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# Comparative Efficacy of *Trichoderma Harzianum* Application Methods for Controlling *Rhizoctonia Solani* in Peas (*Pisum Sativum* L.)

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**ABSTRACT**

Trichoderma has been widely used to control R. *Solani*. 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; seed bio-priming, seed coating and soil treatment to control R. solani in peas was conducted. The main objective of the study was to evaluate the efficacy of T. *harzianum* application methods for controlling root rot and damping off caused by R. *solani* in peas. There were significant differences ($p<0.001$) between different T. *harzianum* application methods in reducing pre and post emergence damping off. Seed biopriming recorded the lowest pre emergence damping off incidence of 2.64 % compared to 7.16 % recorded on untreated control. Seed coating had pre emergence damping off percentages and root rot severity scores of (4.22%; 1.87) respectively. Soil treatment recorded the highest population increase of T. *harzianum* ($2.855 \times 10^4$ CFU) 6 weeks after sowing.



## Introduction

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 a 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 (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 eco-friendly 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*, *Bacillus*, and *Pseudomonas* species have been widely used in controlling a wide range of plant pathogens (Moller *et al.*, 2003). Among these BCAs, *T. Harzianum* species are extensively studied and recommended for use in management of many fungal diseases (Kumar *et al*., 2012). They are 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).

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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 bio-priming, 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. Therefore, the main objective of the study was to compare the efficacy of *T. Harzianum* application methods; seed biopriming, seed coating and soil treatment for controlling root rot and damping off caused by *R. solani* in peas.

## Materials and methods

### Study site

The study was carried out in the greenhouse at Kutsaga Research Station. The greenhouse had average day and night temperature of 25 $^{o}$C.

### 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 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$^2$**

### Source of *R. solani* and *T. Harzianum* inocula

R. *solani* (AG 4) used in this study was isolated from pea roots. R. *solani* inoculum was multiplied using the Czapeks growth medium. T. *Harzianum* (T77) for the study was multiplied using brewery pressings growth medium.

### T. *harzianum* application and fungicide seed dressing

Seed Bio-priming was done using solid matrix method (Kowalska, 2015). 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 direct application into the soil. Fungicide seed dressing was done using Thiram 50WP at the dose of 4 g/kg seeds.

### 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.

### Data collection

### Pre emergence damping off

Pre-emergence damping off in pea seeds 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.

### Post emergence damping off

Post-emergence damping off was recorded 5 weeks after sowing. It was determined as percentage of the number of plants showing the disease symptoms in relation to the number of emerged seedlings.

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

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

### Root rot severity

Root rot severity was assessed 42 days after sowing. 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.

### 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. Analysis was done using Analysis of Variance (ANOVA) and the GenStat 17$^{th}$ edition. Means were separated using the least significance difference (LSD) test at 5% significance level.

## Results

### 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 % compared to 7.161 % recorded on untreated control. No statistical differences have been noted between seed coating and soil treatment application methods."Fig I" below shows performance of different *T. harzianum* application methods in reducing pre emergence damping off.

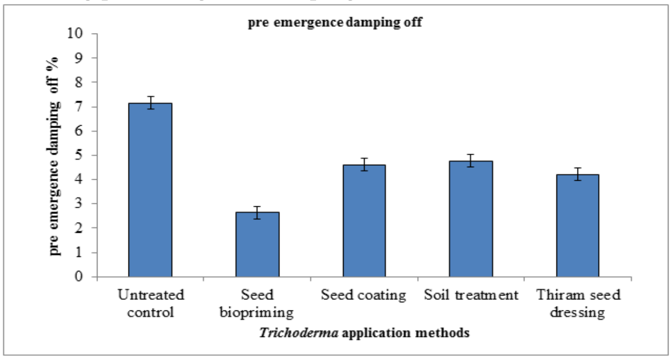


**Fig I. Effects of *T. harzianum* application methods on pre emergence damping off.**

### 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 3.058% respectively while untreated control recorded the highest post emergence damping off incidence of 8.969% ("Fig II").

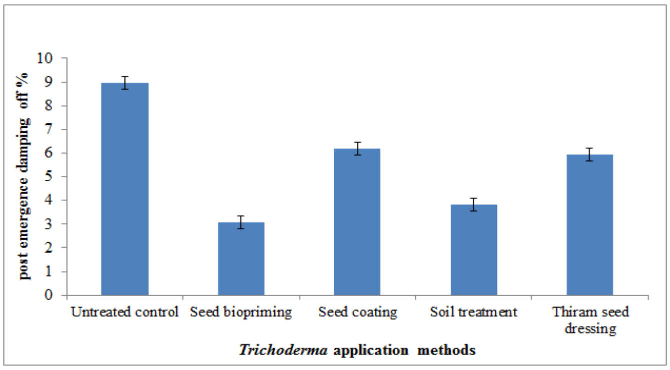


**Fig II: Effect of *T. harzianum* application methods on post emergence damping off.**

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

There were significant differences (p<0.001) on the different *T. Harzianum* application methods at 6 weeks after sowing ("Table II"). Soil treatment method had the highest concerntration of *T. Harzianum* (2.855x$10^4$ CFU) in the root rhizosphere whilst seed dressing had the least (0.707x$10^4$ CFU).

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

| Treatments | Mean CFU x $10^4$ after 6 weeks |
|---|---|
| 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 |

[a,b,c] Numbers with different letters show that there is significant difference.

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

**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 amongst the *T. harzianum* application methods. Soil treatment recorded the lowest root rot severity score of 1.225 while untreated control recorded the highest severity score of 2.233 (Fig III).

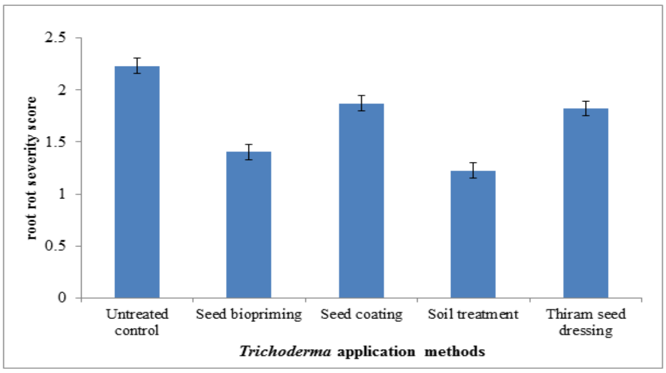


**Fig III . Effect of different *T. Harzianum* application methods on *R. solani* root rot severity.**

**Discussion**

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

The least pre emergence damping incidence (2.64 %) 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 be 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 could 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 on bio-primed seed surface and be able to colonise rhizosphere of the emerging seed therefore protecting the emerging seedling.

**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 significantly suppressed post emergence damping off. The reduction in post emergence damping off confirmed the results 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.

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

Highest CFU concentration (2.855x$10^4$ CFU) were recorded on *T. harzianum* soil treatment . These results are consistent with the studies by Kumar *etal.*, (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 an increase in the biological control agent population in the soil can increase its efficacy. Barakat and Al-Masri (2009) observed an increase in *T. Harzianum* CFU and a decrease in *F. oxysporum* in the soil which they attributed to 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.

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

All the application methods significantly reduced root rot severity caused by *R. solani.* Soil treatment showed the lowest mean root rot severity score (1.225). This low severity score can be attributed to the increase in *T. Harzianum* soil population in the root rhizosphere due to competence against *R. solani* therefore out competing it for nutrients and space (Patel *etal*., 2011). Izzati and Abdullah (2008) reported that an increase in the biological control agent population in the soil can increase its efficacy. More so, *T. harzianum* spp might have colonized plant roots protecting them from plant pathogens, increasing nutrient uptake and increasing drought resistance. Fayad (2013), also indicated that *T. harzianum* can release some compounds which induces systemic resistance against pathogens.

In conclusion, results obtained from the study indicated that *T. Harzianum* application is not only an eco-friendly strategy of disease control but an effective method in disease control in order to maximise yield and quality of the produce. All application methods of the biological control agent significantly reduced pre emergence and post emergence damping off and root rot severity caused by *R. Solani* in peas. Seed biopriming demonstrated significantly reduced pre emergence and post emergence damping off whilst soil treatment significantly suppressed *R. Solani* root rot severity and increased biological control agent concerntration in the root rhizosphere. However, further studies to test the efficacy of the application methods of *T. harzianum* in the field under different climatic conditions is necessary. There is also need to test the shelf life of bio primed seeds as prolonged storage can reduce seed viability of legumes.

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