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Optimization of Biological Control of Phytophthora Root Rot of Avocados

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ABSTRACT

Choosing a delivery system is important when optimizing a biological control agent, as it plays an important role in the effectiveness of the antagonist. Four carrier substrates (millet seed, peat, composted citrus waste and composted pine bark) were tested for sustaining antagonist growth at an optimum moisture level and controlling *Phytophthora cinnamomi* root rot in avocados. Highest colonization was obtained with millet seed at 60 % water content.

INTRODUCTION

Avocado root rot caused by *Phytophthora cinnamomi* Rands is considered the most important root disease in avocados throughout the world (Broadbent & Baker, 1974; Kotzé *et al.,* 1987; Zentmyer, 1980). Although root rot is under control due to the use of fungicides, the Avocado Plant Improvement Scheme (Partridge, 1984) and resistant root stocks (Kotzé *et al.,* 1987), the pressure against the use of fungicides and fear of resistance to phosphonates, have resulted in a shift towards biological control (be) (De Waard *et al.,* 1993; Duvenhage & Kotzé, 1993; Van der Merwe, 1992). Duvenhage *et al.,* (1991) showed that micro-organisms isolated from suppressive soils can suppress the disease in avocado seedlings (Duvenhage & Kotzé, 1993). The next step is finding a suitable method for delivering the potential be agents (Campbell, 1989) to the rhizosphere and allowing them to proliferate in the soil (Cook & Baker, 1983). The be agents must then establish themselves and advance with the feeder roots (Chao *et al.,* 1986; Coffey, 1987). Choosing an effective delivery system is important in optimizing biological control. Using a carrier substrate as food base has been shown to play an important role in the effectiveness of the antagonist (Papavizas, 1985). It is important to ensure that a viable agent, which is able to proliferate, can be incorporated in the soil (Lewis & Papavizas, 1984; Papavizas, 1985).

The aim of this study was:

- to find a suitable carrier substrate for *Trichoderma harzianum* that supports maximum growth of the antagonist; and
- to evaluate the uncolonized carrier substrates for their intrinsic ability to suppress root rot.

MATERIALS AND METHODS

Colonization of carrier substrates

Each carrier substrate was tested for its ability to sustain antagonist growth at the optimum moisture level. Four carrier substrates were tested, namely millet seed, peat, composted citrus waste and composted pine bark (Bark Enterprises, Brits). The moisture levels tested on each carrier were 20, 40, 60 and 80 % water content, and each treatment was replicated three times. After sterilization the medium was inoculated with a spore suspension of *T. harzianum* (2,1 x 10^7 conidia/ml water). After three weeks' incubation at room temperature, growth of *T. harzianum* was determined by means of a tenfold serial dilution on half-strength potato dextrose agar (Biolab). Colonies were counted and tabulated.

Root rot control

Uncolonized carrier substrates were evaluated for suppression of *P. cinnamomi* root rot on avocado seedlings in the greenhouse.

Combinations of the following carrier substrates were used: millet seed, peat, composted citrus waste and composted pine bark (table 2). For each treatment 1,7 % w/v carrier substrate was mixed with nursery mix (3 : 1, composted pine bark : sand). Five replicates were used for each treatment. One week old cultures of *T. harzianum* were used to inoculate carrier substrates. After inoculation with the antagonists, substrates were incubated for four weeks at room temperature. *P. cinnamomi* inoculum used for this study was prepared as described by Duvenhage *et al* (1991) and was macerated for 10 seconds in an Ultra Turrax and added at a rate of 0,1 % v/v *P. cinnamomi* mycelium to the nursery mix. *Persea americana* seedlings cv Edranol were planted in the inoculated and uninoculated nursery mix and watered twice a week for 15 weeks. After 15 weeks, the seedlings were evaluated for root rot, new root growth, as well as fresh and dry mass of roots and leaves. Root rot and new growth were both rated on scales of 0100 (0 = zero root rot/zero new growth; 100 = total root rot/total new growth).

RESULTS

Colonization of carrier substrates

Millet seed rendered the highest number of colony-forming units/ml at 60 % water content, followed by composted pine bark, which showed the widest range of growth with the maximum number of colony-forming units/ml at both 60 % and 80 % water content.

Table 1

Growth of *T. harzianum* measured on various carrier substrates (number of colony-forming units/ml) at various moisture levels

Carrier substrate	*Moisture levels (% water)*			
	20	*40*	*60*	*80*
Composted pine bark	$4,9 \times 10^4$	$2,0 \times 10^3$	$3,6 \times 10^6$	$2,9 \times 10^6$
Peat	$1,9 \times 10^4$	$3,0 \times 10^2$	$4,5 \times 10^3$	$1,0 \times 10^4$
Composted citrus waste	$4,0 \times 10^2$	zero	$1,2 \times 10^5$	$2,7 \times 10^5$
Millet Seed	$5,4 \times 10^4$	$1,0 \times 10^2$	$5,5 \times 10^6$	$4,0 \times 10^4$

Root rot control

Although there was no significant control of root rot with uncolonized carrier substrates, there was a tendency for uncolonized composted citrus waste as well as millet seed and peat to suppress root rot (table 2). Peat on its own stimulated the greatest amount of new root development, followed by sterile nursery mix.

Table 2

Effect of uncolonized carrier substrates on root rot caused by *P. cinnamomi* (*Pc*) on Edranol seedlings in the greenhouse

Uncolonized carrier substrates	*Fresh mass*		*Dry mass*		*% Root rot*[1]	*% New root growth*[2]
	Roots	*Leaves*	*Roots*	*Leaves*		
Composted citrus waste	$7,62^{def3}$	$14,7^{bcd}$	$6,72^{efg}$	$8,48^{bcde}$	46^{bc}	43^{cd}
Millet seed	$18,2^{abc}$	$17,2^{bcd}$	$16,46^{abcd}$	$12,38^{abc}$	$28,2^{cde}$	56^{abcd}
Peat	$15,96^{abcde}$	$20,1^{ab}$	$13,64^{abcdef}$	$10,44^{abcde}$	$6,8^{e}$	78^{a}
Composted citrus waste + peat	$17,02^{abcd}$	$17,62^{bcd}$	$9,56^{cdefg}$	$8,94^{bcde}$	28^{cde}	62^{abc}
Millet seed + peat	$13,44^{bcdef}$	$19,44^{abc}$	$11,64^{bcdef}$	$13,3^{ab}$	33^{cde}	54^{abcd}
Sterile nursery mix	$19,08^{ab}$	$19,72^{abc}$	$16,86^{abc}$	$11,94^{abcd}$	$8,8^{e}$	64^{abc}
Composted citrus waste + Pc	$17,16^{abcd}$	$26,4^{a}$	$13,52^{abcdef}$	$14,76^{a}$	84^{a}	$8,4^{e}$
Millet seed + Pc	$7,44^{def}$	$10,92^{cd}$	$6,42^{efg}$	$6,6^{de}$	100^{a}	0^{e}
Peat + Pc	$10,66^{bcdef}$	$11,58^{bcd}$	$9,38^{cdefg}$	$7,76^{bcde}$	100^{a}	0^{e}
Composted citrus waste + peat + Pc	$7,66^{def}$	$14,26^{bcd}$	$6,96^{efg}$	$11,4^{abcde}$	100^{a}	0^{e}
Millet seed + peat + Pc	$12,38^{bcdef}$	$19,02^{abc}$	$10,48^{cdefg}$	$10,98^{abcde}$	$88,6^{a}$	7^{e}
Sterile nursery mix + Pc	$6,84^{def}$	$9,76^{d}$	$5,98^{efg}$	$7,78^{bcde}$	$95,2^{a}$	$0,8^{e}$

[1]Assessed on a 0–100 scale (0 = healthy; 100 = total root rot)
[2]Assessed on a 0–100 scale (0 = no new root growth; 100 = all new root growth)
[3]Numbers followed by the same characters do not differ statistically according to Duncan's multiple range test (P = 0,05). Treatments for each column were analysed separately.

DISCUSSION

The carrier substrates used for cultivation of the antagonist and application in the greenhouse was ineffective when used for control of *P. cinnamomi* on avocado seedlings. The general tendency for better performance of composted citrus waste and millet seed + peat can be attributed to an inherent ability of compost to have beneficial effects on plant growth (Hoitink, *et al.,* 1991; Lumbsden, *et al.,* 1983; Spencer & Benson, 1982). Hadar, *et al., (*1979) tested various substrates of which wheat bran proved to be the best medium for growth and sporulation of *T. harzianum.* However, wheat bran induced phytotoxic symptoms in avocado seedlings (Hadar *et al.,* 1979).

The role of pH needs to be investigated; because Sivan *et al.,* (1984) found that pH of the carrier substrate can have an effect on effectivity. Lewis & Papavizas (1984) grew T. hamatum in 1 ℓ fermentors containing molasses yeast medium simulating industrial conditions were the fermentor product is mixed with the carrier substrate. Preliminary experimentation with *T. harzianum* in a small-scale fermentor is being conducted now.

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