Control of *Rhizoctonia* and *Pythium* on soybeans (*Glycine max*) using *Trichoderma* and silicon

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Received________.

Acknowledgements

The authors would like to thank the Protein Research Foundation (PRF) and the NRF for funding this research.

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Control of *Rhizoctonia solani* and *Pythium* on soybeans using *Trichoderma* and silicon

Rhizoctonia solani and *Pythium* spp. are the causal organisms of seed and seedling diseases of many crops. Silicon (Si) has been shown to act as a component in plant resistance against abiotic and biotic stresses and to activate host defence responses. The aim of this trial was to investigate the growth promotion activities of Si and *Trichoderma* (Eco-T®) on soybeans. The control of *R. solani* and *Pythium* was also investigated. Five treatments were investigated, i.e., 200 mg l^{-1} Si , Eco-T®, 200 mg l^{-1} Si plus Eco-T®, a control and KOH (to investigate the possible effects of potassium in the potassium silicate). Pot trials were used to measure the shoot biomass of the plants, while rhizotrons were used to measure root area, root biomass and root length. Growth promotion studies showed that the application of 200 mg l^{-1} Si plus Eco-T® resulted in a significant increase in shoot biomass (22.45 g), root area (14.66 cm^2), root biomass (0.22 g) and root length (143.25 cm) compared to the control (14.64 g, 7.32 cm^2, 0.15g and 105.17 cm), respectively. Similar results were found in the *Pythium* control trials where the application of 200 mg l^{-1} Si plus Eco-T® also showed a significant increase in shoot biomass (19.74 g), root area (9.77 cm^2), root biomass (0.093 g) and root length (140.93 cm) compared to the inoculated control (9.09 g, 5.74 cm^2, 0.047 g and 81.75 cm), respectively. For the *Rhizoctonia* control trials this pattern was repeated with similar results as application of 200 mg l^{-1} Si plus Eco-T® also showed a significant increase in shoot biomass (20.45 g), root area (8.98 cm^2), root biomass (0.094 g) and root length (131.2 cm) compared to the control (7.45 g, 5.05 cm^2, 0.032 g and 65.89 cm), respectively. It appears that Eco-T® protects the seed against *Rhizoctonia* and *Pythium* before root development, but once roots develop, the Si taken up by the plant prevents pathogens penetrating the root area as well as possibly inducing host defence reactions.

Keywords: *Pythium*, *Rhizoctonia*, soybeans, silicon, *Trichoderma*

Introduction

Soybeans (*Glycine max* L. Merrill) are legumes that are native to East Asia and have been used for thousands of years as a high protein food source. Various *Trichoderma* spp. have been reported to effectively control several plant pathogens (Koch, 1999; Zhang *et al*., 1999). Findings by Koch (1999) and Lewis & Lumsden (2001) demonstrated that *Trichoderma* spp. formulations were able to effectively reduce damping-off caused by *Rhizoctonia* on peas and cucumber.

Rhizoctonia solani and *Pythium* cause reduced seed germination and damping off of many crops and infect seedlings or the tips of roots belonging to older plants (Hendrix & Campbell, 1973). *Rhizoctonia* causes a much higher incidence of damping-off than *Pythium* and has the ability to cause stem and root rot which may result in patchy growth as well as yield losses in many crops.

Silicon can act as a component in plant resistance against abiotic stress e.g. nutrient toxicities, drought, cold and biotic stresses, e.g. fungi, nematodes and pests (Epstein, 1999). This enhanced resistance is associated with a higher deposit of silicon in the leaf which forms a physical barrier to impede pathogen penetration and the activation of host defence responses (Kim *et al*., 2002;.Kunzheng *et al*., 2009). Silicon has been seen to help with *Diaporthe phaseolorum* (Cooke & Ellis) (soybean stem canker) as resistance occurred in soybeans with the addition of calcium silicate (Juliatti *et al*., 1996).

The aim of this trial was to determine whether Si and *Trichoderma* (in the commercial form of Eco-T®), applied individually or in combination, have growth promotion properties and if they are able to control *Rhizoctonia* and *Pythium* on soybeans.

Material and methods

Seeds & seed treatments

Soybean seeds (cv. LS 6161RR) used for both the growth promotion and the biological control studies, were obtained from Link Seeds (Pty) Ltd, P.O. Box 755, Greytown, 3250, KwaZulu-Natal, South Africa. To ensure adhesion of the biocontrol agent, *Trichoderma* (Eco-T®) onto the seed, 1.5 g of Gumgar sticker suspension was placed in 1 L of water and stirred for approximately 1 hr. Of this solution, 0.5 ml was removed and poured over 76 g of soybean seeds, onto which approximately 0.12 g of the commercial strain of *T. harzianum* (Eco-T®) was added. The treated seeds were air dried on a lamina flow bench for 18 hrs.

Growth promotion studies

Pot trials

Soybean seeds were planted in 15 cm diameter pots and filled with sterile composted pine bark. To ensure the soil was thoroughly wet, pots were watered before and after planting. Five treatments were used (Table 1). Potassium hydroxide (KOH) was applied as a control because silicon (Si) was used in the form of potassium silicate. Potassium chloride (KCl) was added to the Si solution to balance the potassium ions and the pH.

Pots were placed in 2 L plastic containers that contained the treatments, and transferred to a greenhouse maintained at 22°C, with no overhead irrigation system. Pots were placed in a complete randomised block design, each treatment having four replicates with four plants per replicate. Treatments were placed in the plastic containers three times a week, together with NPK soluble fertiliser [3:1:3(38) complete].

After 5 weeks, aerial parts of the seedlings were cut at soil level, placed in brown paper bags and dried at 70°C in an oven for 48 hrs (Labotec, Model number 385, South Africa) in order to determine the total dry mass of the seedlings.

Rhizotron trials

Rhizotrons were constructed from two plexiglass (100 x 150 mm) plates that were separated by a silicon tube spacer (15 mm) and held together with butterfly nuts and filled with perlite. Seeds were treated as described for the pot trials. Rhizotrons were covered with aluminium foil in order to prevent daylight from reaching the roots of the seedlings, but the tops were kept open for seedling emergence. Rhizotrons were left in a glasshouse maintained at 22°C for 3 weeks.

(a) Root area and root length

Seedling roots were harvested at the soil surface and finely spread onto white paper and scanned into a computer. The images were captured and the averages of three root area and length measurements were taken per sample and the mean recorded using Assess 2.0 (Anonymous, 2008a).

(b) Root dry biomass

Roots were dried at 70°C for 48 hrs in an oven (Labotec, Model number 385, South Africa) and their dry weight determined.

Control of *Rhizoctonia* and *Pythium* using silicon and Eco-T®

Growth and preparation of *Rhizoctonia* and *Pythium*

Rhizoctonia and *Pythium* were sub-cultured onto potato dextrose agar (PDA) and stored at ± 25°C. Barley seeds were soaked overnight in distilled water in 500 ml Erlenmeyer flasks, drained and autoclaved at 121°C for 15 min on two consecutive days. Three squares (4 mm x 4 mm) of agar inoculated with either *Rhizoctonia* or *Pythium* were cut and placed onto the seeds. Flasks were incubated at 25°C until the seeds were fully colonised.

Inoculation

An inoculated *Rhizoctonia* barley seed was placed next to individual soybean seeds while one *Pythium* inoculated barley seed was placed 2 cm below each soybean seed.

Pot trials

Pot trials were the same as described for the growth promotion trials. Treatments used are listed in Table 2.

Rhizotron trials

Rhizotron trials were the same as described for the growth promotion trials. Treatments used are listed in Table 2.

Statistical analyses

All trials were repeated and results analysed using Genstat, 11th edition (Anonymous, 2008b).

Results

Pot trials

There was a significant increase in dry weight of the Si and Eco-T® combination, followed by individual applications of Si and Eco-T®, i.e., 22.45 g, 20.62 g and 19.44 g, respectively. The un-inoculated control and KOH treatments had the lowest biomass, 14.64 g and 16.04 g, respectively (Figure 1). Similar results were seen in the *Rhizoctonia* and *Pythium* trials, where the combination of Si and Eco-T® produced the highest biomass, 20.05 g and 19.74 g, respectively, and the inoculated control produced the lowest biomass, 7.45 g and 9.09 g, respectively. The KOH control had a lower dry weight than the Si treatments for the growth promotion as well as the *Rhizoctonia* and *Pythium* trials (Figure 1).

Rhizotron trials

(a) Root area and root length

Si plus Eco-T® produced the greatest root area in the growth promotion trials, 14.66 cm^2, while the control had the smallest root area, 7.32 cm^2 (Figure 2). The same pattern was seen for both the *Rhizoctonia* and *Pythium* trials where Si plus Eco-T® produced the highest root area, 8.98 cm^2 and 9.77 cm^2, respectively. The lowest root areas were seen for the inoculated control of *Rhizoctonia* (5.05 cm^2) and *Pythium* (5.74 cm^2) (Figure 2).

Silicon plus Eco-T® resulted in the highest root lengths in the growth promotion trial, as well as in the *Rhizoctonia* and *Pythium* trials with lengths of 143.25 cm, 131.20 cm and 140.93 cm, respectively (Figure 3). There were no statistically significant differences between any of the other treatments in the growth promotion trial. The KOH treatment did not show any statistically different results from the Si treatment for the *Rhizoctonia* trial and resulted in lower root areas and lengths for the *Pythium* trial.

(b) Root biomass

In the growth promotion trial, the application of Si plus Eco-T® produced the highest biomass, 0.22 g and the *Rhizoctonia* and *Pythium* trials had a statistically higher biomass, than the inoculated controls which had the lowest biomass, 0.032 g and 0.047 g, respectively (Figure 4). The KOH treatments were either statistically the same or lower than the Si plus Eco-T® treatments in both the growth promotion and *Rhizoctonia* and *Pythium* trials (Figure 4).

Discussion

The main objective of this trial was to investigate whether Si and *Trichoderma*, as individual treatments or in combination, had growth promotion properties as well as whether they could control *Rhizoctonia* and *Pythium* on soybeans. When Si plus Eco-T® were applied, the highest shoot biomass, root area, root length and root biomass were obtained.

This trial showed that Si plus Eco-T® have the potential to reduce damping-off and stunting caused by *Rhizoctonia* and *Pythium*. This treatment was more effective than when Si and Eco-T® were applied as individual treatments. The individual application of Si had a greater effect on growth promotion and control of *Rhizoctonia* and *Pythium* when compared to the KOH treatment, showing that Si caused the effect and not the potassium.

Different mechanisms of action of Si and *Trichoderma* may explain why the combination promoted plant growth as well as the control of *Rhizoctonia* and

Pythium. Silicon's mechanism of action is a result of both physical and physiological means. It can be deposited into the epidermal layer of plant cells, forming a mechanical barrier against pathogen penetration (Kim *et al*., 2002). It has also been shown to play a part in plant resistance to both biotic and abiotic stresses (Ma & Yamaji, 2006; Keeping & Kvedaras, 2008). Samuels *et al*. (1991) and Marschner (1995) showed that Si is able to alter signals in the host and activates the plant defence mechanisms much faster and more extensively.

Trichoderma has a wide range of applications due to its various antagonistic mechanisms to pathogens (Elad, 2000). *Trichoderma* spp. have been reported to effectively control several plant pathogens (Koch, 1999; Zhang *et al*., 1999). The results from the biological control trials in this study supported the findings of Koch (1999), Lewis & Lumsden (2001) and Tronsmo & Hjeljord, (1998) that *Trichoderma* spp. formulations are able to effectively reduce damping-off of *Rhizoctonia* as well as act as a growth promoter.

Silicon plus *Trichoderma,* in the commercial form of Eco-T®, were able to increase root area, root length and root biomass as well as shoot biomass of the soybean plants that were infected with *Rhizoctonia* and *Pythium*, as well as enhance plant growth in the absence of either pathogen.

This trial was also conducted on canola and lupins. Silicon (200 mg l^{-1}) in combination with Eco-T® once again produced the highest shoot biomass, root area, root length and root biomass (unpublished results).

Field trials are needed to calculate the effectiveness of *Trichoderma* and Si for growth promotion and control of *Rhizoctonia* and *Pythium* on soybeans to help increase yields.

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Tables

Table 1 Treatments used to determine growth promotion of soybeans

Treatments
200 mg l^{-1} Si
Eco-T®
200 mg l^{-1} Si and Eco-T®
Control
KOH

Si – silicon

Eco-T® - *Trichoderma harzianum*

KOH – Potassium hydroxide

Table 2 Treatments used to determine the effect of silicon and *Trichoderma* (Eco-T®) on soybean infected with *Rhizoctonia* and *Pythium*

Treatments
200 mg l^{-1} Si and *Rhizoctonia/Pythium*
Eco-T® and *Rhizoctonia/Pythium*
200 mg l^{-1} Si and Eco-T® and *Rhizoctonia/Pythium*
Rhizoctonia/Pythium (inoculated control)
KOH and *Rhizoctonia/Pythium*
Un-inoculated control

Si – silicon

Eco-T® - *Trichoderma harzianum*

KOH – Potassium hydroxide

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Figure 2 Root area of three week old soybean seedlings after the application of six treatments. Means followed by different letters are significantly different at $P \leq 0.05$.

Figure 3 Root length of three week old soybean seedlings after the application of six treatments. Means followed by different letters are significantly different at $P \leq 0.05$.

Figure 4 Dry root biomass of three week old soybean seedlings after the application of six treatments. Means followed by different letters are significantly different at $P \leq 0.05$.

Figures

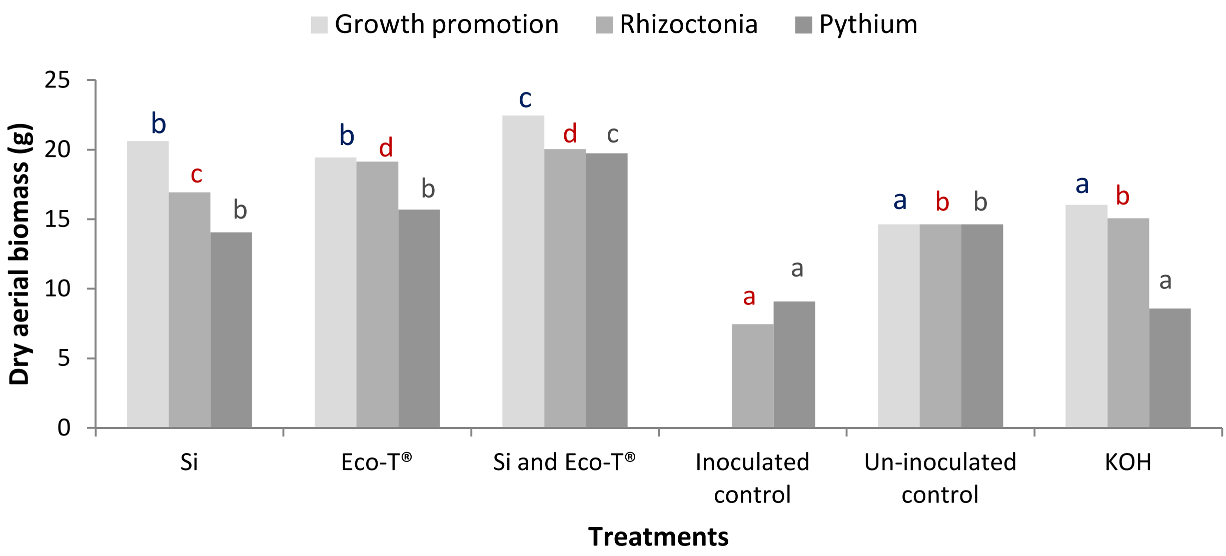
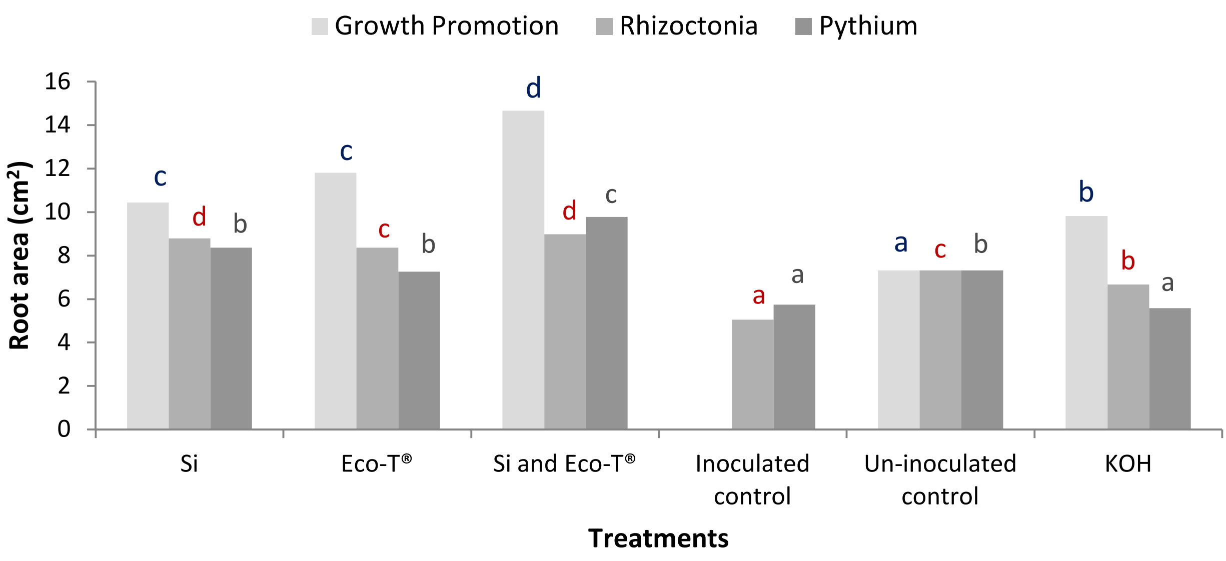


Figure 1.



Growth Promotion
Rhizoctonia
Pythium
Root area (cm²)
16
14
12
10
8
6
4
2
0
c
d
b
c
c
b
d
d
c
a
a
a
c
b
b
b
a
Si
Eco-T®
Si and Eco-T®
Inoculated control
Un-inoculated control
KOH
Treatments

Figure 2.

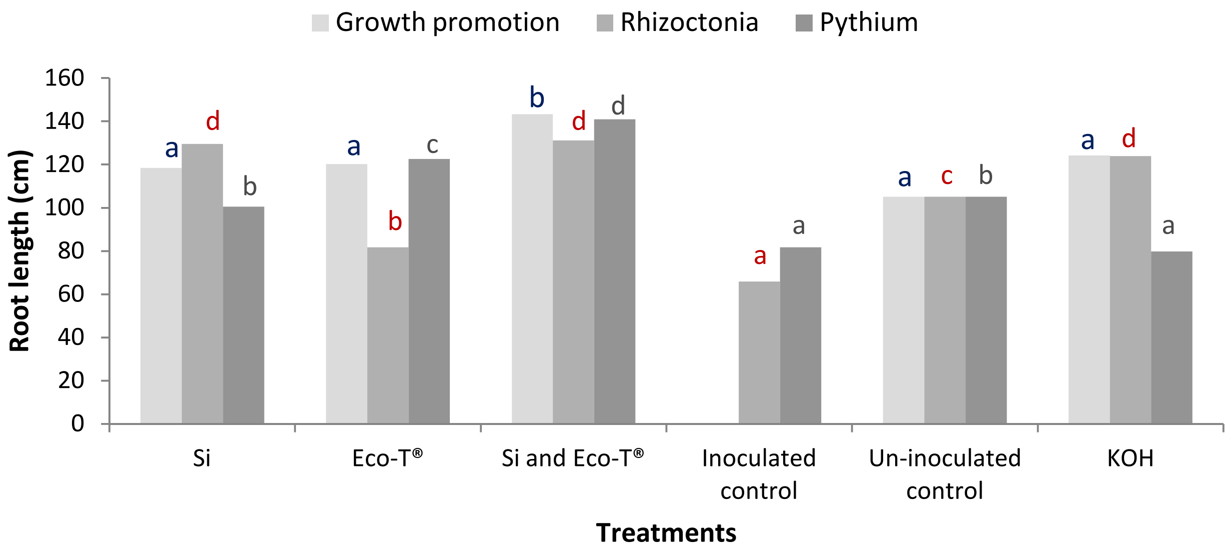
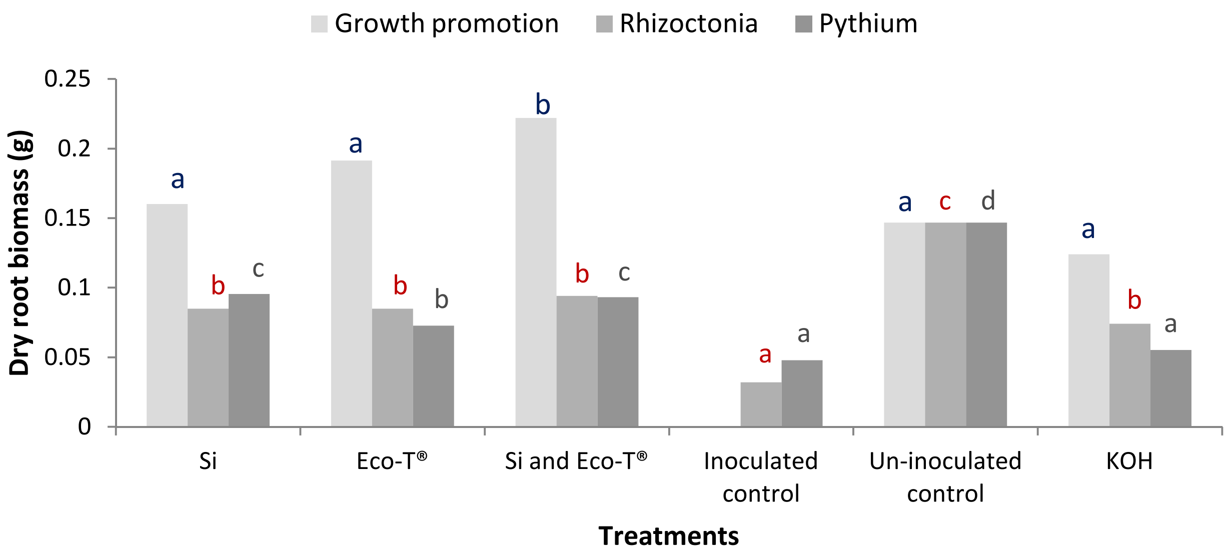

Growth promotion
Rhizoctonia
Pythium
Root length (cm)
160
140
120
100
80
60
40
20
0
a d b
a b c
b d d
a a
a c b
a d a
Si
Eco-T®
Si and Eco-T®
Inoculated control
Un-inoculated control
KOH
Treatments

Figure 3.



Growth promotion
Rhizoctonia
Pythium
Dry root biomass (g)
0.25
0.2
0.15
0.1
0.05
0
a
b
c
a
b
b
b
b
c
a
a
a
c
d
a
b
a
Si
Eco-T®
Si and Eco-T®
Inoculated control
Un-inoculated control
KOH
Treatments

Figure 4.