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Full Length Research Paper

Application of selected biological control agents in conjunction with tolclofos-methyl for the control of damping-off caused by *Rhizoctonia solani*

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Selected *Trichoderma* and *Bacillus* isolates were tested as seed treatments with reduced concentrations of tolclofos-methyl against *Rhizoctonia solani* damping-off using cucumber as test plants. *In vitro* bioassays using filter paper disc infused with six concentrations [active ingredient (ai)] of tolclofos-methyl (0.005, 0.05, 0.125, 0.25, 0.5 and 1.5 g ai/l) showed that growth of *Trichoderma* isolates on agar plates were less inhibited at 0.005 and 0.05 g ai/l than 0.125 – 1.5 g ai/l tolclofos-methyl concentrations while *Bacillus* isolates were not affected by any of the six concentrations. In a greenhouse study with a 0.05 g ai/l tolclofos-methyl concentration, *Trichoderma* and *Bacillus* isolates applied alone or in combination to cucumber seeds resulted in a better disease control than the *Trichoderma* and *Bacillus* treatments used alone. As high as 86% disease control was achieved by combining 0.05 g ai/l tolclofos-methyl with *T. harzianum* Isolate SYN which was significantly better (P = 0.001) than the 0.05 g ai/l tolclofos-methyl control. In all cases, additive effects of combining 0.05 g ai/l tolclofos-methyl with *Trichoderma* and *Bacillus* treatments were observed.

Key words: Active ingredient (ai), *bacillus*, damping-off, tolclofos-methyl, *Trichoderma.*

INTRODUCTION

Rhizoctonia solani Kühn is a soil borne plant pathogen and has a propensity for attacking agricultural horticultural crops throughout the world. It is one of the most important pathogens causing pre- and post-emergence damping-off (Kloepper, 1991), brown-girdling and seedling blight (Benhamou and Chet, 1993) in several crops including vegetables, ornamentals, nursery and greenhouse crops causing huge economic losses to growers (Baker, 1970).

Diseases caused by *R. solani* are usually controlled by the use of fungicides. Examples include pentachloronitrobenzene (PCNB), metalaxyl and toclofos-methyl. These are mostly used as seed treatments to increase seed germination and to protect young seedlings. However, tolerance to some of these fungicides, example PCNB, has been reported in some *R. solani* isolates (Shatla and Sinclair, 1963). In addition, it has also been reported that some fungicides vary in effectiveness, among and within anastomosis groups of *R. solani* isolates (Kataria et al., 1991a,b). This suggests that extensive use of fungicides could result in development of resistance as has been reported to occur in other plant pathogen populations.

Biological control agents (BCAs) are continuously being tested, developed and used in an effort to control various soil-borne plant pathogens. Various reports in the literature indicate that a number of biological control agents can control plant pathogens with efficiencies equal to that of available chemical pesticides (Koch, 1999). However,

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Abbreviations: ai, Active ingredient; **CFU**, colony forming unit; **PCNB**, pentachloronitrobenzene; **BCAs**, Biological control agents.

one of the most serious short-falls of biological control is that in most cases the control rendered is not equivalent to chemical control or is inconsistent (Guetsky et al., 2001). In order to overcome these effects, combinations of chemical control (reduced levels of chemicals) and biological control has been sought. The combined use of biological control agents (BCAs) and fungicides has attracted much attention in order to obtain synergistic or additive effects against target organisms (Locke et al., 1985; Elad et al., 1993; Raupach and Kloepper, 2000; Clarkson et al., 2006).

Tolclofos-methyl has been reported to control bottom rot disease of lettuce caused by *R. solani* (Coley-Smith et al., 1991). It is also currently being used in countries such as South Africa to control damping-off caused by *R. solani* on vegetable seedlings and tuber crops such as potato (*Solanum tuberosum* L.) (M.D. Laing, Discipline of Plant Pathology, University of KwaZulu-Natal, South Africa, personal communication). However, it is known to have long residual in soils, especially in clay soils. Due to the difficulty in effectively controlling diseases caused by *R. solani*, alternative control measures such as the use of lower doses of fungicides in conjunction with BCAs has been sought. These are applied simultaneously or in an alternating application of BCAs and fungicides. Consequently, the aim of this study was to determine the efficacy of single and combined inoculations of selected *Trichoderma* and *Bacillus* isolates in conjunction with tolclofos-methyl as a unified system to control damping-off caused by *R. solani*. Cucumber was used as a benchmark vegetable in this study due to its susceptibility to a wide range of soil-borne pathogens.

MATERIALS AND METHODS

Sources of fungal, bacterial isolates and fungicide

Four *Trichoderma* isolates, *T. atroviride* P. Karsten (Isolates SY3A and SYN6), *T. harzianum* Rifai (Isolate SYN) and a commercial strain, Eco-T® (active ingredient *T. harzianum*) and two *Bacillus* isolates (B69 and B81) were used in this study. The *Trichoderma* and *Bacillus* isolates were used due to their biological control effect in previous greenhouse studies (Kubheka, 2003; Yobo et al., 2004). The fungicide, tolclofos-methyl (500 g ai/kg of tolclofos-methyl) obtained from Philagro South Africa [Pty] Ltd., Republic of South Africa was used in this study. A virulent strain of *R. solani*, isolated from diseased cabbage seedling was identified and used for this study.

Preparation of *Trichoderma* and *Bacillus* inoculants

The *Trichoderma* isolates were all grown and formulated by Plant Health Products, Nottingham Road, South Africa, according to a protocol used for the commercial production of Eco-T® (*T. harzianum*). Kaolin was used as a carrier and each of the formulated isolates contained approximately 10^8 spores/g (M. Morris, Plant Health Products, South Africa) personal communication.

Each of the *Bacillus* isolates was grown separately in 250 ml conical flasks containing 100 ml of sterilized tryptone soy broth (Merck, Darmstadt Germany) medium. Each flask was inoculated with a loopful of the respective *Bacillus* sp. isolate cultured on tryptone soy agar (Merck) (30°C, 48 h). Inoculated flasks were incubated at 30°C for 72 h in a water bath shaker at 150 rpm (GFL® 1083, Germany). Cell suspensions were centrifuged at 9000 g for 20 min (Beckman J2 HS centrifuge). Resulting pellets were washed twice by resuspending in sterile distilled water and centrifuged two times. The final pellets were each dissolved in sterile distilled water to approximately 500 ml. Cell numbers were determined by dilution plate technique and adjusted to approximately 10^9 cfu/ml for each of the *Bacillus* isolates.

Source of cucumber seeds

Seeds of cucumber (*Cucumis sativus* L.) cv. Ashley were obtained from Starke Ayres Seed Company Ltd., Republic of South Africa. An appropriate number of cucumber seeds (which had previously been treated with the fungicide thiram) were washed with distilled water seven times to considerably reduce fungicide residues on seeds. The washed seeds were air-dried under laminar flow bench overnight and tested with the pathogen in the greenhouse before use.

In vitro effect of tolclofos-methyl on growth of *Trichoderma* and *Bacillus* isolates

Six concentrations of tolclofos-methyl (0.005, 0.05, 0.125, 0.25, 0.5 and 1.5 g ai/l) were used to evaluate the effect of tolclofos-methyl on growth of the *Trichoderma* and *Bacillus* isolates and *R. solani*. One mycelial plug (4 mm diameter, cut from the actively growing edge of a 3-day-old mycelial mat on V8 agar) of the respective *Trichoderma* isolates and *R. solani* were placed in the centre in each of seven plastic Petri dishes (90 mm diameter) containing V8 agar medium and carefully inverted. The six tolclofos-methyl concentrations (calculated g ai/l), were separately prepared in distilled water. Whatman No.1 filter paper discs (90 mm diameter) were separately dipped in each of the six tolclosfos-methyl concentrations and placed on the lids of the inverted plastic Petri dishes. Plates were sealed with Parafilm and incubated for 5 days at 28°C in the dark after which diameter of the colony was measured for each treatment. The seventh plate was used as a control lacked tolclosfos-methyl impregnated filter paper discs but were provided with filter paper disk dipped in distilled water. There were four replicates for each isolate x tolclofos-methyl concentration and the experiment was repeated once.

For the *Bacillus* isolates, 0.2 ml of each bacterial suspension was evenly spread on the surface of tryptic soy agar (TSA, Merck, Darmstadt Germany) in disposable Petri dishes using a sterile bent glass rod. The inoculated plates were allowed to dry on a laminar flow bench for 1 h. They were then exposed to tolclosfos-methyl as described above. Control plates were provided with filter papers dipped in sterile distilled water. There were four replicates for each treatment and the experiment was repeated once. Plates were incubated at 30°C for 3 days and compared to control plates. Bacterial growth was scored as equal to or less (percentage) than the control by comparing colony counts to the distilled water control plates.

Seed treatments procedure: *Trichoderma, Bacillus* isolates and their combinations

Kaolin formulations of each *Trichoderma* isolate (4 g) were separately mixed with 2% (w/v) sterile carboxymethyl cellulose (CMC) sticker suspensions (20 ml) a slurry. Approximately 200 seeds were mixed into each slurry suspension, allowing for a 30 min contact period before being removed and air-dried on a laminar

flow bench for 12 - 18 h prior to use. Inoculant densities were determined using a dilution plating technique with an average of 10^6 cfu/seed being achieved.

Bacillus isolate seed treatments were prepared as described above for the *Trichoderma* isolates. An average inoculant density 10^6 - 10^7 cfu/seed for each *Bacillus* isolate was achieved.

For each of the *Trichoderma*-*Bacillus* combination treatment, *Trichoderma* formulations were separately mixed with the respective *Bacillus*-CMC suspension prior to seed treatment.

Seedling Trial 1: Control of *R. solani* damping-off using three tolclofos-methyl concentrations in combination with *Trichoderma* and *Bacillus* isolates

Three tolclofos-methyl concentrations chosen from the *in vitro* studies were tested in combinations with *Trichoderma* and *Bacillus* isolates in the greenhouse. Based on the results obtained, one of the lower concentrations which completely inhibited *R. solani* growth *in vitro* was chosen for further greenhouse studies.

A completely randomized design with three replicates and 48 treatments was used to determine seedling survival. Seedling trials were carried out in Speedling® trays (24 cells per tray; 37 x 37 mm wide and 61 mm deep). The trays were half filled with composted pine bark (Potting Mix, Gromed, Republic of South Africa) and infested with *R. solani* by placing 4 mm square V8 agar plugs (completely colonized with the pathogen) in the centre of each cell directly on top of the growth medium. The trays were filled and the treated seeds planted. Prior to covering of seeded trays, they were drenched with the three tolclofos-methyl concentrations (three seeded trays per concentration per BCA treatment, each cell receiving 1 ml of tolclofos-methyl). Seeds treated with combinations of *Trichoderma* and *Bacillus* isolates also received tolclofos-methyl applications. Controls were seeds coated solely with kaolin and were treated as follows:

1) Tolclofos-methyl control infested with *R. solani* and drenched with the three different fungicide concentrations (0.005, 0.05, 0.125 g ai/l);
2) Manufacturer's recommended tolclofos-methyl concentration (0.5 g ai/l) infested with *R. solani* and drenched with 1 ml per cell tolclofos-methyl;
3) Non-infested tray that received 4 mm agar plugs without *R. solani* or tolclofos-methyl application but drenched with 1 ml of water.
4) Infested tray that received *R. solani* plugs and 1 ml of water in place of a tolclofos-methyl drench.

The trays were left in a germination room at 20 - 24°C (70 - 75% rh) for 2 days and then moved to a polycarbonate greenhouse tunnel maintained between 22 - 26°C. Trays were irrigated three times a day by microjet overhead irrigation (Inverted mini wobbler, Sennenger, U.S.A). Irrigation water contained NPK soluble fertilizer [3:1:3 (38)] at a rate of 1 g/l and maintained at 20°C using a temperature controlled heating system (Pro Heat 2000 Plus, Republic of South Africa). Number of surviving seedlings was counted after 4 weeks. Plants were cut at the soil line and dried at 70°C for 48 h and after which the total dry weight of seedlings per tray was determined. The experiment was repeated twice.

Seedling Trial 2: Control of *R. solani* damping-off using one tolclofos-methyl concentration in combination with *Trichoderma* and *Bacillus* isolates

A completely randomized design with three replicates and 18 treatments was used. Infestation of the growth medium and planting procedure were as described for Seedling Trial 1. Each treatment was planted into six Speedling® trays. Three seeded trays were drenched with tolclofos-methyl (0.05 g ai/l) as described for Seedling Trial 1 and the remaining three trays were drenched with 1 ml of water. Four control treatments were established:

1) Tolclofos-methyl control infested with *R. solani* and treated with 1 ml per cell of 0.05 a.i g/l tolclofos-methyl concentration. The other three controls were as established for Seedling Trial 1 in (ii), (iii) and (iv).

The trays were further treated as described under Seedling Trial 1. Number of surviving seedlings was counted after 4 weeks, after which the total dry weight of seedlings per tray was determined. The experiment was repeated twice.

Statistical analysis

SAS (SAS, 1987) was used to run linear regression on data for Seedling Trial 1. The three fungicide concentrations were regressed against percentage seedling survival for the various treatments. A general linear model (GLM) was used to run an Analysis of Variance (ANOVA) on all data collected. If the ANOVA was significant, ($P \leq 0.05$), means were separated using the Students Newman Keul's (SNK) test.

RESULTS

In vitro effect of tolclofos-methyl on growth of *Trichoderma* and *Bacillus* isolates

All *Trichoderma* isolates were less sensitive than *R. solani* to the active ingredient of the six tolclofos-methyl concentrations tested (Table 1). However, colony growth of the *Trichoderma* isolates were reduced as concentrations of tolclofos-methyl was increased. *T. atroviride* SY3A and *T. atroviride* SYN6 both exhibited tolerance to tolclofos-methyl at 0.05 g ai/l. Concentrations as high as 1.5 g ai/l tolclofos-methyl did not completely inhibit the *Trichoderma* isolates tested (Table 1). At a concentration of 0.005 g ai/l, tolclofos-methyl reduced colony growth of *R. solani* by 53.7% compared to the *R. solani* control, whereas concentrations of 0.05 and 0.125 g ai/l completely inhibited *R. solani* growth. None of the tolclofos-methyl concentrations, compared to water controls affected growth of the *Bacillus* isolates (data not presented).

Seedling Trial 1: Control of *R. solani* damping-off using three tolclofos-methyl concentrations in combination with *Trichoderma* and *Bacillus* isolates

A significant ($P = 0.001$) percentage increase (54.88 – 75.71%) in the number of surviving cucumber seedlings was recorded for the tolclofos-methyl controls as concentrations were increased (Table 2).

The overall general linear relationship of surviving seedlings across all treatments shows a significant increase ($P = 0.0001$) in the number of surviving seedlings with increase in tolclofos-methyl concentration (Table 2). Of all single and combined *Trichoderma* and *Bacillus* treatments, only Eco-T® in combination with tolclofos-methyl concentrations showed a significant

Table 1. *In vitro* effects of tolclofos-methyl concentrations (g ai/l) on growth of *Trichoderma* and *R. solani* after 5 days of incubation at 28°C.

Isolates	Tolclofos-methyl concentrations (g ai/l)/diameter of colony growth (mm) of respective fungal isolates						
	0	0.005 g ai/l	0.05 g ai/l	0.125 g ai/l	0.25 g ai/l	0.5 g ai/l	1.5 g ai/l
Eco-T®	87.0 b	65.8 c	60.3 b	49.3 b	37.0 c	36.5 b	33.3 c
T. atroviride SY3A	90.0 a	90.0 a	63.3 b	52.5 b	46.5 b	43.5 a	40.3 b
T. atroviride SYN6	90.0 a	90.0 a	77.3 a	60.3 a	62.5 a	43.5 a	40.3 b
T. harzianum SYN	85.5 b	73.5 b	58.0 b	56.0 ab	45.8 b	47.8 a	47.8 a
R. solani	90.0 a	48.3 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 d
P – level	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F – ration	8.71	64.14	85.55	147.47	311.74	205.57	166.45
% CV	1.62	5.98	12.48	9.33	6.89	7.96	9.00

Values followed by the same letters within a column are not significantly different (Students-Newman-Keuls test at α = 0.05).

increase (P = 0.04) in the number of surviving seedlings as concentrations were increased (Table 2). The non-infested and recommended tolclofos-methyl controls recorded the highest percentage of surviving seedlings (94.4 and 84.0%, respectively). These were significantly different from the *R. solani* infested control where only 35.4% of the seedlings inoculated with the pathogen survived (Table 3). Although percentage seedling survival at 0.005 g ai/l was not significantly different from the pathogen infested control, increases at 0.05 and 0.125 g ai/l concentrations (68.0 and 75.7%, respectively) were (Table 3). At 0.125 a.i g/l tolclofos-methyl concentration, percentage seedling survival was not significantly different from the full strength tolclofos-methyl (0.5 g ai/l) control treatment (Table 3).

Seedling Trial 2: Control of *R. solani* damping-off using one tolclofos-methyl concentration in combination with *Trichoderma* and *Bacillus* isolates

Seedling survival within the non-infested control was significantly better than the infested and tolclofos-methyl (0.05 g ai/l) controls (Table 4). No significant difference was found between the non-infested control and the recommended full strength tolclofos-methyl (0.5 g ai/l).

Trichoderma and *Bacillus* treatments and their respective combinations without toclofos-methyl (0.05 g ai/l) were significantly better (between 60 – 81%) than the infested control [except *Bacillus* B81 (50.71%)] but were not as effective as the recommended full strength tolclofos-methyl control (86%), with the exception of Eco-T® (81%) (Table 4).

A general trend was that combined applications of microbial inoculants plus 0.05 g ai/l tolclofos-methyl resulted in an additive effect with a corresponding increase (4 – 22%) in percentage seedling survival. The increase in percentage seedling survival was comparable to the recommended full strength tolclofos-methyl control (Table 4). Moreover, percentage seedling survival for the integrated control was better than the *Trichoderma* and *Bacillus* and the 0.05 g ai/l tolclofos-methyl control. For example, *T. harzianum* Isolate SYN and Eco-T® plus tolclofos-methyl (0.05 g ai/l) gave 86.7 and 84.7% seedling survival, respectively, which was comparable to the recommended full strength tolclofos-methyl control. Percentage seedling survival was better than either of the individual components used in isolation (Table 4).

The pathogen infested control exhibited a significantly lower seedling dry biomass compared to all the other treatments (Table 4). Percentage dry biomass of *Trichoderma* and *Bacillus* treatments without 0.05 g ai/l tolclofos-methyl were not significantly different from *Trichoderma* and *Bacillus* treatments with 0.05 g ai/l tolclofos-methyl (Table 4).

DISCUSSION

Chemical residue in foods is of major concern to the consumer leading to criticisms of the chemical crop protection industry (Urech, 2000). Hence, the main objective of this study was to ascertain whether reduced concentrations of tolclofos-methyl, in combination with selected *Trichoderma* and *Bacillus* isolates could be used to achieve effective control of *R. solani* damping-off comparable to the manufacturer's dosage recommendation for tolclofos-methyl. It has been reported that some *R. solani* vary in their sensitivity to fungicides among and within, anastomosis groups of and among different species (Kataria et al., 1991a; Kataria et al., 1991b). Kataria et al. (1991b) found that tolclofos-methyl was effective against different species and anastomosis groups of *R. solani*. The *R. solani* isolate used in this study was sensitive to tolclofos-methyl. In Seedling Trial 2, it was evident that combination(s) of lowered concentrations of tolclofos-methyl and some BCAs gave equal levels of control compared to the use of the recommended full strength tolclofos-methyl (0.5 g ai/l).

Table 2. Effects of single and combined applications of selected *Trichoderma* and *Bacillus* isolates in conjunction with three tolclofos-methyl concentrations (0.005, 0.05 and 0.125 a.i g/l) on survival of cucumber seedling (cv. Ashley) against *R. solani* damping-off after 4 weeks of growth in the greenhouse.

Treatment	Slope[a]	R^2	RSD	% Seedling survival at 0.005 g ai/l	% Seedling survival at 0.05 g ai/l	% Seedling survival at 0.125 g ai/l	P-value[b]
Overall	5.55 (1.129)	0.13	3.263	-	-	-	0.0001**
Rizolex® controls (reduced concentrations)	19.95 (3.769)	0.77	1.119	54.88	68.04	75.71	0.001**
T. atroviride SY3A	2.44 (6.346)	-0.12	1.885	73.63	63.21	74.29	0.71[ns]
T. atroviride SYN6	1.587 (6.385)	-0.13	1.896	52.08	60.42	54.88	0.81[ns]
T. harzianum SYN	1.19 (4.825)	-0.13	1.433	63.21	69.46	65.29	0.81[ns]
Eco-T®	10.43 (4.083)	0.411	1.213	68.04	76.37	79.17	0.04 *
Bacillus B69	5.61 (3.649)	0.15	1.084	52.08	58.33	58.33	0.17[ns]
Bacillus B81	- 3.12 (2.993)	0.01	0.889	65.29	61.79	61.79	0.33[ns]
T. atroviride SY3A + *Bacillus* B69	10.66 (7.832)	0.10	2.326	54.88	61.79	65.95	0.22[ns]
T. atroviride SYN6 + *Bacillus* B69	3.68 (5.277)	-0.07	1.567	51.38	51.38	54.88	0.51[ns]
T. harzianum SYN + *Bacillus* B69	1.87 (1.655)	0.03	0.492	60.42	62.50	62.50	0.30[ns]
Eco-T® + *Bacillus* B69	11.85 (7.637)	0.15	2.268	52.08	56.96	56.96	0.16[ns]
T. atroviride SY3A + *Bacillus* B81	0.62 (4.905)	-0.14	1.457	75.00	75.70	75.70	0.90[ns]
T. atroviride SYN6 + *Bacillus* B81	2.78 (6.326)	-0.11	1.879	56.25	52.79	58.33	0.67[ns]
T. harzianum SYN + *Bacillus* B81	9.52 (5.169)	0.23	1.535	60.42	65.29	70.13	0.11[ns]
Eco-T® + *Bacillus* B81	14.46 (8.205)	0.21	2.437	54.12	55.54	63.88	0.12[ns]

[a] Slope (Regression coefficient) describing the linear relationship of percentage seedling survival rate and concentration of tolclofos-methyl for each treatment over the 4 week growth period in the greenhouse.
[b] Significance level of percentage seedling survival of each treatment over a period of 4 weeks of growth in the greenhouse.
RSD = Residual Standard Deviation.
R^2 = Coefficient of determination.
***, ** = Significantly different at P = 0.0001 and P = 0.001 respectively.
ns = Not significant (P > 0.05).

For instance, the control achieved with 0.05 g ai/l tolclofos-methyl in combination with selected *Trichoderma* and *Bacillus* treatments (*T. harzianum* SYN, Eco-T® and *T. atroviride* SY3A + *Bacillus* B81 and *T. harzianum* SYN + *Bacillus* B81) were comparable to that of the prescribed tolclofos-methyl dosage (0.5 g ai/l). The significance of this result is that fungicide application rates could feasibly be reduced resulting in a possible decrease in costs per season. Similar experiments by Henis et al. (1978) used a combination of pentachloronitrobenzene (PCNB) and an isolate of *T. harzianum* to control *R. solani* damping-off on radish. In a separate study, Kaur and Mukhopadhyay (1992) successfully controlled "chickpea wilt complex" by combining each of the following three fungicides Vitavax-200, Bavistin and Ziram with an isolate of *T. harzianum*.

The *in vitro* bioassay had a predictive value on whether lowered concentrations of tolclofos-methyl and the selected *Trichoderma* and *Bacillus* isolates could be used in an integrated system. At concentrations ranging from 0.05 - 1.5 g ai/l, tolclofos-methyl did not exhibit fungicidal or bactericidal action *in vitro* towards the *Trichoderma* or *Bacillus* isolates indicating that the two disease control systems could be used together for management of *R. solani* diseases in the greenhouse. Integrated control with this concentration proved successful and much better seedling performance was achieved than a 0.05 g ai/l tolclofos-methyl control or selected *Trichoderma* and *Bacillus* isolates used alone. This additive response supports the hypothesis made by Kaur and Mukhopadhyay (1992) and Hjeljord and Tronsmo (1998). Lowered doses of

Table 3. Seedling survival of cucumber (Ashley) as influenced by integration of single and dual inoculations of selected *Trichoderma* and *Bacillus* isolates with three tolclofos-methyl concentrations (0.005, 0.05 and 0.125 g ai/l) to control *R. solani* damping-off in the greenhouse.

Isolates/Treatments/Combinations	Tolclofos-methyl concentrations (a.i g/l)		
	0.005 g ai/l (mean number of surviving seedlings after 4 weeks)	0.05 g ai/l (mean number of surviving seedlings after 4 weeks)	0.125 g ai/l (mean number of surviving seedlings after 4 weeks)
Non-infested control	22.67 a (94.46)	22.67 a (94.46)	22.67 a (94.46)
R. solani infested control	8.50 g (35.42)	8.50 f (35.42)	8.50 f (35.42)
Rizolex® controls (reduced concentrations)	13.17 cdefg (54.88)	16.33 cde (68.04)	18.17 bcd (75.71)
Rizolex® Full strength control (0.5 g ai/l)	20.17 ab (84.04)	20.17 ab (84.04)	20.17 b (84.04)
T. atroviride SY3A	17.67 bcd (73.63)	15.17 cde (63.21)	17.83 bcd (74.29)
T. atroviride SYN6	12.50 defg (52.08)	14.50 cde (60.42)	13.17 e (54.88)
T. harzianum SYN	15.17 cdef (63.21)	16.67 cde (69.46)	15.67 cde (65.29)
Eco-T®	16.33 bcde (68.04)	18.33 bc (76.37)	19.00 bc (79.17)
Bacillus B69	12.50 defg (52.08)	14.00 cde (58.33)	14.00 e (58.33)
Bacillus B81	15.67 cdef (65.29)	14.83 cde (61.79)	14.83 de (61.79)
T. atroviride SY3A + *Bacillus* B69	13.17 cdefg (54.88)	14.83 cde (61.79)	15.83 cde (65.95)
T. atroviride SYN6 + *Bacillus* B69	12.33 efg (51.38)	12.33 e (51.38)	13.17 e (54.88)
T. harzianum SYN + *Bacillus* B69	14.50 cdef (60.42)	15.00 cde (62.50)	15.00 de (62.50)
Eco-T® + *Bacillus* B69	12.50 defg (52.08)	13.67 de (56.96)	13.67 e (56.96)
T. atroviride SY3A + *Bacillus* B81	18.00 bc (75.00)	18.17 bcd (75.70)	18.17 bcd (75.70)
T. atroviride SYN6 + *Bacillus* B81	13.50 cdef (56.25)	12.67 e (52.79)	14.00 e (58.33)
T. harzianum SYN + *Bacillus* B81	14.50 cdef (60.42)	15.67 cde (65.29)	16.83 bcde (70.13)
Eco-T® + *Bacillus* B81	13.00 cdefg (54.12)	13.33 e (55.24)	15.33 de (63.88)
F-ratio	10.32	11.31	15.98
P-level	0.0001	0.0001	0.0001
% CV	12.67	10.58	8.69

Values followed by the same letters within a column are not significantly different (Students-Newman-Keuls test at $P \leq 0.05$.

PCNB have also been found to enhance the efficiency of *T. harzianum* (Chet et al., 1979). Weakening of the pathogen could also mean less competition for resources between the pathogen and the biological control agents.

The results presented here indicate the possibility of combining chemical and biological control systems to curb *R. solani* damping-off in the greenhouse. Further trials are needed to ascertain the combined use of reduced tolclofos-methyl concentrations and biological control agents such as *Trichoderma* and *Bacillus* as a disease control system in different soils, growth media and under different sets of environmental conditions.

With legislation concerning the use of fungicides becoming more stringent, the need to apply reduced amounts of fungicides while still maintaining proper control of plant diseases has become imperative. For this reason, we believe BCAs that are resistant or less sensitive to a range of agrochemicals would be beneficial in integrated disease management such as the one reported in this study. This work suggests the need for development and implementation of proper disease management strategies and mention of tolclofos-methyl and EcoT® as commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation by the authors.

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Table 4. Survival and dry biomass of cucumber seedlings (Ashley) as a result of integration of *Trichoderma* and *Bacillus* treatments with tolclofos-methyl (0.05 g ai/l) to control damping-off caused by *R. solani* under greenhouse conditions.

Isolates/Treatments/Combinations with or without tolclofos-methyl	Mean number of surviving seedlings after 4 weeks	Mean dry biomass after 4 weeks (g)
Non-infested control	23.67 a (98.63)	9.21 a
R. solani infested control	9.83 g (40.96)	4.51 d
Tolclofos-methyl control (0.05 g ai/l)	15.83 cdef (56.96)	6.34 bc
Full strength Tolclofos-methyl control (0.5 g ai/l)	20.67 abc (86.13)	6.88 bc
T. atroviride SY3A	16.17 bcdef (67.38)	7.85 abc
T. atroviride SY3A (R)	19.17 abcde (79.88)	7.16 abc
T. harzianum SYN	15.83 cdef (65.96)	7.71 abc
T. harzianum SYN (R)	20.83 ab (86.79)	9.25 a
Eco-T®	19.50 abcde (81.25)	6.73 bc
Eco-T® (R)	20.33 abc (84.71)	6.48 bc
Bacillus B81	12.17 fg (50.71)	6.02 c
Bacillus B81 (R)	16.67 bcdef (69.46)	6.99 bc
T. atroviride SY3A + *Bacillus* B81	15.50 def (64.58)	7.88 abc
T. atroviride SY3A + *Bacillus* B81 (R)	20.67 abc (86.13)	8.32 ab
T. harzianum SYN + *Bacillus* B81	14.83 ef (61.79)	7.53 abc
T. harzianum SYN + *Bacillus* B81 (R)	20.17 abcd (84.04)	8.23 ab
Eco-T® + *Bacillus* B81	14.50 f (60.42)	7.40 ab
Eco-T® + *Bacillus* B81 (R)	19.33 abcde (80.54)	8.31 abc
F-ratio	12.28	6.91
P-level	0.0001	0.0001
% CV	9.90	10.23

Values followed by different letters within a column are significantly different (Student Newman Keul's test, $P \leq 0.05$).
(R) indicates *Trichoderma* and *Bacillus* and their respective combinations supplemented with 1 ml of 0.05 g ai/l tolclofos-methyl.

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