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Short communication

Application method and rate of *Trichoderma* species as a biological control against *Pythium aphanidermatum* (Edson) Fitzp. in the production of microgreen table beets (*Beta vulgaris* L.)

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ABSTRACT

Seed balls of 'Early Wonder Tall Top' table beet (*Beta vulgaris* L.) were incubated for 2 days at 21 °C in moist exfoliated grade 3 vermiculite (3 g seed balls [168 seed balls] and 1.1 g vermiculite) containing equal weights of *Trichoderma harzianum* Rifai strain KRL-AG2 G41 and *T. virens* strain G-41 (ThTv) at 0, 0.25, 0.50, 0.75 or 1.00 mg ThTv per seed ball. ThTv also was applied to the peat-lite growth medium 14 days before planting, at the same rates per seed ball as the seed ball treatments. Four days before planting, the peat-lite was inoculated with *Pythium aphanidermatum* (Edson) Fitzp. (*Pa*) at 0, 0.5 and 1.0× the rate that resulted in 96% damping-off when non-ThTv-treated dry seed balls were sown in peat-lite containing 1.0 *Pa*. Increasing ThTv level per seed ball decreased percentage damping-off, with 1.0 *Pa* giving greater percentage damping-off than 0.5 *Pa*. At 1 mg ThTv per seed ball, damping-off was 5% and 19% at 0.5 and 1.0 *Pa*, respectively. Including Agro-Lig UF (mostly humic acids) in the incubating seed-ball-ThTv mixture further decreased damping-off by an average 13 and 10 percentage points in 0.5 and 1.0 *Pa*, respectively. Increasing ThTv per seed ball in growth media decreased percentage damping-off, but not to the extent achieved with seed ball treatment, with 1 mg ThTv per seed ball giving 20% and 55% damping-off in 0.5 and 1.0 *Pa*, respectively. Decreasing incidence of damping-off with increasing ThTv application to seed balls or growth media was associated with increasing shoot fresh weight m^{-2} at 14 days after planting, a response attributable to increased percentage plant survival and not to a ThTv growth-promoting effect.

1. Introduction

Microgreens are defined as salad crop shoots harvested for consumption within 10–20 days of seedling emergence (Lee et al., 2004). Microgreens are susceptible to pre- and post-emergence damping-off caused by several soil-bourne pathogenic fungi including species of *Pythium* (Agrios, 1997). Having a biological (non-chemical) protection against damping-off is important for microgreen crops due to the lack of an assigned pre-harvest interval.

Many fungal biocontrol agents, including *Trichoderma*, are applied to seed as conidia or resting spores which must become active before interaction with the pathogen. Pill et al. (2009) showed that the combination of *Trichoderma harzianum* (*Th*) and *T. virens* (*Tv*) strains slurry-treated on cucumber (*Cucumis sativus*) seeds was more effective than either species alone in reducing damping-off caused by *Pythium aphanidermatum* (*Pa*). Whilst *ThTv* was not used in their study, Murphy et al. (2010) found that sowing table beet seed balls that were incubated in grade 3 exfoliated vermiculite moistened with 150% water for 5 days at 20 °C (50% germination, radicles 6.5 mm long) before sowing onto peat-lite resulted in 26% greater shoot fresh weight m^{-2} at 15 days after sowing than from sowing dry seed balls. Our hypothesis for the present study was that *ThTv* colonization of table beet seed balls during the pre-sowing moist incubation period would afford greater subsequent protection against damping-off than *ThTv* application to the growth medium. A further hypothesis was that adding an acidic compound rich in humic acids may enhance *ThTv* colonization of the seed balls since such a compound (Agro-Lig) enhanced the effectiveness of *Trichoderma* species in protecting cucumber seeds against damping-off caused by *Pythium ultimatum* (Harman and Taylor, 1988).

The objectives of this study were to evaluate the effectiveness of five rates of *ThTv* (0, 0.25, 0.50, 0.75 or 1.00 mg) per seed ball of 'Early Wonder Tall Top' table beet against three rates of *Pa* inoculation in peat-lite in controlling damping-off. The *ThTv* was applied either during a 2-day incubation of the seed balls in moist vermiculite or to the growth medium. A further objective was to determine

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whether inclusion of Agro-Lig in the seed-ball-vermiculite-*ThTv* mixture affected *ThTv*-efficacy against damping-off.

2. Materials and methods

2.1. ThTv application to seed balls

Seeds balls of 'Early Wonder Tall Top' table beet (Johnny's Selected Seeds, Winslow, ME) without surface sterilisation, were mixed in grade 3 exfoliated vermiculite (W.R. Grace, Columbia, MD; 3 g of seed balls [168 seed balls] and 1.1 g vermiculite in 28.4 ml soufflé cups). An equal weight of *T. harzianum* Rifai strain KRL-AG2 G41 (*Th*, PlantShield®) and *T. virens* strain G41 (*Tv*, Root Mate®; both from BioWorks, Victor, NY) were mixed (*ThTv*). *ThTv* powder was added at 0, 7.63, 15.27, 22.90, and 30.53 g per 0.5 l of reverse osmosis water. These suspensions when added to each seed ball-vermiculite mixture at 2.75 ml, delivered 0, 0.25, 0.50, 0.75 or 1.00 mg *ThTv* per seed ball, respectively (150% water per vermiculite dry weight). Each seed ball-vermiculite-*ThTv* concentration mixture received 0 or 0.7 g of Agro-Lig UF (75% humic acids; Northwest Agricultural Products, Pasco, WA). Cup contents were stirred thoroughly, lids secured on the cups, and the cups were placed in constant 21 °C for 2 days in darkness. Non-treated (dry) seed balls were included in the study as a comparison with those incubated in moist vermiculite.

2.2. ThTv application to growth medium

At 14 days before planting, media were drenched with *ThTv*. *Th* and *Tv* at 1 g each were added to 1 l of reverse osmosis water and magnetically stirred. To each 4 l of peat-lite growth medium (Sunshine Mix No. 1; Bellevue, WA), equal to the capacity of 4 replicate 17 cm × 12 cm × 6 cm plastic flats, was added 0, 84, 168, 252 or 336 ml of *ThTv* suspension diluted to 0.5 l which provided, respectively, 0, 0.25, 0.50, 0.75, or 1.00 mg *ThTv* per seed ball. Seed balls imbibed in vermiculite without *ThTv* and dry seed balls were sown in media prepared with the 5 *ThTv* rates. After thorough mixing of the *ThTv* into the media, the four flats were filled. Flats remained in a greenhouse set at 25/22 °C (day/night) under natural light (November, 2008) and were watered daily. Ten days later (and 4 days before planting), these flats were inoculated with *Pa*.

2.3. Pythium aphanidermatum preparation and inoculation of growth medium

Pa growing on acidified potato dextrose agar in three 10-cm diameter Petri dishes was blended into 300 ml of reverse osmosis water and then stirred into 4 l of grade 3 horticultural vermiculite. To achieve 1.0 *Pa*, the vermiculite-*Pa* mixture was mixed into the peat-lite at 40 ml l^{-1}. For 0.5 *Pa*, the 1.0 *Pa* inoculum was diluted with an equal volume of fresh vermiculite, and then applied at 40 ml l^{-1} to the peat-lite. Zero *Pa* was achieved by adding fresh vermiculite to peat-lite at 40 ml l^{-1}.

2.4. Crop planting and culture

Once seed balls of each treatment were broadcast on the peat-lite surface within each flat, they were covered with 3 mm of Redi-Earth (SunGro Horticulture, Bellevue, WA) and watered. The flats were watered twice daily (0800 and 1500 h) in a greenhouse set at 25/22 °C (day/night) under natural light (November, 2008). At 7 days after planting, seedlings in all flats received a liquid application of 200 mg N l^{-1} from 21 N–5P_2O_5–20K_20.

2.5. Experimental design and data analysis

The experiment was a 3 (*Pa* level) × 2 (*ThTv* application to seed balls or growth medium) × 5 (*ThTv* rate) factorial with dry seed balls not treated with *ThTv* included for comparison. These treatments (flats) were arranged in randomised blocks with four replications, flats being 50 cm apart to avoid *Pa* or *ThTv* contamination between flats. From daily counts of seedling emergence (when cotyledons were free of the growth medium surface), days to first emergence (when at least 20 seedlings had emerged) was calculated. At 14 days after planting, percentage damping-off was visually estimated as the percentage of seedlings that was wilted or lodged within a flat; and, all healthy seedlings were cut at the growth medium surface within each flat, and the combined shoot fresh weight determined. From this value, shoot fresh weight m^{-2} was calculated. Shoot fresh weight of a counted subsample of shoots (20 when possible) from each flat was used to calculate fresh weight per shoot. Days to first emergence, percentage damping-off and its angular transformation (arcsine$\sqrt{\%}$), and shoot fresh weight (m^{-2} and per shoot) were subjected to analysis of variance. Subsets of data were selected according to logical treatment comparisons. Only main effect means are presented if factor interactions were not significant.

3. Results

In media without *Pa* inoculum there was no damping-off (Table 1). Incubating seeds in moist vermiculite for 2 days at 21 °C with 0 mg *ThTv* per seed ball resulted in 42% and 65% damping-off in 0.5 and 1.0 *Pa* compared to 96% damping-off when dry seeds were sown in 0.5 or 1.0 *Pa* media. Increasing *ThTv* per seed ball decreased damping-off percentage but to a greater extent in 0.5 *Pa* than in 1.0 *Pa*l. *ThTv* at 1 mg per seed ball, resulted in 5% and 19% damping-off in 0.5 and 1.0 *Pa*, respectively. Agro-Lig UF mixed with the seed balls incubated in *ThTv* and moist vermiculite reduced percentage damping-off by 13 and 10 percentage points (averaged over all levels) in 0.5 and 1.0 *Pa*, respectively. Days to first emergence increased as *ThTv* increased from 0.25 to 1.00 mg per seed ball, although the range was only 0.8 days. Days to first emergence from dry seed balls were one day later than from seed balls incubated in moist vermiculite containing 1 mg *ThTv* per seed ball.

In the absence of *Pa*, shoot fresh weight m^{-2} or per shoot was unaffected by *ThTv* level per seed ball (Table 2). Increasing *ThTv*, however, from 0 to 1.0 mg per seed ball increased shoot fresh weight m^{-2} by 192% and 321% in 0.5 and 1.0 *Pa*, respectively. In 0.5 *Pa*, fresh weight per shoot was increased only by 1.0 mg *ThTv* per seed ball compared to lower *ThTv* levels. In 1.0 *Pa*, however, fresh weight per shoot was increased by incubating seeds in vermiculite containing 1 mg *ThTv* per seed ball, compared to 0.25–0.75 mg *ThTv*, which, in turn, gave greater shoot fresh weight than 0 mg *ThTv*. Agro-Lig UF had no effect on shoot fresh weight m^{-2} or per seedling.

ThTv was less effective in reducing percentage damping-off when applied to growth medium (Table 3) than to seed balls (Table 1). As *ThTv* increased from 0.25 mg to 1.00 mg per seed ball in 0.5 *Pa*, damping-off decreased from 26% to 5% as a seed ball application (Table 1); with corresponding values of 64% and 20% for growth medium application (Table 3). As with seed ball application, growth medium application with *ThTv* was less effective against damping-off in 1.0 *Pa* level than in 0.5 *Pa*. As *ThTv* increased from 0.25 mg to 1.00 mg per seed ball in growth medium, damping-off decreased from 91% to 55% (Table 3). Corresponding values for seed ball applications were 42% and 19% (Table 1).

Applying *ThTv* to seed balls (Table 2) rather than to growth medium (Table 3) resulted in greater shoot fresh weight m^{-2}, a response that was more pronounced as *Pa* increased from 0.5