani* as a disease causing pathogen in peas

R. solani Kühn is the imperfect stage of basidiomycete fungus *Thanatephorus cucumeris.* It is a necrotrophic soil inhabitant plant pathogenic fungus which have a wide host range (Babiker, 2012). In Zimbabwe, *R. solani* was reported to affect more than 75 plant species including peas (Masuka *et al.*, 1999). The fungus has the ability to remain for long period in soil as a saprophyte or can overwinter as sclerotia. Due to wide host range, *R. solani* is divided into different Anastomosis Groups (AGs) based on the ability of hyphal fusion *in vitro*. Anastomosis can be defined as the affinity of hyphal fusion *in vitro* between the isolates (Yang and Li, 2012). The *R, solani* AG 4 affect a wide range of crops in solanaceous family, brassica spp and legumes including peas causing root rot and damping off (Yang and Li, 2012). Xue (2002) reported that *R. solani* can account for up to 70 % yield loss when the conditions are favourable.

2.1.1.1 *R. solani* seedling damping-off

Seedling damping off is among the most common symptoms caused by *R. solani*. Agrios (2005) defined damping-off as symptoms caused by *R. solani* which may result in poor seed germination and seedling emergence and crop stand. Seedling damping off is accelerated by persistence of cool soil temperatures and dumpy soils (Swartz *et al*., 2007). After seedling emergence, the fungus attacks seedling stem causing water soaked stems which can be soft and incapable of supporting the seedling and the seedling can eventually fall over and die (Burrows, 2013). When invading older seedlings, the fungus is restricted to the outer cortical tissues causing reddish-brown lesions. These lesions may increase in length and width until they finally girdle the stem, and the plant may die (Agrios, 2005).

2.1.1.1 *R. solani* root and stem rot

R. solani root and stem rot disease is caused by the fungus *R. solani* AG 4 mostly develops soon after emergence. Infection can cause early inhibition of root growth which impedes nutrient and water uptake by the plant roots resulting in stunted growth as stunted growth (Oyarzun, 1993). Disease development is favoured by wet weather and low soil temperatures. A brown to black lesion develops on the stem near the soil line and may enlarge until the stem is girdled. The stem breaks easily at infected areas while roots usually remain healthy with some brown discoloration. If stalk of old plant is split open, the decay pith is dry and brown with patches of light grey fungi (Bost, 2006). Reddish-brown lesions usually appear first just below the soil line, but in cool, wet weather the lesions enlarge in all directions and may increase in size and number to include the whole base of the plant and most of the roots. This results in weakening, yellowing, and sometimes death of the plant (Roberts, 2010).

2.1.2 *R. solani* damping off and root rot disease cycle

R. solani species are strong saprophytes (Huang e*t al.*, 1990) which can be able to survive can be able to survive a long time in the absence of the living hosts by feeding on decaying organic matter (Goodwin e*t al.*, 1994). The fungus can be spread by rain water, contaminated tools (Agrios, 2005). The most important means of disease dissemination is through infected seeds. *R. solani* may infect seeds of a number of plants at the time or just prior to maturation (Adams *et al.*, 1983). The pathogen can overwinter as mycelium or sclerotia in the soil or plant debris, on alternative hosts or propagative material such as seed. The fungus can be dispersed by raining water, irrigation water or can be spread by tools and carrying contaminated soil and with infected seed. Infection is favoured by temperatures ranging between 15°C to 18°C, however some isolates can be active at much higher temperatures, up to 35°C (Agrios, 2005).

Adams *et al.,* (1983) reported that the rate of infection depends on the prevailing temperature and also varies within *Rhizoctonia* species. *R. solani* can colonize the surface of the host plant cell by forming long unbranched runner hyphae. The runner hyphae may branch and penetrate through stomata or give rise to structures forming infection cushions (Agrios, 1988).

2.1.3 Pathogenesis and disease development

R. solani species are necrotrophic pathogens, i.e. they kill host cells before colonizing them (Ceresini, 1999). *R. solani* is reported to be more aggressive over a broad range of, soil pH, soil types, fertility levels and soil moisture levels (Bost, 2006). Agrios (2005) reported that infection of young plants by *R. solani* is more severe when plant growth is slow due to adverse environmental conditions for the plant such as poor nutrients and moisture availability. This is accomplished through the secretion of enzymes and toxins in advance of fungal growth. The fungus is attracted to the plant by chemical stimulants or exudates released by actively growing plant cells and decomposing plant residues which stimulate the formation of infection cushions prior to penetration. As the attraction proceeds, the fungal hyphae come in contact with the plant and become attached to its external surface. The infection process is promoted by the production of different extracellular enzymes that degrade various components of plant cell walls. Under favourable conditions the sclerotia germinate by producing delicate hyphae that grow through the soil. It invades the roots directly, through wounds or natural openings and initiates infection resulting in plant death by causing root rot or stem rots (Roberts, 2010). The fungus grows quickly in the dying plants and reaches a high population which then attacks the new seedlings (Burrows, 2013).

2.2 Main practices used in *R. solani* management and their setbacks

In order to attain high quality produce and higher yield, timely disease control is important. Crop losses can be minimized by adoption of the best control measures (Mahato, 2005). R. solani root rot and damping off can be controlled by chemical, cultural and biological control. More so, it is important to use a cost effective method in disease control in order to maximise yield and quality of the produce.

2.2.1 Cultural management practices

Several cultural practices were reported to prevent and control *R. solani* root rot and damping off. Sanitation is very important starting from choice of planting site, uses of high quality seedlings as well as sterilization of tillage and planting equipment (Olsen, 1998). A 4-5 year crop rotation may be of some benefit in reducing the *R. solani* inoculum level (Milleretal, 2005). Growing resistant or tolerant varieties, decreasing plant population are also practices which reduce incidence and severity of root rot and damping off (Kikkert *et al.*, 2011).

2.2.1.1 Setbacks of Cultural management practices

Use of cultural control methods has not successfully controlled the persistence of *R. solani* root rot and damping off. Several control strategies such as crop rotation have been reported to have of low significance in controlling *R. solani*. Due to its wide host range, crop rotation is of little value in controlling *R. solani* (Bost, 2006). More so, land shortages among small holder farmers make it impractical to practice these long rotations.

2.2.2 Chemical control

Agrios (1997) reported that chemical control is the most widely used practice for disease control. *R. solani* is currently controlled by fungicides. Mahato (2005) defined a fungicide as natural or synthetic compound which can protect plants against fungal invasion, suppresses and eradicate established fungal pathogens. These fungicides can be applied as foliar sprays or seed treatments. Seed treatment is the most effective method of controlling *R. solani* root rot and damping off in peas (Gaurilčikienė *et al.*, 2012). Goggi (2011) defined seed treatment as the application of physical, chemical or treatments to planting materials in controlling disease causing pathogens or pests. Seed treatment can protect the seed for up to six weeks after sowing (Hawthorne *et al*., 2010).

2.2.2.1 Setbacks of chemical control

Despite the effectiveness of these synthetic fungicides, their continuous use may not always be desirable. Their toxicity on non-target organisms and the undesirable changes they inflict upon the environment result in their discouragement (Arcury, 2003). This led researchers to shift their attention towards alternative eco-friendly approaches for disease management. Biological control is a viable and eco-friendly way of disease management (Sharma et al., 2012).

2.2.3 Biological control

The toxicological effects on the environment and on non-targeted organisms together with development of resistance to pesticides compelled a search for alternatives to chemical pesticides. Extensive studies of BCAs in plant disease control have been reported as early as the 1930s (Weindling, 1934). Biological disease control is a promising and sustainable strategy for managing seed-borne, soil-borne and foliar diseases in a wide range of crops. The efficacy of biological control depends on the methods used to produce, formulate and application methods of these BCAs (Nayaka, 2008). More than 80 BCAs are reported to control soil borne pathogens worldwide. However *T. harzianum* is reported to be most effective and widely used BCA for the management of several soil borne pathogens including *R. solani* spp (Lenteren, 2000).

2.2.3.1 Benefits enjoyed from BCA use in disease control

BCA is an eco-friendly strategy way of disease control. Low resistance development cases have been reported after use of BCAs. Apart from diseases suppressing ability of BCAs, they are also growth promoters, and compatible with different diseases management models in agriculture (Monte, 2003).

2.2.4 *T. harzianum* as a BCA

T. harzianum is a genus of anaerobic, endophytic, facultative soil inhabitant saprophytic fungi which found in forests and agricultural soils (Howell, 2002). *T. harzianum* is classified as imperfect fungi, due to the absence of the known sexual stage. Harman (2004) reported that *T. harzianum* can adapt to a wide range of environmental conditions earning them the ability to be used in different soil, crops, climates and technological processes. It also have rapid growth rate in culture and the production of numerous conidiospores that are varying shades of green characterize fungi in this genus compete well with other soil microorganisms, show resistance to chemical pesticides and produce various antibiotics (Howell, 2002). T. harzianum strains have long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients (Ranasingh *et al*., 2006).

2.2.5 *T. harzianum* mode of action in plant disease control

Herman (2003) reported that it is important to understand the mode of action of the BCAs for an effective bio control of plant diseases. Herman (2000) reported that *T. harzianum* have evolved numerous mechanisms which it can use in attacking plant pathogen as well as for plant growth enhancement. *T. harzianum* is reported to control pathogens through mycoparasitism, antibiosis by enzymes and secondary metabolites and induction of plant resistance (Mancini 2013). The BCAs can individually or synergistically use some of the above mentioned mode of action.

2.2.5.1 Mycoparasitism and Antibiosis

Mycoparasitism is a mechanism which involves direct attack of plant pathogenic fungi by the BCAs. It involves, the recognition of the pathogen by the antagonist BCA, attack through penetration resulting in killing the pathogen by the BCA. *T. harzianum* spp. may exert direct bio control by parasitizing a range of fungi, detecting other fungi and growing towards them (Agrios 2005). McIntyre (2004) reported that mycoparasitism can result in morphological changes of the BCA. These changes can include the formation of appressorium structures which they use to penetrate the host. Weindling (1932) reported that *T. lignorum* parasitize *R. solani* by coiling around pathogen hyphae, penetration and subsequently leading to dissolution of the host cytoplasm. Most *T. harzianum* spp has the ability to produce volatile and non-volatile toxic metabolites that impede colonization by antagonized microorganisms (Vey *et al*., 2001). *T. harzianum* is reported to produce several secondary metabolites which can be used in disease control. The BCA can produce antibiotics such as acetaldehydes gliotoxin and viridin (Dennis and Webster 1971), to alpha-pyrones (Keszler *et al*., 2000), terpenes, polyketides, isocyanide derivatives, piperacines, and complex families of peptaibols (Sivasithamparam and Ghisalberti 1998). Lorito *et al*., (1996) reported that these compounds can individually of synergistically with cell degrading enzymes have a strong antifungal inhibitory effect in several soil borne fungi.; Schirmböck *et al*., 1994).

2.2.5.2 Rhizosphere competence

T. harzianum species exhibit competition through rhizosphere competence by growing rapidly along with the developing root system of the treated plant (Howell, 2011). Howell (2013) reported that creation of a zone of protection against soil borne pathogens after seed treatment is important for rhizosphere competence. T. harzianum applied as soil or seed treatments, can grow along the developing plant root system (Ahmad, 1987).

2.2.5.3 Enzyme production

Some species has the ability to produce enzymes, which breaks down the polysaccharides, chitin and beta- glucans that are responsible for the rigidity (Reese, 1989). Metcalf (2001) reported that the colonisation of onion roots, infected by *T. koningii* was due to the production of endo- and exo-chitinases by *T. koningi*. *T. harzianum* was found to produce proteases enzymes that inactivate the hydrolytic enzymes produced by *B. cineria* on bean leaves. Secretion of protease enzyme on plant surfaces by *T. harzianum* was reported to suppress the action of pectinases, cutinase, glucanase and chitinase in *Botrytis cineria*. The presence of *T. harzianum* was also reported to decrease the secretion of *endo- PG* by *A. Alternate* (Roco, 2001).

2.2.5.4 Induced Resistance

Some *T. harzianum* spp can colonize and penetrate the plant root tissues and causes a series of biochemical and morphological changes in the plant. These morphological and biochemical changes results in induced systemic resistance (Bailey, 1998). Treatment of roots with *T.*

harzianum T-203 was reported to exhibited higher chitinase, *ß*-1, 3-glucanase, cellulase and peroxidase activities 72 hours (Yedidia *et al.*, 1991).

2.2.6 *T. harzianum* application methods and disease control

Kumar *et al*., (2014) reported that the application method of *T. harzianum* into the plant environment is very important for a successful disease control. The BCA can be applied as a foliar, seed treatment or soil treatment. These application methods play an important role in disease control as they determine the mode of action of the bio control in suppressing the disease.

2.2.6.1 Seed coating

Seed coating is when seeds are soaked in *T. harzianum* spore solution before sowing. The spores of the BCA germinate on the surface of seed and colonize roots of germinated seedlings and rhizosphere (Kumar *et al*., 2009). Seed treatment with *T. harzianum* spp has suppressed a wide range of seed borne fungal pathogens including *R. solani* of oil seed crops (Jat, 2013). The application of *T. harzianum* as seed treatment was effective against *Pythium* spp. and *R. solani* (Mukherjee, 1995). More so, T. *viride* and *T. harzianum* were found to be synergistically effective in controlling sheath blight when applied as seed treatments (Das, 2000). El-Mohamedy (2008) discovered that coating pea seed with *T. harzianum* have significantly reduced pea root rot.

2.2.6.2 Seed biopriming

Seed biopriming is the integration of physiological and biological treatment for control of seed and soil borne disease (El-Mohamedy, 2008). In comparison to simple coating, bio priming technique results in a rapid and uniform emergence of the seedlings. A reduction in sowing to emergence period of bioprimed seeds can lead to pre emergence damping off diseases escape (Olsen, 1998). The conidiospores can germinate on the seed surface. Seed biopriming can also help in seed tolerance to adverse soil conditions (Kumar *et al*., 2014). Seed biopriming technique was successfully used to control seedling damping off in several crops including tomato, soybean and chickpea (Mishra *et al*., 2001). Hydropriming, osmopriming and solid matrix are some of the priming methods used (Girolamo, 2012). Taylor *et al.*, (1998) reported that these methods can be grouped based on water uptake during the priming process i.e. non-controlled water uptake and controlled water uptake.

2.2.6.2.1 Solid matrix priming

Solid matrix priming is a technique which involves the use an inorganic material imitating the natural imbibition processes in the soil (McDonald, 2000). The inorganic material should have a low matric potential; higher ability to adhere on the seed surface; high water retention capacity (Khan, 1991). Peat and vermiculite are among the commonly used materials (Girolamo, 2012). The seed is mixed with hydrating substance and the BCA spore solution in a bottle jar. The mixture is then placed on a rotary shaker for 48-72 hours to ensure uniform coating of the seed with the BCA.

Despite the advantages enjoyed in seed priming, there is a problem in seed storability and longevity. The drying-back method is very important in seed longevity. A rapid drying back method is reported to alter the soluble carbohydrate content which result in reduced desiccation tolerance and seed longevity (Gurusinghe, 2001). Compared to fungicide seed treatment, a significant reduction in pre- and post-emergence damping off was recorded on bioprimed seeds with *T. harzianum* in peas. More so, higher seedling survival percentages seedling survival was recorded with these treatments (El-Mohamedy, 2008).

2.2.6.3 Soil treatment

This is the direct application of *T. harzianum* spp. into the soil. This increases the population of augmented fungal antagonists and thereby suppressing the establishment of pathogenic microbes in the rhizosphere (Kumar *et al*., 2012). Several reports review that application of the BCA as a soil treatment prior to sowing or at planting controls a wide range of soil-borne fungal pathogens (Kumar *et al*., 2009). Root rot, stem rot and seedling blight in jute can be suppressed by soil treatment of *T. viride* (Srivastava *et al*., 2010). *T. harzianum* can grow on Farm Yard Manure (FYM) and application of the colonized FYM is more beneficial. Soil treatment is the most effective *T. harzianum* application method in the management of root rot and damping off (Kumar *et al.*, 2015). Soil treatment of *T. harzianum* in naturally-infested soil resulted in a significant reduction in the *R. solani* inoculum density. More so it was noted that applying *T. harzianum* reduces damping-off of snap bean at a soil pH of 3.5 (Marshall, 1982).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Site description

The study was carried out in the greenhouse at Kutsaga Research Station. Kutsaga Research station is located 15 km south east of Harare central business district. The site lies in Natural Region IIb (Vincent and Thomas, 2004) at an altitude of 1 479 metres above sea level and is found on latitude 17`55``S, longitude 31`08``E (FAO, 2006). The greenhouse has average day and night temperature of 25 ^{o}C.

3.2 Experimental design and treatments

The experiment was laid in a Randomised Complete Block Design (RCBD) with 5 treatments in 4 blocks. Sunlight was the blocking factor in the green house.

Table 3.1. *T. harzianum* application methods and the application rates

Application method		Application rate (g/kg seed)
1	Untreated control	0
2	Seed biopriming	20
3	Seed coating	20
4	Soil treatment	20^{*}
5	Thiram seed treatment	4

N.B * applied per m^2

3.3 Experimental procedure

3.3.1 Source of *R. solani* and *T. harzianum* inocula

R. solani AG 4 used in this study was obtained from Kutsaga Research Station Pathology Laboratory. The pathogen was isolated from pea roots. *R. solani* inoculum was multiplied using the Czapeks growth medium. *T. harzianum* (T77) was obtained from Kutsaga Research Station Plant Protection Division in the Pathology laboratory. The BCA was multiplied using brewery pressings growth medium.

3.4 *T. harzianum* application and fungicide seed dressing

Seed Bio-priming was done using solid matrix method. In this method, *T. harzianum* spore solution was added to the seed and vermiculite mixture at a rate of 20g/kg of seed in 500 ml glass bottles. The bottles were shaken on a rotary shaker set at 150 rounds per minute for 48 hours and the seeds were dried at room temperature 12 hours before sowing. Seed coating was done by soaking the seeds for 30 minutes in suspension of *T. harzianum*. After 30 minutes, excess water was drained and the seeds were allowed to dry at room temperature for 12 hours before sowing. Soil treatment with *T. harzianum* inoculum was done by directly applying into the soil and incorporating it in the soil at sowing. Fungicide seed dressing was done using Thiram 50WP at the dose of 4 g/kg seeds.

3.5 Sowing of pea seeds in the green house

Pot size of 22.5 cm wide were filled with sterilized sandy loam soil. *R. solani* inoculum was inoculated in each pot at a rate of 25g per pot and was thoroughly mixed. The pots were watered 72 hours prior to sowing. Ten seeds were sown per pot in the green house.

3.6 Data collection

3.6.1 Pre emergence damping off

Peas at pre-emergence was recorded 14 days after sowing (DAS). It was determined as the number of non-emerged seeds in relation to the number of sown seeds. Germination test were conducted before the experiment so as to assess the seed germination percentages.

3.6.2 Post emergence damping off

Post-emergence determined was recorded 5 WAS. It was determined as percentage of the number of plants showing the disease symptoms in relation to the number of emerged seedlings.

3.6.3 *T. harzianum* CFU determination in the root rhizosphere

Soil samples of 10 g from each treatment were collected at 6 WAS. *T. harzianum* CFU was determined by the procedure described by Izzati and Abdullah (2008). The CFUs counted and recorded after 5 days of incubation at 25^{o}C.

3.6.4 Root rot severity

Root rot severity was assessed 42 DAS. In root rot severity assessment, seedlings were visually classified according to a Kutsaga *R. solani* Root Rot severity scale which range from 0-5 by Cole and Cole, (1998). Where 0 = No damage, 1 = 0 - 1% slight damage on stem, 2 = 1.1 - 10% lesions on stem, slight root discoloration, 3 = 11 - 25% several lesions on stem, about one third of root discolored, 4 = > 26% Extensive lesions on stem, remains of root discolored and 5 = Plant dead. Dead plants were 5_s on the severity scale.

3.7 Data analysis

Data on pre and post emergence damping off, root rot severity and *Trichoderma* CFU concentration in soil were transformed using the Square Root Transformation was analysed using Analysis of Variance (ANOVA) using the GenStat 17^{th} edition. Means were separated using the least significance difference (LSD) test at 5% significance level.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of *T. harzianum* application methods on pre emergence damping off.

There were significant differences (p<0.001) between different *T. harzianum* application methods in reducing pre emergence damping off. All application methods have greatly reduced pre emergence damping off. Seed biopriming recorded lowest pre emergence damping off incidence of 2.64 % (6 %) compared to 7.161 % (51 %) recorded on untreated control. No statistical differences have been noted between seed coating and soil treatment application methods. The fig 4.1 below shows performance of different *T. harzianum* application methods in reducing pre emergence damping off.

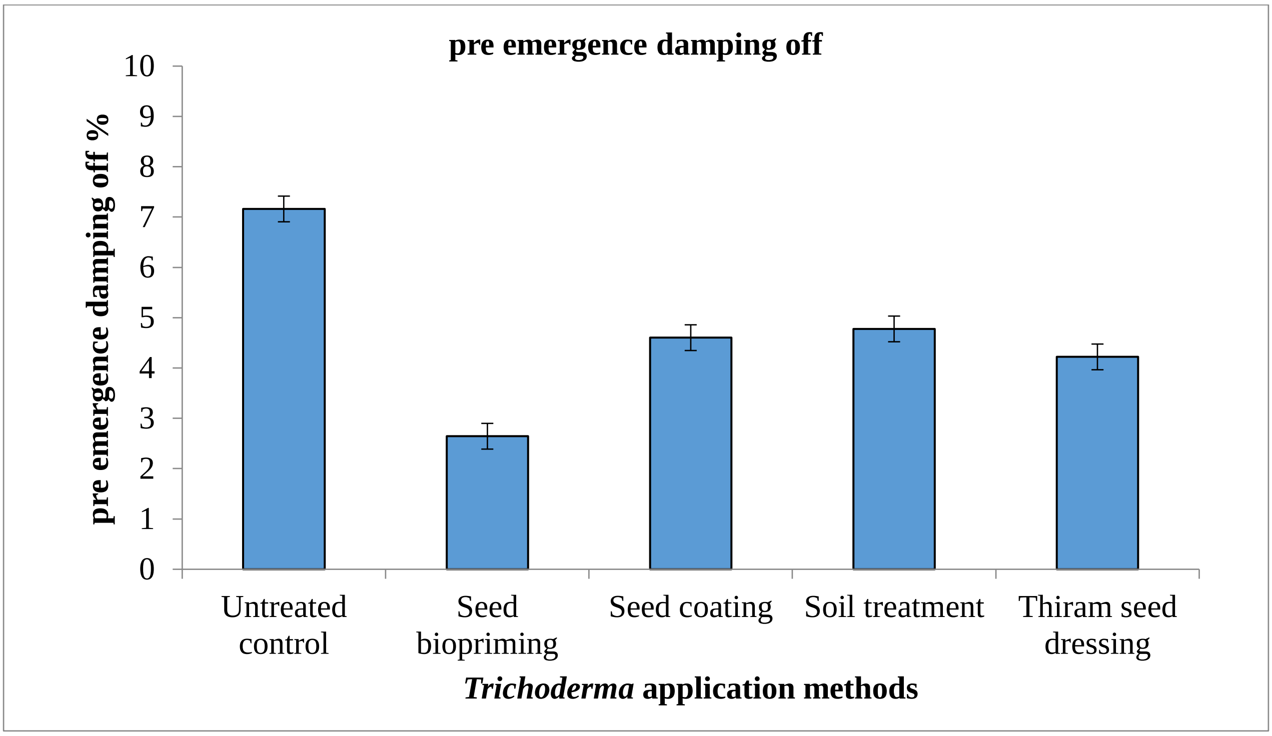

Fig: 4 1 Effects of *T. harzianum* application methods on pre emergence damping off.

4.2 Effect of *T. harzianum* application method on post emergence damping off.

All application methods have shown significant differences ($p<0.001$) in reducing post emergence damping off. *T. harzianum* seed biopriming significantly recorded the least post emergence damping off of 3.054% (8%) respectively while untreated control recorded the highest post emergence damping off incidence of 8.969% (75%). Fig 4.2 show the efficacies of different application methods in reducing post emergence damping off.

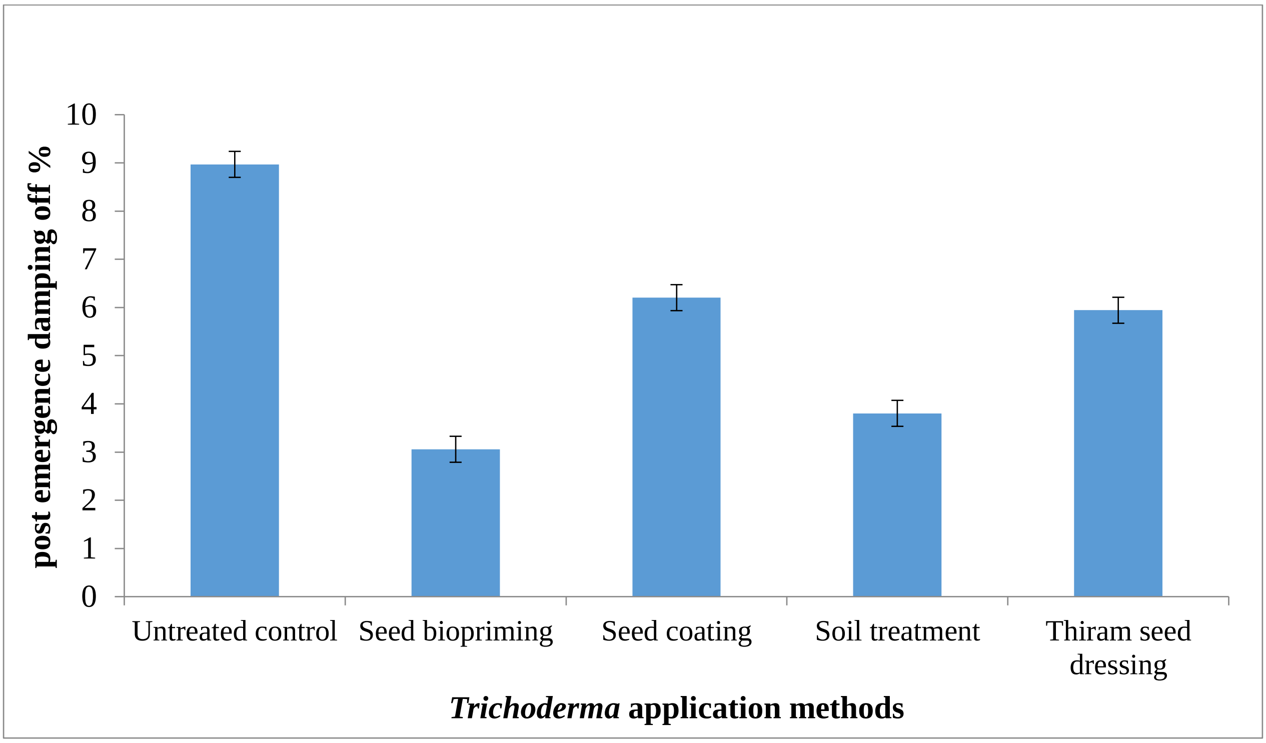

Fig: 4.2. Effect of *T. harzianum* application methods on post emergence damping off.

4.3 Estimation of the CFU of *T. harzianum* in the root rhizosphere.

In estimating the CFU concentration in root rhizosphere, there were significant differences ($p<0.001$) on the different *T. harzianum* application methods at 6 WAS. Highest CFUs were recorded in soil treatment method with 2.855×10^{4} (7.14×10^{4}) at 6 WAS. There was statistical differences between seed biopriming (2.39 (5.223)) and seed coating (2.365 (5.108)) with *Trichoderma* as shown on the table below.

Table 4.1. Estimation of CFU concentration of *T. harzianum* in the root rhizosphere.

Treatments	6 WAS
Untreated control	0.707^{a} (0.000)
T. harzianum Seed bio-priming	2.39^{b} (5.223)
T. harzianum seed coating	2.365^{b} (5.108)
T. harzianum soil treatment	2.855^{c} (7.657)
Thiram seed dressing	0.707^{a} (0.00)
Grand mean	1.805 (3.598)
P value	<0.001
cv %	5.0
LSD	0.1088

* Numbers with different letters show that there is significant difference.

** Numbers in brackets show the original mean before data transformation.

4.4 Effect of different *T. harzianum* application methods on *R. solani* root rot severity

There were significant differences ($p<0.001$) in *R. solani* root rot severity damage on the *T. harzianum* application methods. *T. harzianum* soil treatment recorded the lowest root rot severity score of 1.225 (0.833) while untreated control recorded the highest severity score of 1.225 (4.5) as shown on the fig below.

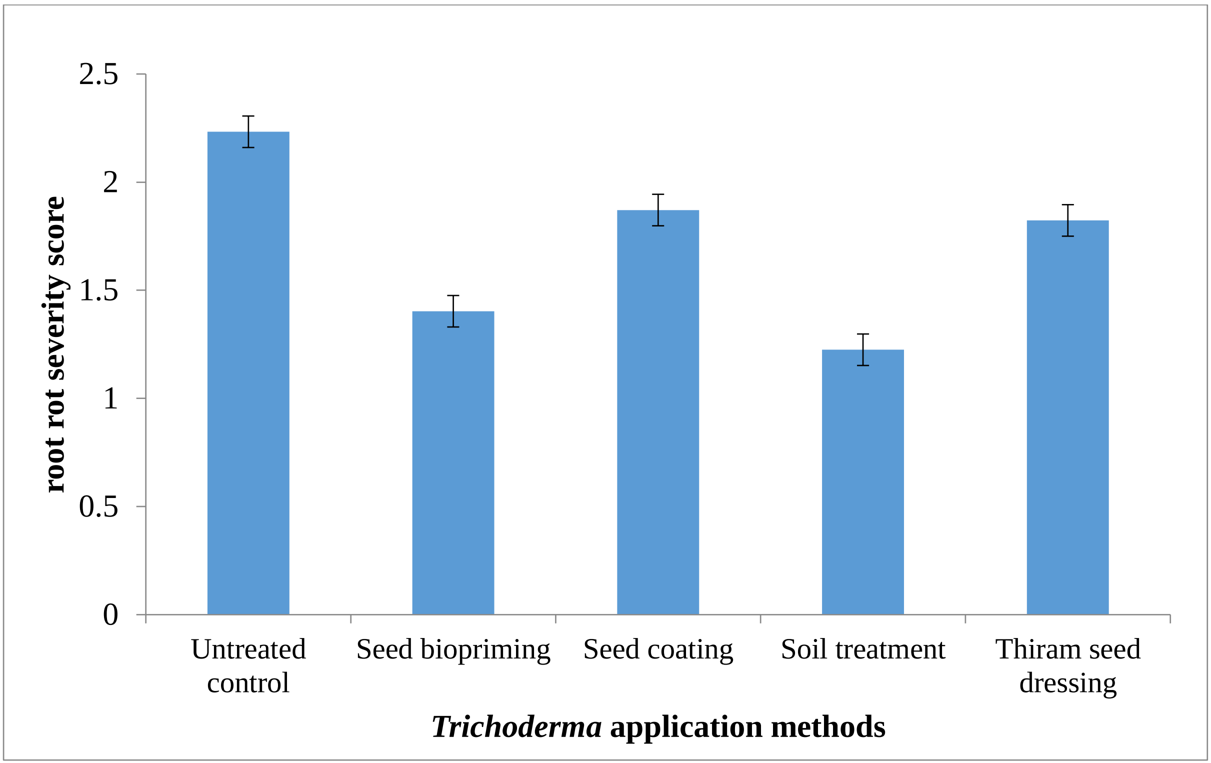

Fig: 4.3. Effect of different *T. harzianum* application methods on *R. solani* root rot severity

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of different *T. harzianum* application methods on pre emergence damping off.

The least pre emergence damping incidence was recorded in the *T. harzianum* seed bio-priming treatment. These results are consistent with the findings of El-Mohamedy (2008) who reported that *T. harzianum* seed biopriming can significantly reduce pre emergence damping off in peas. Khan *et al.,* (1992) reported that the reduction in disease incidence may due to exclusion of the disease causing pathogens by reduction of exude excretion by the germinating seed. Furthermore, low incidence may have been due to direct attack by pathogen resulting in parasitism of *R. solani* (Osburn and Scharoth 1989).

Brocklehurst and Dearman, (1983) reported that seed priming reduces time taken between sowing to emergence. Shorter sowing to emergence period of the bioprimed seeds can have resulted in pre emergence damping off disease escape. Jungles *et al*., (2012) reported that the combination of priming and seed treatment can greatly reduce pre emergence damping off. Nelson (1988) reported that *T. harzianum* can induce systematic resistance to the emerging seedlings. Pill *et al.,* (2009) observed that *T. harzianum* spores can grow of bio-primed seed surface and be able to colonise rhizosphere of the emerging seed therefor protecting the emerging seedling.

5.2 Effect of different *T. harzianum* application methods on post emergence damping off.

Compared to untreated control, all *T. harzianum* application methods have significantly reduced post emergence damping off. Seed biopriming have significantly suppress post emergence damping off. The reduction in post emergence damping off showed the same results as reported by El Mohammed *et al*., (2006) who observed a significant reduction in post emergence damping off in seed biopriming treatment. El-bab and El-Mohamedy, (2012) also showed that biopriming with *T. harzianum* have greatly reduced post emergence disease incidence in green bean plants. El-bab, and El-Mohamedy (2013), concluded that seed bio-priming integrates the biological and physiological aspects which can be employed in disease control. Therefore the reduction in post emergence damping off is due to resistance induction by *T. harzianum*. Furthermore, Howell (2011), observed that *T. harzianum* spps can grow along the growing root there exhibiting competition through rhizosphere competence. The creation of zone of protection against phytopathogens through seed treatment plays a crucial role for rhizosphere competence (Howell 2013).

5.3 Estimation of the CFU of *T. harzianum* in the soils

Highest cfu concentration were recorded on *T. harzianum* soil treatment. These results are consistent with the studies by Kumar *et al*., (2014) who reported a significant increase in *T. harzianum* populations in the soil treatment compared with corresponding seed coat treatment in the root rhizosphere. Izzati and Abdullah (2008) reported that increase in the BCA population in the soil can its efficacy. Barakat and Al-Masri (2009) observed an increase in *T. harzianum* CFU and a decrease in *F. oxysporum* in the soil. They reported that the increase in *T. harzianum* can be attributed to its competitive ability to out compete *F. oxysporum* for nutrients and space. *T. harzianum* is reported to grow rapidly when inoculated in the soil resulting in the rhizosphere competence for the nutritional factors and rhizosphere colonization.

According to Patel *et al*., (2011) an increase in soil applied *T. harzianum* population in the soil can increase is ability to suppress plant pathogens.

5.4 Effect of different *T. harzianum* application methods on *R. solani* root rot severity

All the application methods have significantly reduced root rot severity caused by *R. solani. T. harzianum* soil treatment showed the lowest root rot severity score. This low severity score be attributed to the *T. harzianum* soil population in the root rhizosphere as observed in this study. The increase in population of the BCA recorded in this study may also be attributed to rhizosphere competence against *R. solani* therefore out competing it for nutrients and space (Patel *et al*., 2011) The BCA might have out competed *R. solani* for host surface and attacked the pathogen development through antibiosis thereby reducing root rot severity (Hamid et al., 2012). Izzati and Abdullah (2008) reported that increase in increase in the BCA population in the soil can increase its efficacy. More so, *T. harzianum* spp might have colonize plant roots protecting them from plant pathogens, increasing nutrient uptake and increasing drought resistance, Fayad (2013). Fayad (2009) also indicated that *T. harzianum* can release some compounds which induces systemic resistance against pathogens.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Basing on the results obtained from the present study, compared to untreated control, all application methods have significantly reduced pre emergence and post emergence damping off and root rot severity caused by *R. solani*. Among the application methods, *T. harzianum* seed biopriming have significantly reduced both pre emergence and post emergence damping off. *T. harzianum* soil treatment have significantly suppressed *R. solani* root rot severity and recorded a significant increase in *T. harzianum* CFU in the root rhizosphere.

6.2 Recommendations

Farmers may use seed bio-priming in root rot and damping off control. However further studies to test the efficacy of the application methods of *T. harzianum* in the field is necessary. More so, there is need to evaluate these application methods in different growing regions since the BCA's efficacy can be affected by climatic conditions and ph. There is also need to test the shelf life of bio primed seeds i.e. prolonged storage can reduce seed viability of legumes.

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APPENDICES

Appendix 1: Materials required for serial dilution to determine soil CFU of *T. harzianum*

Sterile water

Sterile bottles

PDA

Pipette

Incubator

Appendix 2: Disease assessment scale for *R. solani* stem and root rot by Cole and Cole (1998)

Disease assessment was done at 5 weeks after sowing using 0-5 scale:

0: 0% damage

1: 0.1 –1.0 % = slight damage on stem

2: 1.1 – 10.0 % = two lesions on stem, slight root discolouration

3: 10.1 –25.0%= several lesions on the stem about one third of root discoloured

4: > 25.0%= extensive lesions on the stem, remains of root discoloured

5: plant dead

Appendix 3: ANOVA for pre emergence damping off

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	5	0.5277	0.1055	0.54	
TREAT	4	63.1608	15.7902	80.61	<.001
Residual	20	3.9178	0.1959		
Total	29	67.6063			

Appendix 4: ANOVA for post emergence damping off

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	5	2.2359	0.4472	2.05	
TREAT	4	129.1440	32.2860	148.14	<.001
Residual	20	4.3587	0.2179		
Total	29	135.7387			

Appendix 5: ANOVA for CFU concentration 6 WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	5	0.021850	0.004370	0.54	
TREAT	4	25.014391	6.253598	766.30	<.001
Residual	20	0.163215	0.008161		
Total	29	25.199456			

Appendix 6: ANOVA for R. solani root rot severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	5	0.01755	0.00351	0.22	
TREAT	4	3.85255	0.96314	60.56	<.001
Residual	20	0.31810	0.01590		
Total	29	4.18820			