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Evaluation of Trichoderma harzianum, Bacillus megaterium, *Composted Citrus Waste and Soil Solarization on Avocados with and without* Phytophthora cinnamomi *Preliminary Results*

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ABSTRACT

This progress report constitutes preliminary findings on the application of Trichoderma harzianum *and* Bacillus megaterium *evaluated as multiple applications for the control of* Phytophthora *root rot of avocado seedlings in the greenhouse. These antagonists were also applied in various combinations with composted citrus waste and soil solarization in young avocado orchards to evaluate their effect on trees. Multiple applications were made with the purpose of simulating continuous application of antagonists through the irrigation system, in contrast with single application of bio-control agents. Multiple applications of* T. harzianum *eliminated the effect of* Phytophthora *in the greenhouse, whereas* B. megaterium *was ineffective. In the field, the best effect on plant growth was obtained with* T. harzianum *in combination with solarization, followed by application of 4kg composted citrus waste per tree*

UITTREKSEL

Hierdie verslag gee die voorlopige resultate van die effek van Trichoderma harzianum *en* Bacillus megaterium *in meervoudige toedienings vir die beheer van* Phytophthora *wortelvrot op avokado saailinge. Hierdie antagoniste is ook saam met gekomposteerde sitrus skil en solarisasie in 'n nuwe avokado aanplanting in die veld toegedien, om die effekte op Jong borne te evalueer. Die doel van die meervoudige toediening van antagoniste was om die moontlikheid van toediening in die besproeiing sisteem te simuleer. Veelvoudige toediening vans* T. harzianum *het die effek van* Phytophthora *cinnamomi in die glashuis onderdruk.terwyl* B. megaterium *geen effek getoon het nie. In die veldproef het* T. harzianum + *solarisasie die beste plant groei gelewer, gevolg deur 4kg kompos per boom.*

INTRODUCTION

At present, root rot control of avocados is heavily dependent on the use of phosphonates. In view of the danger of possible breakdown of efficacy, it is imperative that alternative methods of control be developed. Biological control is one possibility. This does not only imply application of antagonists, but also augmentation of the soil with organic material such as compost which stimulates plant growth in addition to the suppression of pathogens (Hoitink & Fahy, 1986; Hoitink *et a*l., 1991; Lumbsden *et al,*

1983). Furthermore compost can act as a carrier substrate for antagonists to proliferate and survive (Papavizas, 1985; Lewis & Papavizas, 1985).

The aim of this study was to:

• evaluate multiple applications of antagonists, thereby simulating continuous application via the irrigation system, and

• evaluate citrus waste compost and soil solarization in comparison with antagonist applications.

This progress report only reflects preliminary results showing the effect of treatments on plant growth.

MATERIALSAND METHODS

Greenhouse study

For each treatment carrier substrate was mixed with nursery growth media (3:1 composted pine bark: sand). Five replicates were used for each treatment. *P. cinnamomi* inoculum, used for this study was prepared as described by Duvenhage *et al.* (1991). The mycelium was macerated for 10 seconds in an Ultra Turrax and added at a rate of 0,1% v/v *P*. *cinnamomi* to the nursery mix. *Persea Americana* seedlings cv. Duke 7 were planted in the inoculated and uninoculated nursery mix and watered twice a week for 15 weeks. *T. harzianum* (C4) and *B. megaterium* (B4) (on their own) were applied weekly for four weeks and thereafter every consecutive week (total of eight applications). The mixture of *T. harzianum* and *B. megaterium* were applied in the same manner as the individual antagonists by alternating every consecutive week (total four applications for each antagonist). After three months the seedlings were evaluated for shoot length and stem thickness.

Treatments were as follows:

A *T. harzianum*

B *T. harzianum* and *B. megaterium* (Mixture)

C *B. megaterium*

D *T. Harzianum* + *P. cinnamomi*

E Mixture + P *cinnamomi*

F *B. megaterium*+ *P. cinnamomi*

G Composted pine bark + *P. Cinnamomi*

H Composted pine bark

Field study

T. harzianum and *B. megaterium* were cultured in a fermentar tank for three weeks, in a liquid medium comprising 30g/l molasses (Ferminore) and 5g/l yeast extract (Bio Lab) according to Papavizas, *et al,* 1984. *Trichoderma* was cultured at pH 4 and *Bacillus* at

pH 6. The final concentration of *Trichoderma* was 2 x 10^6 colony forming units/ml and *Bacillus 2,6* x 10^8 colony forming units/µm. Solarization was done for three weeks under clear poly-ethylene plastic sheets of 30 µm thickness (Plastall Gundle, P. 0. Box 746, Germiston 1400). *T. harzianum* and *B. megaterium* were poured around each tree at one ℓ/tree and mixed with the soil.

Citrus waste was obtained from Letaba Citrus Processors, Tzaneen. The waste comprised the residue remaining after juice extraction and consists of citrus peel, membranes, juice vesicles and seeds. Composting was performed on a semi-commercial scale under shelter. Calcium hydroxide was added at the rate of 15 kg/ton, and mixed thoroughly with the citrus waste before piling. Active aeration and moisture were provided when the moisture content dropped below 40%. The composted citrus waste was added at the various rates/tree as indicated below, and incorporated into the soil.

The treatments were as follows:

A 2kg composted citrus waste + Solarization

B 4kg composted citrus waste + Solarization

C 8kg composted citrus waste + Solarization

D Control + Solarization

E *T. harzianum* + Solarization

F *B. megaterium* + Solarization

G 2kg composted citrus waste

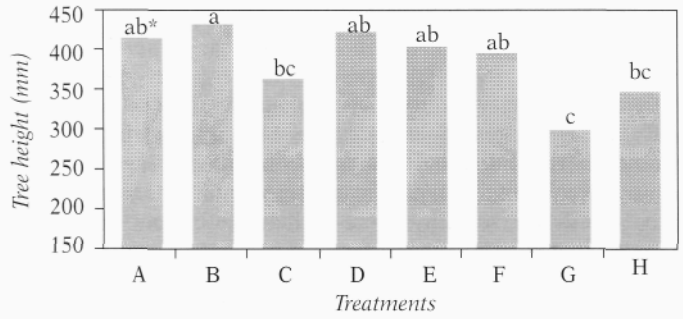
H 4kg composted citrus waste

I 8kg composted citrus waste

J Control (untreated)

K *T. harzianum*

L *B. megaterium*



* Values followed by the same letter do not differ significantly according to the Manova test (P = 0.05)

A *T. harzianum*
B *T. harzianum* and *B. megaterium*
C *B. megaterium*
D *T. harzianum* + *P. cinnamomi*
E *T. harzianum* and *B. megaterium* + *P. cinnamomi*
F *B. megaterium* + *P. cinnamomi*
G *P. cinnamomi*
G Control (uninoculated)

Figure 1
Effect of multiple applications of antagonists for control of *Phytophthora cinnamomi* on avocado seedlings in the greenhouse

RESULTS and DISCUSSION

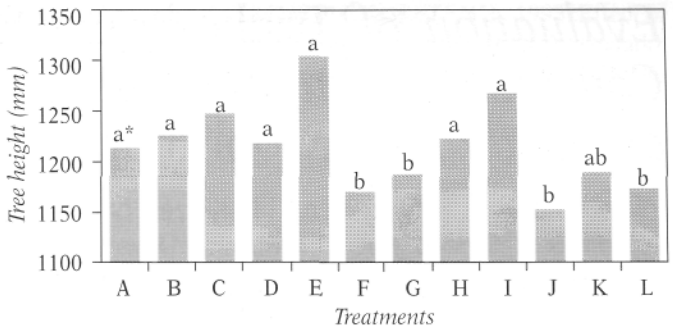
Greenhouse study

The data (figure 1) indicates that *T. harzianum* on its own and in combination with *B. megaterium* eliminated the effect of *P. cinnamomi* on the trees, whereas *B. megaterium* on its own was ineffective. There was also a tendency for the *T. harzianum* to stimulate plant growth although this was not statistically significant.

Field Study

The greatest effect on plant growth was obtained with *T. harzianum* in combination with Solarization (figure 2). The second best effect was obtained with 4kg composted citrus waste per tree. Poorest growth (equal to untreated control) was observed with 8kg of composted citrus waste, and the treatments with *B. megaterium.* Poor growth with the high rate of composted citrus waste might indicate phytotoxicity.

The growth stimulation obtained with *T. harzianum* in this study corresponds with previous reports by Hoitink *et al.,* 1991;



* Values followed by the same letter do not differ significantly according to the Manova test (P = 0.05)

A 2kg composted citrus waste + solarization
B 4kg composted citrus waste + solarization
C 8kg composted citrus waste + solarization
D Control + solarization
E *T. Harzianum* + solarization
F *B. Megaterium* + solarization
G Control (intreated)
H 2kg composted citrus waste
I 4kg composted citrus waste
J 8kg composted citrus waste
K *T. Harzianum*
L *B. Megaterium*

Figure 2
Effect of antagonists and organic amendments on the growth of avocado seedlings (Duke 7) in the field

Lumbsden *et al., 1983;* Spencer & Benson, 1982; and Chang *et al.,* 1986. The multiple application of antagonist during this study was effective, indicating potential for field application through the irrigation system.

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