Full Length Research Paper

Negative effect of three commonly used seed treatment chemicals on biocontrol fungus 
*Trichoderma harzianum*

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Successful integration of biocontrol organisms into agricultural production systems requires knowledge of their compatibility with commonly used pesticides. The compatibility of the biocontrol fungus *Trichoderma harzianum* (Eco-T®) with a pesticide mix containing imidacloprid, metalaxyl and thiram (Product X) was assessed for maize seed treatment. Laboratory evaluations demonstrated that Product X caused up to 97.6% mortality of *T. harzianum* in a period as short as 3 days after seed treatment. Under greenhouse conditions, combined treatment of *T. harzianum* and Product X resulted in a significant reduction in percentage germination, dry shoot biomass and maize vigour when compared to single treatment with *T. harzianum*. Seed treatment with *T. harzianum* significantly enhanced maize germination, dry shoot biomass and vigour. In contrast, treating maize seed with Product X showed no actual benefit towards optimizing maize seedling growth.

Key words: Biological control, compatibility, maize, pesticides, *Trichoderma harzianum*.

INTRODUCTION

Treating seeds with fungicides is a common practice that is expected to prevent seed borne and soil borne diseases caused by pathogenic fungi. Moreover, to avoid damage caused by insect pests, combinations of fungicides with insecticides have been employed, resulting in a considerable increase in the number of chemical components used in seed treatments. In Africa, pesticide use accounts for 4% of the global pesticide market of 31 billion US dollars (Agrow, 2006; PAN UK, 2006).

The need for better seed treatments has led to the formulation of different chemical cocktails containing several active chemical ingredients in a single seed treatment (Williamson et al., 2008). One such mix, called ‘Product X’ for anonymity, contains the insecticide imidacloprid and the fungicides metalaxyl and thiram. This product has been sold in some countries in Africa without formal registration, and limited labelling and documentation on its correct use. Inappropriate use of such products may have consequences not only for operator and consumer health but also for farm livestock, soil microflora, bees, wildlife and vegetation (Pretty and Hine, 2005; Williamson et al., 2008).

Imidacloprid is a systemic insecticide belonging to the...
chemical class of neonicotinoids, which acts by specifically blocking the microinergic neuronal pathway of insects (Jemec et al., 2007). This systemic property combined with high toxicity to sucking insects allows neonicotinoid soil treatment and seed coatings to provide long lasting protection of the whole plant (van der Sluijs et al., 2013). However, neonicotinoid insecticides are currently the subject of intense debate stemming from their link to an increase in diabetes incidence in humans, ecotoxic effects on earthworms, soil and ground water, and large-scale losses in honey bee populations (Ratnieks et al., 2010; Alves et al., 2012; Tapparo et al., 2012; Anderson et al., 2013; Kim et al., 2013). Seed-coating with neonicotinoid insecticides used worldwide on maize crops have been linked to the rapid decline in honeybee colonies.  

This phenomenon is known as colony collapse disorder and represents a worldwide crisis that has adverse effects both on crop production and ecosystems (vanEngelsdorp et al., 2009; Di Prisco et al., 2013). In January 2013, the European Food Safety Authority (EFSA) stated that neonicotinoids pose an unacceptably high risk to the global honey bee population. As a result, several countries have restricted the neonicotinoids, including imidacloprid (EFSA, 2013).

Biocontrol can provide an alternative approach to plant disease control and avoid the detrimental effects from chemical pesticides. Research activities have pointed to the potential of isolates of Trichoderma sp. to control soil borne plant pathogens effectively with no harmful effects to the environment. They are present in nearly all agricultural soils (Harman et al., 2004) and are efficient in controlling plant pathogens through several antagonism mechanisms such as antibiosis, antibiotic production, competition, and induction of resistance in addition to growth promotion of some plants (Howell, 2003). Trichoderma spp. has been shown to be antagonistic to some soil-borne pathogens, such as Rhizoctonia, Sclerotium, Fusarium, Pythium (Deng et al., 2007). It is use in plant disease control is expanding as an increasing number of studies have reported its antagonistic effect on other plant pathogens (Gomathinayagam et al., 2012; Zhang et al., 2014; Larralde-Corona et al., 2008; Abdel-Fattah et al., 2007).

A critical impediment to the application of biological control in the field is lack of knowledge of the compatibility between seed treatments with commonly used pesticides and biological agents. Previous studies have reported the compatibility of biocontrol agents with chemical pesticides (Bhal and Thomas, 2010).

However, little information is available on the compatibility of Trichoderma harzianum, a well-known bio-control agent against several plant pathogens, with current chemical pesticide mixes in Africa. Given the rapid adoption of a variety of pesticide seed treatments in Africa, this study evaluated the efficacy of Product X and its compatibility with the biocontrol fungus T. harzianum as treatments of maize seed.

### MATERIALS AND METHODS

#### Seed and products

Untreated seed of the white maize cultivar, Mac’s Medium Pearl was obtained from McDonalds Seed Company (Pty) Ltd., Pietermaritzburg, Republic of South Africa. Trichoderma harzianum Eco-T® is a registered, formulated biocontrol product, known for its inhibitory action on certain root diseases and growth promoting effects on a wide variety of vegetables, crops and ornamental species. It was provided by Plant Health Products (Pty) Ltd (Estcourt, RSA, 3280) and applied as a seed treatment at the recommended rate of 1 g.kg⁻¹ seed.

Product X has the active ingredients of 10% imidacloprid (insecticide), 10% metalaxyl (fungicide) and 10% thiram (fungicide), and was applied as a seed treatment at the recommended rate of 3.3 g.kg⁻¹ seed.

#### Seed treatments

The seed was coated with either single or combined treatments of T. harzianum (Strain Eco-T®) and an insecticide/fungicide seed treatment Product X. The seeds were surface sterilized by washing 8 times in sterile distilled water before being immersed in 10% sodium hypochlorite for 1 min. Seeds were then washed 3 times with sterile distilled water and blotted dry on sterile filter paper. They were coated with the recommended amount of seed treatment together with a small amount of an organic adhesive (2% guar gum solution). After treatment, seeds were left overnight to dry in a laminar flow cabinet.

#### Experimental design

The experiment was arranged in a randomized complete block design consisting of four treatments and five replications per treatment. Treatments included: (1) control: untreated (treated with adhesive only), (2) Thz: treated with T. harzianum only, (3) Product X: treated with Product X only, and (4) Thz + Product X: treated with both T. harzianum and Product X. Each treatment consisted of five Speedling® 49 trays. One seed per cell of the tray was sown and trays were filled with composted pine bark medium (Growmor, Cato Ridge). Trays were maintained at 25-30°C in a tunnel (CERU, UKZN-PMB), at 60 to 80% relative humidity, and fertilized weekly with 3:1:3 (nitrogen: phosphate: potassium) soluble fertilizer. The experiment was repeated three times.

#### Assessment of growth parameters

**Germination percentage and dry shoot biomass**

At six days post planting, emerging seedlings were counted and counts were presented as the percentage seedlings that had germinated in one Speedling® 49 tray. The dry shoot biomass was assessed at 21 days post planting. Shoots of seedlings in a tray were carefully cut from roots at soil level and placed in a paper bag. The shoot biomass was dried in an oven at 65°C for 72 h. Dry shoots were weighed and the values were expressed in grams dry shoot biomass.

**Vigour index**

At 21 days post planting, root and shoot length of seedlings were measured. Mean values per tray were calculated and expressed in millimetres. Vigour index values were computed using the following
Table 1. Single and combined effect of *Trichoderma harzianum* Eco-T® and Product X seed treatments on the germination percentage, dry shoot biomass and vigour of maize (*Zea mays* L.).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination (%)</th>
<th>Dry shoot biomass (g)</th>
<th>Vigour index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>93.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51333&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thz</td>
<td>97.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63987&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Product X</td>
<td>94.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53634&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thz &amp; Product X</td>
<td>94.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54555&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Effects**

<table>
<thead>
<tr>
<th>P value</th>
<th>l.s.d</th>
<th>s.e.d</th>
<th>%cv</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.01</td>
<td>1.84</td>
<td>0.82</td>
<td>1.4</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>1.89</td>
<td>0.869</td>
<td>4.9</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>1925.7</td>
<td>864.3</td>
<td>2.4</td>
</tr>
</tbody>
</table>

- Means within a column not following by the same letter are significantly different (P= 0.05).

**Assay of antifungal activity of Product X on *T. harzianum***

An inhibition index was calculated to determine the degree of antifungal activity of Product X on the growth of *T. harzianum*. A sub-sample of 10 seeds of the Thz and Thz + Product X treated lots was incubated on moist sterile filter paper for 3 days at 30°C. Individual seeds were placed in an Eppendorf tube containing 1 ml sterile distilled water and vortexed to remove any conidia from the seed. The resultant 1 ml suspension was serially diluted to count *T. harzianum* colony forming units (CFU) on Trichoderma Selective Media (TSM, Elad et al., 1981). Plates were incubated at 30°C and colony counts were made 72 h post inoculation. There were three replicates for each plate. The antifungal index was calculated as follows: Inhibitory index (%) = ((A-B)/A) x 100. Where A is the colony count of seeds treated with *T. harzianum* only and B is the CFU count of seeds treated with *T. harzianum* and Product X. Each experiment was performed three times and the data were averaged.

**Statistical analysis**

Data was analysed using the statistical analysis software GenStat<sup>®</sup> 14<sup>th</sup> Edition. A one way analysis of variance (ANOVA) was used to determine treatment effects. Significant difference between treatments at 5% probability level was determined using Fisher’s unprotected least significant difference (LSD) test.

**RESULTS**

**Germination percentage and dry shoot biomass**

Single treatment of seed with *T. harzianum* significantly enhanced maize germination (P<0.01) and dry shoot biomass (P<0.001), (Table 1). In contrary, seed treatment with Product X did not significantly improve germination percentage or dry shoot biomass. Combined treatment of *T. harzianum* and Product X significantly reduced the effect of *T. harzianum*.

**Vigour index**

As shown in Table 1, seed treatment with *T. harzianum* caused the highest seedling vigour, with a vigour index of 63987 (P<0.001). Figure 1 depicts the influence of each treatment on maize seedling growth. Treatment with Product X also enhanced seedling vigour (53634). Combined treatment of *T. harzianum* and Product X (54555) significantly diminished the effect of *T. harzianum*.

**Assay of antifungal activity of product X on *T. harzianum***

Viable cell counts resulted in *T. harzianum* population densities of 528,571 CFU.ml<sup>-1</sup> and 12,867 CFU.ml<sup>-1</sup> for the Thz and Thz + Product X treatments respectively. A total of 97.6% of *T. harzianum* cells were inhibited by Product X (Table 2).

**DISCUSSION**

*T. harzianum* is an effective biocontrol agent that is known to suppress certain soil borne diseases (Pal and Kumar, 2013). In addition, seed treatments with *T. harzianum* have shown to enhance plant growth (Howell, 2003). Our studies have shown that germination and vigour of maize can be improved by seed treatment with a selected *T. harzianum* strain. However, factors that reduce the active population of *T. harzianum* on the seed will directly reduce the efficiency of the biological activities involved in enhanced maize growth. In Africa, seed treatment with pesticides has been broadly practiced as an insurance against seed and soil-borne pests, although the toxicity of most pesticides to *T.
Trichoderma harzianum has often been underestimated. A lack of knowledge of the compatibility of a biocontrol agent with pesticides may contribute to the failure of the biocontrol agent to perform as expected (Fravel et al., 2005). This is the first study in Africa to report the negative effect of commonly used seed treatment chemicals on biocontrol fungus *T. harzianum*.

In this study, the active ingredients in Product X dramatically reduce the viability and biological control abilities of *T. harzianum*. Results from viable cell counts using the antifungal assay showed that Product X caused 97.6% mortality to *T. harzianum* in a period as short as 3 days after seed treatment (Table 2). As a result, combined treatment of *T. harzianum* and Product X resulted in a significant reduction in germination, dry shoot biomass and seedling vigour when compared to treatment with *T. harzianum* only (Table 1). *T. harzianum* and Product X are not compatible treatments for use in an integrated pest management system. When compared to the untreated control (93.74%), seed treatment with Product X (94.66%) did not significantly improve germination percentage or dry shoot biomass. Hence, the practice of applying Product X as a seed treatment to maize provides no immediate benefit to maize seedlings. Previous investigations into the compatibility of biocontrol and pesticide treatments have reported a similar trend. Campo et al. (2009) evaluated the compatibility between seed treatment with fungicides and bradyrhizobial inoculated on soybean under laboratory, greenhouse and field conditions. Not only did the study demonstrate fungicide toxicity to the bradyrhizobial population, but it also noted that fungicide seed treatment did not enhance either plant emergence or yield compared to the fungicide-free treatments (Campo et al., 2009). Sarkar et al. (2008) also reported inhibition of *T. harzianum* by systemic fungicides hexaconazole (87.7%), propiconazole (56.4%) and triflumizole (36.2%). Similarly, Malathi et al. (2002) showed incompatibility of fungicides thiophanate methyl and carbendazim when tested with six *Trichoderma* strains in the integrated management of red rot disease of sugarcane. Considering the poor performance of Product X, the use of *T. harzianum* should be considered as a more efficient and environmentally-friendly alternative for seed treatment of maize.

In Africa, a large maize industry faces numerous pest and pathogen attacks, demanding pesticide usage with mixtures of active ingredients (Williamson et al., 2001). The results of this study offer new insight into the toxicity and efficacy of pesticide cocktails used to treat a range of seed in Africa. This cocktail drastically hinders biological inoculant survival and activity on treated seed. Such seed treatments may provide no benefits to the treated seed in practice. In contrast, *T. harzianum* provided

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**Table 2. Effect of seed coating agents of Product X on Trichoderma harzianum Eco-T® colonization of treated maize (Zea mays L.) seed.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colony forming units.ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thz</td>
<td>528,571</td>
</tr>
<tr>
<td>Thz &amp; Product X</td>
<td>12,867</td>
</tr>
<tr>
<td>Inhibitory index (%)</td>
<td>97.6</td>
</tr>
</tbody>
</table>

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**Figure 1.** Effect of singular and combined *Trichoderma harzianum* Eco-T® and Product X seed treatments on the growth of maize (*Zea mays* L.).
measurable growth enhancement. The information presented in this study will be of benefit to farmers in Africa when choosing a suitable seed treatment for maize.

Conflict of Interest

The authors have declared that they have no conflict of interest.

ACKNOWLEDGEMENT

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REFERENCES


