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# Efficacy of *Trichoderma harzianum* against *Fusarium oxysporum* and *Rhizoctonia solani* on bean and tomato plants

**Gwendolyn Ban[1*], Shamsul Akanda[2] and Macquin Maino[3]**

## ABSTRACT

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Experiments were conducted in the laboratory, greenhouse, and field at the Papua New Guinea University of Technology (PNGUT) to assess the efficacy of *Trichoderma harzianum* against *Rhizoctonia solani* and *Fusarium oxysporum.* The dual culture of *T. harzianum* with *R. solani* and *F. oxysporum* isolated from the diseased bean and tomato plants under laboratory conditions showed 60.1% and 63.3%, and 54.9% and 61.6% growth reduction for *R. solani* and *F. oxysporum*, respectively. In greenhouse fungal inoculation experiments, bean and tomato plants showed relative germination index ranging from 0.56 to 1, 0.83 to 1, and disease reduction ranging from 64.8 to 96.1, and 20.3 to 83.7%, respectively. Field experiments involved tests with *T. harzianum* against one pathogenic fungus or against a combination of both *R. solani* and *F. oxysporum*, applied simultaneously as the pathogenic fungi or five days before application of pathogenic fungi. The results for bean and tomato plants showed relative germination index ranging from 0.42 to 0.94, and 0.63 to 0.94, and disease reduction recorded at 63.8 to 96.1%, and 11.3 to 63.9%, respectively. 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.

**Keywords**: *Trichoderma*, *R. solani, F. oxysporum,* biological control

## INTRODUCTION

Soil-borne plant pathogens, more specifically *F. oxysporum* and *R. solani,* can be a significant limitation to the yield and quality of vegetable crops (Koike et al 2003). These pathogens are predominantly challenging because they often survive in the soil for many years and have a wide host range. As a result, each vegetable crop may

[1,2,3]Department of Agriculture, Papua New Guinea University of Technology, PMB, Lae 411, Morobe Province, Papua New Guinea

***Corresponding Author.** Address: Department of Agriculture, Papua New Guinea University of Technology, PMB, Lae 411, Morobe Province, Papua New Guinea; Email: gwendolyn.ban@pnguot.ac.pg

be susceptible to several pathogen species and are often difficult to control with conventional strategies. The use of chemicals in controlling plant diseases has contributed significantly to improved crop productivity and quality over the years (World Bank 2007). However, excessive use of agrochemicals has resulted in environmental pollution, which is toxic to humans and animals (Pal and Gardener 2006). Biological control could be a suitable alternative to the use of chemicals as these are cost-effective, self-sustaining, unlikely to develop pathogen resistance and any adverse effects on human health or environment, and compatible with other crop disease control techniques (Benitez et al 2004).

*Trichoderma* is an antagonistic, saprophytic, soil-borne fungus found in all soil types that has been widely used as an effective biological control agent. *Trichoderma* works through mycoparasitism, antibiosis, competition, plant growth promotion, and induced resistance (Cai et al 2013). *Trichoderma* produces and releases various compounds that provoke localized or systemic resistance responses and, therefore, explains their lack of pathogenicity to plants (Rao et al 2015, Motesharrei and Salimi 2014). *Trichoderma* spp. is also known to provide plants with useful molecules, such as glucose oxidase and growth stimulating compounds, that can increase their vigor and resistance to pathogens (Brunner et al 2005). Another characteristic of this fungus is its highly competitive saprophytic ability. It competes with other fungi or pathogenic fungi for available substrates through high growth rate, proficient enzyme production, antibiotic production, tolerance to antibiotics, and effects of other microorganisms (Motesharrei and Salimi 2014). *Trichoderma* spp. also has rhizosphere competence. They compete for space and nutrients when added to the soil or applied as seed treatments as they grow readily with the developing root system of the treated plant (Harman 2000). *Trichoderma* produces antibiotics, such as gliotoxin, viridian and cell wall degrading enzymes, and biologically active heat-stable metabolites, such as ethyl acetate, which is known to be involved in disease suppression (Cai et al 2013, Gajera et al 2016). *Trichoderma* spp. were found to be very effective in controlling diseases, such as wilting of tomato, chili, peanut, potato, coffee, and black pepper; root rot of citrus, tobacco, pineapple, durian, rubber, black pepper, and lychee; damping-off of tomato, chilli, peanut, potato, soybean, maize, cabbage, and Chinese cabbage; southern stem rot of tomato, chilli, peanut, potato, and soybean; cottony rot of cabbage, Chinese cabbage and soybean and sheath blight of rice and maize (Ha 2010).

Most of the studies done elsewhere with *Trichoderma* against the pathogenic fungi have used single biocontrol agents as antagonists against a single pathogen. However, in reality, crop species are affected by multiple pathogens at the same time. Control of a wide spectrum of pathogens by applying antagonists remains a challenge and unfulfilled goal of biocontrol (Raupach and Kloepper 1998). Moreover, in most biological control studies, the biological control agent is inoculated in the field 5-7 days before the pathogenic fungi to provide the advantage in population growth and substrate colonization, but the reality is different in that the pathogens are already established in the soil, which has to be challenged with *Trichoderma*.

Despite the importance of *Trichoderma* as an effective biological control agent, no studies were done on the use of *Trichoderma* in Papua New Guinea (PNG) against multiple pathogens. Under these circumstances, experiments were conducted with the following objectives: (i) To test the effectiveness of *T.*

*harzianum* as a biological control agent against *R. solani* and *F. oxysporum* through dual culture under laboratory conditions, (ii) To test the effectiveness of *T. harzianum* as a biological control agent against *R. solani* and *F. oxysporum* separately or in combinations using bean and tomato plants under greenhouse condition, and (iii) To test the effectiveness of *T. harzianum* as a biological control agent against *R. solani* and *F. oxysoprum* separately or in combinations using bean and tomato plants under field condition.

## MATERIALS AND METHODS

### *Laboratory Experiment*

#### *Isolation of T. harzianum (strain LIPIMCO548), R. solani and F. oxysporum*

*Trichoderma harzianum* (strain LIPIMCO548) used in this experiment was isolated using *Trichoderma* specific medium (TSM) and later identified using DNA-based molecular technique (Ban et al 2017). The *F. oxysporum* and *R. solani* were isolated and cultured from diseased bean and tomato plants using a potato dextrose agar (PDA) medium. The diseased samples were collected from the Eastern Highlands Province, which is one of the major vegetables producing provinces in PNG. The isolates were purified using hyphal tip culture, and their identity was confirmed using molecular techniques.

#### *In vitro Testing of Trichoderma Isolates Against R. solani and F. oxysporum*

*Trichoderma harzianum (*strain LIPIMCO548), *R. solani* and *F. oxysporum* were multiplied on PDA. The *T. harzianum* was cultured together with *R. solani* and *F. oxysporum* in dual culture (Rekha et al 2012) as follows:

A 5mm disc of actively growing mycelial agar block of the *T. harzianum* was placed 30mm from the edge of the petri dishes containing 20mL of PDA medium. To the plates containing *T. harzianum,* 5mm disc of either *R. solani* or *F. oxysporum* were placed at the opposite end 30mm away from the edge of the petri dish. Separate plates were also inoculated only with the pathogenic fungal cultures alone to serve as a control treatment. The plates were incubated for three days at room temperature (22°C) at varying light and dark conditions. As soon as the *Trichoderma* isolates grew and met with dual cultured pathogenic fungi, the growth of the selected soil-borne pathogens was measured. The per cent growth reduction was calculated using the following formula:

Growth reduction (%) = (Growth in control – Growth in treatment / Growth in control) x 100 (Vincent 1927).

Each of the *Trichoderma* and pathogen combinations was replicated five times, and the plates were arranged in a completely randomized design (CRD).

### *Greenhouse Experiment*

Two separate greenhouse experiments were conducted at the Agriculture Department of PNG Unitech using tomato and bean plants. First, the soil was

steam-sterilized in a drum for 4h and then allowed to cool before filling the polystyrene pots of 200mm size, which were sterilized using 70% ethanol. Each of the pots was filled with 2kg of sterilized soil and was inoculated according to treatment combinations shown in Table 1.

Table1. Treatment combinations and the amount of inoculum

| Treatments | Amount of inoculum | |
|---|---|---|
| | Greenhouse (g per plot) | Field (g per plot) |
| *R. solani* alone | 5g | 50g |
| *F. oxysporum* alone | 5g | 50g |
| *T. harzianum* applied 5 days before inoculating *R. solani* | 5g of each inoculum | 50g of each inoculum |
| *T. harzianum* applied 5 days before inoculating *F. oxysporum* | 5g of each inoculum | 50g of each inoculum |
| *T. harzianum* applied 5 days before inoculating *R. solani* and *F. oxysporum* | 5g of *Trichoderma* inoculum and 2.5g each for *R. solani* and *F. oxysporum* | 50g of *Trichoderma* inoculum and 25g for each *R. solani* and *F. oxysporum* |
| *T. harzianum* applied together with *R. solani* | 5g of each inoculum | 50g of each inoculum |
| *T.harziaum* applied together with *F. oxysporum* | 5g of each inoculum | 50g of each inoculum |
| *T. harzianum* applied together with *R. solani* and *F. oxysporum* | 5g of *Trichoderma* inoculum and 2.5g each for *R. solani* and *F. oxysporum* | 50g of *Trichoderma* inoculum and 25g for each *R. solani* and *F. oxysporum* |

### *Inoculum Preparation*

Autoclaved rice bran was used to mass produce the inoculum of all three fungi. Glad zip bags were filled with 1kg of sterilized rice bran and were inoculated with five-day-old actively growing *T. harzianum*, *F. oxysporum,* and *R. solani* grown on PDA medium. One petri dish of fungi grown on the PDA was added to 1kg of rice bran and was allowed to grow for seven days before soil inoculation.

### *Inoculum of the Potted Soil*

Inoculation of sterilized soil in pots was done according to treatments presented in Table 1. Each was replicated five times, and the pots were arranged in a randomized complete block design (RCBD). Treatments were applied in a span of 10 days and covered with plastic bags to allow fungal growth before planting bean and tomato seeds.

### *Sowing of Bean and Tomato Seeds*

Five healthy bean seeds were sown in each pot to a depth of about 25mm and 10cm between seeds, but tomato seeds were sown at 10 seeds per pot with two seeds per hole to a depth of about 6mm. The same number of pots were planted with beans and tomato as check to test the germinability of the seeds.

### Data Collection

Seed germination, disease symptom, and mortality resulting from *R. solani* and *F. oxysporum* treatments were recorded daily. Data collection for tomato and bean was completed 35 and 40 days after planting (DAP), respectively.

### Data Analysis

Data on disease incidence were analyzed using statistical software STATISTIX. Preliminary analysis for greenhouse trials showed non-significant interactions between the treatments and season (time) for bean plants, but a significant interaction between treatments and season (t) for tomato plants. Therefore, a separate combined analysis was done for trials with bean plants, whereas two separate analyses were done for the trials with tomato plants. In addition, relative germination indices were also calculated by taking the ratio of seed germinations in treatments to the uninoculated controls (Ranal and Santana 2006).

### Field Experiment

Two separate field experiments were conducted at the Agriculture department farm of PNG Unitech using bean and tomato plants. Plot size of 1mx1m with 0.5m spacing between plots was prepared according to the total number of replicates for all the treatments, as shown in Table 1. The soils of the plots were fumigated with methyl bromide at 48g per $m^3$ and left for two weeks to allow for chemicals to evaporate before sowing the seeds.

### Inoculum Preparation and Application

The preparation of inoculum was the same as that used for greenhouse experiments. Sterilized plots were inoculated with rice bran inoculum according to treatments shown in Table 1. There were eight treatments, each replicated five times and organized into RCBD. Treatments were applied in a period of 10 days before planting bean and tomato seeds.

### Sowing of Bean and Tomato Seeds

Bean and tomato seeds were sown in separate experiments. Bean seeds were sown at nine seeds per plot to a depth of about 25mm and 10cm between seeds, whereas tomato seeds were sown at 18 seeds per plot with two seeds in each hole to a depth of about 6mm and 10cm between seeds. The same numbers of plots were sown with bean and tomato seeds separately to ascertain the germinability of the seeds.

### Data Collection

For both experiments, data were collected on the number of seeds germinated. Daily observations on the incidence of *R. solani* and *F. oxysporum* infection were also recorded. In addition, the mortality of plants due to disease was recorded for each treatment daily. Data collections were completed in 35 and 42 DAP for tomato and bean, respectively.

### *Data Analysis*

Data on disease incidence were analyzed using statistical software, STATISTIX. The preliminary analysis for bean and tomato plant field trials showed non-significant interactions between the treatments and season (time). Therefore, a separate combined analysis was done both for bean and tomato plants. In addition, the relative germination index in relation to control (check) was also calculated.

## RESULTS

### *Laboratory Experiments*

Dual culture of *T. harzianum* with *R. solani* and *F. oxysporum* isolated from diseased bean plants showed 60.1% and 63.3% growth reduction for *R. solani* and *F. oxysporum,* respectively. The dual culture of *T. harzianum* with *R. solani* and *F. oxysporum* isolated from diseased tomato plants showed 54.9% and 61.5% growth reduction for *R. solani* and *F. oxysporum*, respectively (Figure 1).

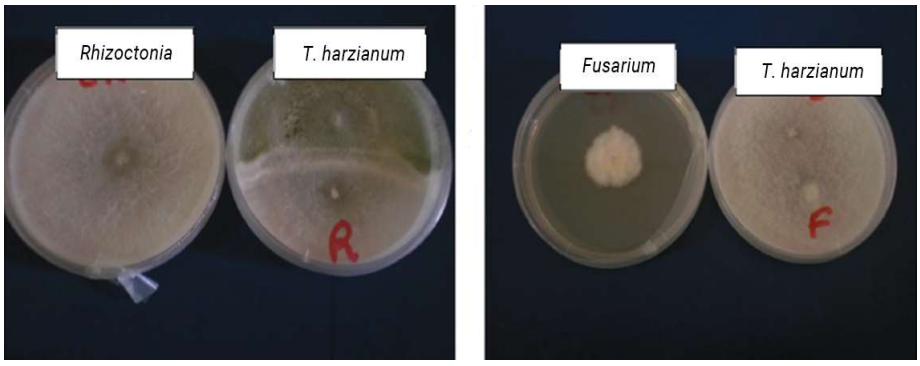


Figure 1. Dual culture of *R. solani* and *F. oxysporum isolated from tomato* with *T. harzianum*

### *Greenhouse Trials*

The results on relative seed germination index and disease incidence of bean trials are presented in Table 2. The results show that the relative germination index ranged from the lowest of 0.56 to 1.0, indicating that the seeds were highly viable and the lower germination may be due to the pathogen effect (pre-emergence germination failure).

The disease incidence ranged from the lowest of 2% when the soil was inoculated with *T. harzianum* five days before inoculating the soil with pathogenic *F. oxysporum* to the highest of 79.3% when the soil was inoculated with *R. solani* (Table 2), and this difference was significant ($p \leq 0.05$, LSD). T. *harzianum* inoculation either at the same time or five days before applying *R. solani* significantly ($p \leq 0.05$, LSD) reduced the disease incidence compared to the pots inoculated only with *R. solani,* and the percent disease reduction ranged from 71.2 to 86.8%. However, the time of

inoculation was not significantly different. The result was similar in the case of *F. oxysporum* and *T. harzianum* combination when compared with *F. oxysporum* inoculation alone. The per cent disease reduction ranged from 67 to 96%. *T. harzianum* inoculation either simultaneously or five days before inoculating the soil with *R. solani* and *F. oxysporum* together, significantly ($p \leq 0.05$, LSD) reduced the disease incidence compared to inoculation with *R. solani* or *F. oxysporum* alone. The time of inoculation of *T. harzianum* against *R. solani* and *F.oxysporum* together was insignificant.

Table 2. Relative germination index and disease incidence for bean trials (combined analysis)

| Treatment | Relative germination index | Disease incidence* | Per cent disease reduction[1] |
|---|---|---|---|
| *R. solani* alone | 0.56 | 79.34a | - |
| *F. oxysporum* alone | 0.68 | 51.5b | - |
| *T. harzianum* applied 5 days before inoculating *R. solani* | 1 | 10.5cd | 86.8 |
| *T. harzianum* applied 5 days before inoculating *F. oxysporum* | 1 | 2.0d | 96.1 |
| *T. harzianum* applied 5 days before inoculating *R. solani* and *F. oxysporum* | 1 | 20.5c | 68.7 |
| *T. harzianum* applied together with *R. solani* | 0.88 | 22.8c | 71.2 |
| *T. harzianum* applied together with *F. oxysporum* | 0.72 | 17.0cd | 67.0 |
| *T. harzianum* applied together with *R. solani* and *F. oxysporum* | 0.8 | 23.0c | 64.8 |

*Means followed by the same letter in a column are not significantly different at $p \leq 0.05$ (LSD)

[1]Percent disease reduction was calculated within the same pathogen group relative to the disease incidence within the single pathogen. For combined inoculation of *R. solani* and *F. oxysporum*, per cent disease reduction was calculated relative to the average in disease incidence of *R. solani* and *F. oxysporum* alone.

The results on the mean relative germination index for the two greenhouse trials with tomato indicate that the germinability of the seeds in different treatment combinations was as good as or better than the controls (Table 3).

The disease incidence for the two greenhouse trials with tomatoes is presented in Table 3. The disease incidence ranged from the lowest of 20.4% with *T. harzianum* inoculated five days before inoculating the pathogenic fungi *R. solani* to the highest of 96% when the soil was inoculated with *F. oxysporum* alone for trial 1. This difference in disease incidence was statistically significant ($p \leq 0.05$, LSD). Irrespective of the time of inoculation, *T. harzianum* significantly reduced the amount of disease compared to inoculation with the pathogenic fungi *R. solani* and *F. oxysporum* alone or together. Moreover, for *R. solani* and *F. oxysporum*, the application of *T. harzianum* five days ahead of the pathogenic fungi showed significant ($p \leq 0.05$, LSD) improvement in terms of disease reduction. For trial 1, the highest percentage of 76.7% disease reduction was recorded for the treatment when *T. harzianum* was inoculated five days before *F. oxysporum*.

For trial 2, the disease incidence ranged from the lowest of 14.2% when *T. harzianum* was applied five days ahead of inoculating the soil with *R. solani* to the highest of 87.3% when inoculated solely with *R. solani* (Table 3). This difference is statistically significant ($p \leq 0.05$, LSD). Similar to results in trial 1, *T. harzianum* application five days before the pathogenic fungi for all the treatment combinations

significantly ($p \leq 0.05$, LSD) reduced the disease incidence compared to applying *T. harzianum* simultaneously with the pathogenic fungi. For trial 2, the highest percentage of 83.7 of disease reduction was observed when *T. harzianum* was inoculated five days ahead of inoculating with *R. solani*. Even with the mean disease incidence of the two trials, there are no changes in the ranking of the treatments.

Table 3. Relative germination index and disease incidence of tomato plants under greenhouse condition

| Treatment | Relative germination index | Disease incidence* | | | Percent disease reduction[1] | |
|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Mean of two trials | Trial 1 | Trial 2 |
| *R. solani* alone | 1 | 86.9ab | 87.3a | 87.1 | - | - |
| *F. oxysporum* alone | 0.98 | 96.0a | 87.1a | 91.6 | - | - |
| *T. harzianum* applied 5 days before inoculating *R. solani* | 1 | 20.4d | 14.2d | 17.3 | 76.5 | 83.7 |
| *T. harzianum* applied 5 days before inoculating *F. oxysporum* | 1 | 22.4d | 20.4d | 21.4 | 76.7 | 76.6 |
| *T. harzianum* applied 5 days before inoculating *R. solani* and *F. oxysporum* | 1 | 26.7d | 35.9c | 31.3 | 70.8 | 58.8 |
| *T. harzianum* applied together with *R. solani* | 1 | 49.4c | 35.7c | 42.5 | 43.2 | 59.1 |
| *T. harzianum* applied together with *F. oxysporum* | 1 | 48.0c | 33.2c | 40.6 | 50.0 | 61.9 |
| *T. harzianum* applied together with *R. solani* and *F. oxysporum* | 0.83 | 72.9b | 50.0b | 61.5 | 20.3 | 42.7 |

*Means followed by the same letter in a column are not significantly different at $p \leq 0.05$ (LSD)
[1]Percent disease reduction was calculated within the same pathogen group relative to the disease incidence within the single pathogen. For combined inoculation of *R. solani* and *F. oxysporum*, per cent disease reduction was calculated relative to the average in disease incidence of *R. solani* and *F. oxysporum* alone.

### *Field Trials*

The results on relative seed germination index and disease incidence of bean trials under field conditions are presented in Table 4. The results show that the relative germination index ranged from the lowest of 0.42 to 0.94, indicating that the seeds were reasonably viable and the lower germination may be due to pre-emergence germination failure due to the infection of *R.solani* and *F.oxysporum*. The higher relative germination indices were recorded with the treatment containing the *T. harzianum* inoculation.

The disease incidence ranged from the lowest of 3.5% when the soil was inoculated with *T. harzianum* five days before inoculating the soil with pathogenic *F. oxysporum* to the highest of 89.9% when the soil was inoculated with *R. solani* alone (Table 4), and this difference was significant ($p \leq 0.05$, LSD). In all the treatment

combinations, the application of *T. harzianum*, five days before the pathogenic fungi had significantly ($p \leq 0.05$, LSD) lower disease than the treatment combinations when *T. harzianum* was applied along with the pathogenic fungi at the same time. The highest of 96.1% disease reduction was recorded when *T. harzianum* was applied five days ahead of *R. solani* application. Per cent disease reduction ranges from 63.8 to 96.1.

Table 4. Relative germination index and disease incidence for bean trials (combined analysis)

| Treatment | Relative Germination index | Disease incidence* | Percent disease reduction[1] |
|---|---|---|---|
| *R. solani* alone | 0.42 | 89.9a | - |
| *F. oxysporum* alone | 0.42 | 88.5a | - |
| *T. harzianum* applied 5 days before inoculating *R. solani* | 0.94 | 5.7d | 96.1 |
| *T. harzianum* applied 5 days before inoculating *F. oxysporum* | 0.92 | 3.5d | 96.0 |
| *T. harzianum* applied 5 days before inoculating *R. solani* and *F. oxysporum* | 0.89 | 15.8c | 82.3 |
| *T. harzianum* applied together with *R. solani* | 0.92 | 20.0c | 77.8 |
| *T. harzianum* applied together with *F. oxysporum* | 0.79 | 21.8c | 75.4 |
| *T. harzianum* applied together with *R. solani* and *F. oxysporum* | 0.81 | 32.3b | 63.8 |

*Means followed by the same letter in a column are not significantly different at $p \leq 0.05$ (LSD)

[1]Percent disease reduction was calculated within the same pathogen group relative to the disease incidence within the single pathogen. For combined inoculation of *R. solani* and *F. oxysporum*, per cent disease reduction was calculated relative to the average in disease incidence of *R. solani* and *F. oxysporum* alone.

The results on relative seed germination index and disease incidence of tomato trials under field conditions are presented in Table 5. The results show that the relative germination index ranges from the lowest of 0.63 to 0.94, indicating that the seeds were reasonably viable. The lower germination might have been due to the pathogenic effect (pre-emergence germination failure). It is interesting to note that the treatment combinations with *T. harzianum* showed higher relative germination indices, except when it was applied with *R.solani* and *F. oxysporium* at the time of seeding.

The disease incidence ranged from the lowest of 32.9% with treatment where the soil was inoculated with *T. harzianum* five days before inoculating the soil with *R. solani* to the highest of 91.1% when the soil was inoculated with *R. solani* (Table 5) alone, and this difference was statistically significant ($p \leq 0.05$, LSD). All the treatment combinations with *T. harzianum*, irrespective of the time of application in the soil, significantly ($p \leq 0.05$, LSD) reduced the disease incidence compared to *R. solani* and *F. oxysporum* alone. For all the treatment combinations, application of *T. harzianum* five days ahead of the *R. solani* and *F. oxysporum* alone or together showed significantly ($p \leq 0.05$, LSD) lower disease incidence. The highest of 63.9% disease reduction was observed when *T. harzianum* was applied five days before the application of *R. solani*. In comparison, the lowest of only 11.3% disease reduction was observed when *T. harzianum* was applied together with *R. solani* and *F. oxysporum* at the same time.

Table 5. Relative germination index and disease incidence for tomato trials (combined analysis)

| Treatment | Relative germination index | Disease incidence* | Per cent disease reduction[1] |
|---|---|---|---|
| *R. solani* alone | 0.74 | 91.1a | - |
| *F. oxysporum* alone | 0.68 | 90.6a | - |
| *T. harzianum* applied 5 days before inoculating *R. solani* | 0.94 | 32.9d | 63.9 |
| *T. harzianum* applied 5 days before inoculating *F. oxysporum* | 0.92 | 39.0d | 57.0 |
| *T. harzianum* applied 5 days before inoculating *R. solani* and *F. oxysporum* | 0.83 | 55.7c | 38.7 |
| *T. harzianum* applied together with *R. solani* | 0.81 | 56.2c | 38.3 |
| *T. harzianum* applied together with *F. oxysporum* | 0.78 | 55.3c | 39.0 |
| *T. harzianum* applied together with *R. solani* and *F. oxysporum* | 0.63 | 80.6b | 11.3 |

*Means followed by the same letter in a column are not significantly different at $p \leq 0.05$ (LSD)
[1]Percent disease reduction was calculated within the same pathogen group relative to the disease incidence within the single pathogen. For combined inoculation of *R. solani* and *F. oxysporum*, per cent disease reduction was calculated relative to the average in disease incidence of *R. solani* and *F. oxysporum* alone.

## DISCUSSION

In the current study, the dual culture of *R. solani* and *T. harzianum* isolated from bean and tomato plants showed significant growth inhibition of 60.1% and 54.9%, respectively. Similar dual culture studies of *R. solani* with *T. harzianum* showed growth inhibition of 55.55 to 65.18% (Naeimi et al 2010) and 80 to 100% (Ali and Nadarajah 2012). Dual culture of *F. oxysporum* with *T. harzianum* isolated from bean and tomato plants in the current study showed 63.3% and 61.5% growth inhibition, respectively. In similar in vitro studies, *T. harzianum* was most effective in inhibiting the mycelial growth of *F. oxysporum* by more than 56.43% (Perveen and Bokhari 2012) and 65% (Hibar et al 2005). Abd-El-Khair et al (2011) also reported significant mycelial growth inhibition *of R. solani* and *F. solani,* causing damping off of beans from in vitro tests with four different species of *Trichoderm*a, including *T. harzianum* in Egypt. Dual culture studies suggest the secretion of diffusible non-volatile inhibitory substances by *Trichoderma* before hyphal contact. It was demonstrated that before the mycelia of fungi interact, *Trichoderma* spp. produces low quantities of extracellular exochitinases (Brunner et al 2005). The cell fragment, in turn, induces the production of more enzymes, which trigger a cascade of physiological changes stimulating rapid and directed growth of *Trichoderma* spp. (Zeininger et al 1999).

With the current greenhouse and field studies, it was observed that bean and tomato seeds planted in treatment combinations with *T. harzianum* had early germination and the improvement in seed germination percentage. The higher relative seed germination indices for trials with bean and tomato under greenhouse and field conditions were observed in all treatment combinations with *T. harzianum* than for *R. solani* or *F. oxysporum* alone. This may be due to the inoculation of *T.*

*harzianum* that improves pre-emergence germination (Ali and Nadarajah 2012). Rapid and improved seed germination due to *Trichoderma* has also been reported on many other plants, such as chilli (Asaduzzaman et al 2010), muskmelon (Kaveh et al 2011), cotton (Hanson 2000), rice (Mishra and Sinha 2000), and silverweed (Oyarbide et al 2011). In addition, *T. harzianum* was found to enhance seed germination, root and shoot length (Dubey et al 2011) and increase in the frequency of healthy plants and higher yield (Rojoa et al 2007). A similar increase was also observed when seeds were separated from *Trichoderma* by a cellophane membrane, indicating that *Trichoderma* produces growth factors that increase the seed germination rate (Benitez et al 2004). *Trichoderma* also competes with other microorganisms; for example, it competes for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil (Howell 2003) and, more generally, competes with soil microorganisms for nutrients and/or space (Bailey et al 2008, Motesharrei and Salimi 2014, Baghani et al 2012, Segarra et al 2010).

A highly significant reduction of disease incidence in trials with bean and tomato plants was observed in this study both under greenhouse and field conditions. Treatments with *T. harzianum* applied five days before applying *R. solani*, *F. oxysporum,* or both pathogenic fungi together performed better than treatments with *T. harzianum* applied at the same time along with the pathogenic fungi. This could mostly be due to the site and/or substrate colonization, as the soil was sterilized before the inoculation of the fungi. *Trichoderma* species are generally very aggressive competitors, grow very fast and rapidly colonize substrates to exclude pathogens, such as *F. oxysporum* spp. (Papavizas 1985). Hadar et al (1979) reported that *T. harzianum* directly attacked the mycelium of *R. solani* when two fungi were grown together. They also reported effective control of damping off of bean, tomato, and eggplant seedlings when *T. harzianum* was applied in the form of wheat bran culture. The antagonistic activity of the *T. harzianum* was also reported by Dennis and Webster (1971) against many fungi, including *R. solani*. The effectiveness of *T. harzianum* as a biological control agent against *R. solani* and *F. oxysporum* was also confirmed by numerous research studies (Abd-El-Khair et al 2011). Moreover, *T. harzianum* has been found to be able to control many other pathogenic fungi, including *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* (Tančić et al 2013), *Alternaria alternate* (Gveroska and Ziberoski 2011), and *Bipolaris oryzae* (Abdel-Fattah et al 2007). *Trichoderma harzianum* protected the bean seedlings against pre-emergence damping-off infection, decreasing disease severity, and increasing plant growth in the presence of *R. solani* (Paula et al 2001). Reduction in disease incidence may be due to a combination of mechanisms, like competition (Bailey et al 2008; Motesharrei and Salimi 2014; Baghani et al 2012; Segarra et al 2010), antibiosis (Benitez et al 2004, Rao et al 2015, Bailey et al 2008, Motesharrei and Salimi 2014), mycoparasitism (Benitez et al 2004, Bailey et al 2008, Motesharrei and Salimi 2014), hyphal interactions, enzyme secretions (Singh 2010), competitive saprophytic ability (Woo et al 2006), induction of defence responses in plants and metabolism of germination stimulants (Howell 2003). Abd-El-Khair et al (2011) reported the accumulation of enzymes, like chitinase, peroxidase, and polyphenol oxidase, which improves plant defense mechanisms against pathogen infection. They also reported increased enzymatic activities in *Trichoderm*a treated bean plants over the untreated controls.

*Trichoderma* actively takes over the root zone, making it difficult for pathogens to compete for space on the roots and nutrients (Lester 2010). Either added to the soil or applied as seed treatments, they readily grow along with the developing root system of the treated plant (Harman 2000). *Trichoderma* produces many antibiotics, such as trichodermin, trichodermol, harzianum A, and harzianolide (Kucuk and Kivanc 2004), as well as some cell wall degrading enzymes, such as chitinases, glucanases that break down polysaccharides, chitins, and glucanase, thereby destroying cell wall integrity (Woo et al 2006). *Trichoderma* can parasitize pathogenic fungi by coiling around pathogen hyphae, penetration, and subsequent dissolution of the host cytoplasm (Weindling 1934). *Trichoderma* hyphae also penetrate infected epidermal and cortical tissue of the root to destroy the hyphae of the pathogen, with little or no damage to uninfected plant tissue (Metcalf and Wilson 2001). It readily colonizes and competes for available substrates as a source of food. As it decomposes substrates, the amount of readily available cellulose decreases, which activates the chitinase of *Trichoderma*, parasitizing pathogenic fungi (Benhamou and Chet 1993). Due to the interactions between *Trichoderma* and plants, a variety of root pathogens and above-ground parts of plants cause less disease in plants in which the roots are colonized by *Trichoderma* (Cai et al 2013, Gajera et al 2016, Amer and Abou-El-Seoud 2008). Even in the absence of disease or pathogens, plants frequently have more extensive roots and higher productivity in the presence of *Trichoderma* (Harman 2000).

## CONCLUSION

This is a significant and in-depth study on biological control of multiple soil borne diseases using *Trichoderma harzianum* conducted in PNG. The application of *T. harzinium* five days before seeding is significantly better in controlling the disease than applying the biocontrol agent simultaneously. The results from this study would significantly improve productivity and quality of bean and tomato production in the major vegetable growing provinces of PNG, especially the Eastern and Western Highlands Provinces and other areas that have similar problems with diseases caused by diseases *R. solani* and *F. oxysporum*.

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