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Effect of *Trichoderma* on Plant Growth: A Balance Between Inhibition and Growth Promotion

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Abstract. The effect of lettuce (*Latuca sativa* L.) germination and growth in nonsterilized potting compost of 0.1% and 1.0% w/w incorporation of fermenter biomass inocula of six strains of *Trichoderma* was investigated. Except for strains WT and T35 at 0.1% w/w, all inocula inhibited germination. Biomass of strains WT, T35, 20, and 47 at 1.0% promoted shoot fresh weight, whereas strains TH1 and 8MF2 were inhibitory. In contrast, when biomass of strains WT, TH1, and 8MF2 was autoclaved and incorporated at 1%, shoot fresh weight was promoted, but the biomass of T35 was inhibitory. None of the strains incorporated at 0.1% w/w increased shoot fresh weight, and autoclaved biomass of TH1, T35, and 20 incorporated at 0.1% w/w resulted in lower shoot fresh weights in comparison with uninoculated controls. The shoot dry weight of lettuce seedlings could be enhanced by germinating seeds in uninoculated compost and after five days' growth transferring them into WT-inoculated compost. Inoculum of strain TH1 when applied using this method was very inhibitory. With WT the degree of increase in shoot fresh weight and germination rate declined as the fermentation time to produce inocula was increased.

Introduction

Trichoderma isolates have been shown to enhance growth and flowering in a range of horticultural crops [1]. We [14] have demonstrated with six different *Trichoderma* strains that, when added to potting compost, the fresh weight of 4-week-old lettuce (*Latuca sativa* L.) seedlings can be consistently increased by up to 54%. Other strains screened were found to lose their initial ability to stimulate plant

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growth following subculture or were ineffective plant growth promoters [11, 14]. The mechanism of growth promotion by *Trichoderma* is unknown but in some instances may involve control of minor pathogens. Thus some soils suppressive to plant pathogens such as *Pythium* and *Rhizoctonia* have been found to contain high levels of *Trichoderma* [15]. This biocontrol activity may involve competition for infection sites or substrates on the plant surface, physical restriction, antibiosis, or production of lytic enzymes [10]. Nevertheless, the direct stimulation of plant growth by *Trichoderma* cannot be ruled out.

Plant growth inhibition has also been reported. In biocontrol trials [8], seeds took longer to germinate and the fresh weight of 2-week-old lettuce seedlings was less in *Trichoderma*-inoculated controls than uninoculated controls. This effect was also observed in radish (*Raphanus sativus* L.), where growth enhancement with respect to *Trichoderma* soil incorporation was not seen until the seedlings were 3–4 weeks old [16].

The basis for the variation in effects on plant growth by different *Trichoderma* strains is unknown but may relate to age and form of inocula. A single report [12] suggests that autoclaving *Trichoderma* inocula does not affect biocontrol activity against *Rhizoctonia*.

This paper describes a series of trials investigating the effect on germination and growth of lettuce by adding a range of nonautoclaved and autoclaved *Trichoderma* inocula to nonsterile potting compost. The strains were chosen for either their ability to promote growth [14] or their potential biocontrol activity [6, 9, 17]. The addition of inocula produced with different fermentation times in molasses/yeast media and time of inoculum application was also considered.

Materials and Methods

Sources of Trichoderma *Strains*

Trichoderma harzianum Rifai strain TH1 (IMI 275950) was isolated from wheat straw [9], *T. harzianum* strain T35 (IMI 298374) was isolated as an antagonist of *Rhizoctonia solani* Kühn [17], and *T. harzianum* strain WT was obtained from Prof. R. Baker at Colorado State University. *T. harzianum* strain 20 (IMI 337473) and *T. viride* Pers. ex. S.F. Gray strain 47 (IMI 337474) were isolated from surface sterilized sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary, and *T. viride* strain 8MF2 (IMI 322663) was isolated from onion tissue at H.R.I. Littlehampton.

All strains were stored on potato dextrose agar (PDA; Oxoid Unipath Ltd, Basingstoke, U.K.) slopes at 20°C.

Preparation of Inocula

Inocula of *Trichoderma* strains were prepared using the method of Lumsden et al [8]. Flasks (1 liter) of medium containing 15 g cane molasses and 2.5 g autolysed yeast granules in 500 ml distilled water were inoculated with approximately one-sixth of a fully colonized PDA plate. After shaking at 150 rpm for 6 days of 25°C in diffuse light in the laboratory, the biomass produced was air dried at room temperature, ground to pass through a 500 μm screen in a Cyclotech sample mill (Tecator, Bristol, U.K.) and stored at 4°C. Autoclaved material was prepared by autoclaving the dried inoculum powder for 30 min at 122°C. In trial 2, *T. harzianum* (WT) inocula were also grown in molasses/yeast medium for 6, 12, 24, and 48 days.

Pot Trials

Three trials were set up using nonsterilized 50:50 peat:sand potting compost mixture with added (g/l) potassium nitrate (0.40), superphosphate (0.77), chalk (0.63), and frit 255WM (Ferro (AB Ltd) Wolverhampton, U.K.) (0.40). The pH of the compost was 4.66, 4.69, 5.02 in trials 1, 2, and 3, respectively.

Trichoderma strains were added autoclaved (A) in trials 1 and 3, or nonautoclaved (NA) in trials 1, 2, and 3 at a rate of 1% (trials, 1, 2, 3) and 0.1% (trials 1 and 2) dry w/w to potting compost in plastic bags and mixed thoroughly by hand. Plastic pots (9 cm diameter) were half filled with uninoculated compost and the remaining half of each replicate pot was filled with *Trichoderma* inoculated compost. The control pots were filled with uninoculated compost only. The compost was tapped down lightly and unpelleted lettuce seeds cv. Ravel (10 in trial 1, 5 in trials 2 and 3) were placed on the compost surface of (5 in trial 1, 8 in trial 2, and 6 in trial 3) replicate pots per treatment. The seeds were covered with a thin layer of the appropriate inoculated compost. The pots were positioned in the glasshouse on capillary matting in a randomized block formation and covered for the first 3 days with polystyrene sheeting to prevent large fluctuations in temperature and to ensure darkness during seed germination. The temperature was set at 15°C at night, 18°C during the day, with the glasshouse vents opening at 23°C.

In trial 3, using the method described above, 6 replicate pots were filled with uninoculated compost and compost inoculated with nonautoclaved or autoclaved WT or TH1. A duplicate set of unseeded inoculated and control pots were also prepared and placed on capillary matting. Five days later, 5-day-old lettuce seedlings grown in uninoculated compost were transferred to these and another set of pots containing fresh inoculated compost.

Seed emergence was recorded after 4 days. Plant stand, shoot fresh weight, and shoot dry weight were recorded at harvest, between 31 and 39 days. Data were analyzed using analysis of variance. A square root transformation was used for number of seeds emerged/plant stand in trial 1, and a logit transformation ($y = \log((x/100 - x))$ was used on all percentage data. For trial 2, a one-way analysis was used, but a factorial structure was incorporated for the other two trials [4].

Results

In trial 1, except for nonautoclaved WT and T35 incorporated at 0.1% w/w, all *Trichoderma* treatments reduced the germination of lettuce seed after 4 days (Table 1).

Strain differences between nonautoclaved *Trichoderma* inocula added to compost at 1% were apparent. Strains WT, T35, 20, and 47 significantly ($P < 0.05$) increased shoot fresh weight, whereas TH1 and 8MF2 produced substantially smaller plants (Table 1). When added autoclaved at 1%, WT (Fig. 1), TH1, and 8MF2 produced significantly ($P < 0.05$) greater shoot fresh weights than the untreated controls. None of the nonautoclaved *Trichoderma* strains applied at 0.1% increased shoot fresh weight and strains, whereas TH1 and T35 applied autoclaved at this concentration resulted in lettuce plants with significantly ($P < 0.05$) lower shoot fresh weights.

In general, most *Trichoderma* strains applied nonautoclaved and autoclaved at both concentrations resulted in lettuce plants with shoot dry weights similar to the control, although there were exceptions. For instance, application of autoclaved TH1 at 1% and 8MF2 at 0.1% resulted in greater shoot dry weights, whereas applications of TH1 at 0.1% A, 1.0% NA, and T35 at 0.1% A and 1.0% A resulted in lower shoot dry weights.

In trial 2, nonautoclaved *Trichoderma* WT inocula at 1% reduced the germination rate of lettuce seed (Table 2). As fermentation time to produce inocula increased, the germination of lettuce was reduced further (Table 2).

The shoot fresh and dry weight was enhanced by nonautoclaved *Trichoderma* WT inocula applied at 1% and produced with a fermentation time of 6 or 12 days,

Table 1. Effect of nonautoclaved and autoclaved *Trichoderma* inocula on lettuce growth

		No. seeds emerged/plant stand				Shoot weight/pot[a] (g)			
		4 days[a]		Harvest[a]		Fresh		Dry	
Trichoderma strain	%w/w	NA[b]	A[c]	NA[b]	A[c]	NA[b]	A[c]	NA[b]	A[c]
Control[d] (no *Trichoderma*)		6.6 [2.56][e]		9.8 [3.13]		18.1		1.07	
WT	1.0	0.4 [0.40]	0.0 [0.00]	9.8 [3.13]	9.4 [3.06]	25.0	25.4	1.04	1.15
	0.1	7.0 [2.64]	2.8 [1.63]	10.0 [3.16]	9.8 [3.13]	21.1	20.2	1.23	1.18
TH1[f]	1.0	0.0	0.2 [0.20]	6.0	10.0 [3.16]	2.2	30.7	0.10	1.53
	0.1	1.2 [1.15]	2.2 [1.40]	10.0 [1.36]	9.6 [3.10]	19.6	11.3	1.09	0.69
T35[f]	1.0	0.8 [0.55]	0.0	10.0 [3.16]	7.8	27.8	6.3	1.32	0.33
	0.1	6.2 [2.46]	3.2 [1.72]	9.8 [3.13]	9.0 [2.99]	19.4	11.9	1.09	0.67
20	1.0	0.4 [0.40]	0.4 [0.40]	10.0 [3.16]	9.6 [3.13]	27.6	19.5	1.18	0.94
	0.1	3.4 [1.77]	2.8 [1.66]	10.0 [3.16]	9.8 [3.13]	20.7	14.0	1.23	0.82
47	1.0	0.0 [0.00]	0.2 [0.20]	9.8 [3.13]	9.8 [3.13]	23.4	18.1	0.98	0.83
	0.1	1.2 [0.68]	2.0 [1.23]	9.8 [3.13]	9.8 [3.13]	18.5	18.6	1.06	1.06
8MF2[f]	1.0	0.0	0.0 [0.00]	8.2	9.6 [3.10]	3.8	23.5	0.16	1.04
	0.1	1.0 [0.88]	3.0 [1.66]	9.8 [3.12]	10.0 [3.16]	17.9	18.6	1.04	1.64
SED[g]		[0.328]		[0.0528]		2.55		0.127	
DF[h] = 88									

[a] Estimate of germination and plant stand 36 days after sowing; values are means of 5 replicate pots containing 10 seeds

[b] Not autoclaved

[c] Autoclaved

[d] Values are means of 10 replicate pots containing 10 seeds, 36 days after sowing

[e] Square root transformation

[f] Strains TH1 NA, T35 A and 8MF2 NA at the 1% level are not included in statistical analyses because they had variable numbers of plants per pot at harvest

[g] Standard error of difference between means

[h] Degrees of freedom

but when the fermentation time was increased to 24 and 48 days the shoot fresh weight was not significantly ($P < 0.05$) different from the untreated controls (Table 2, Fig. 2).

In trial 3, the shoot fresh and dry weights of lettuce plants grown from seed or transferred to fresh or 5-day-old uninoculated compost were similar (Table 3). Lettuce seedlings grown in compost inoculated with nonautoclaved WT had significantly ($P < 0.05$) higher shoot fresh weights than those grown in uninoculated compost, irrespective of transfer. However, when seeds were sown directly into compost inoculated with nonautoclaved WT, the shoot dry weight was not enhanced, whereas seedlings pricked out into compost inoculated with nonautoclaved WT produced significantly higher fresh and dry shoot weights than seeds germinated in compost inoculated with nonautoclaved WT.

Autoclaved WT also increased the shoot fresh weight in comparison with uninoculated controls, but there was no difference in shoot fresh weight between seedlings transferred and seed germinated in situ. Seedlings transferred to 5-day-old compost inoculated with autoclaved WT had an increased shoot dry weight in

Fig. 1. Comparison of shoot growth of 10 lettuce plants per pot grown from seed in peat/sand compost uninoculated and inoculated with WT 1% and WT 1% autoclaved w/w after 36 days.

Table 2. The effect of fermentation time of *T. harzianum* (WT) inocula produced in molasses/yeast medium on lettuce growth

Inocula					
Fermentation time (days)/dry biomass weight (gl^{-1})		Incorporation rate of NA inocula (% w/w compost)	% No. plants after 4 days[b]	Shoot wt/pot[a] (g)	
				Fresh	Dry
Control (no *Trichoderma*)			98 [4.81][c]	15.28	1.35
6	7.86	1.0	85 [3.09]	29.06	2.04
12	6.80	1.0	55 [0.61]	27.44	1.96
24	6.60	1.0	55 [0.61]	17.04	1.26
48	6.12	1.0	30 [−1.30]	16.71	1.19
6	7.86	0.1	95 [4.32]	16.37	1.37
12	6.80	0.1	90 [3.34]	15.42	1.31
24	6.60	0.1	98 [4.81]	16.40	1.41
48	6.12	0.1	93 [3.83]	15.16	1.28
SED[d]			[0.902]	0.752	0.080
DF[e] = 60					

[a] Values are means of 8 replicate pots containing 5 seeds, 31 days after sowing
[b] Estimate of germination; values are mean percentages of 8 replicate pots containing 5 seeds
[c] Logit transformation
[d] Standard error of difference between means
[e] Degrees of freedom

comparison with all uninoculated treatments and with seeds sown into fresh compost inoculated with autoclaved WT.

Most of the seedlings transferred to 5-day-old compost inoculated with nonautoclaved TH1 died, although seedlings transferred into freshly inoculated compost and seeds sown into inoculated compost produced only slightly smaller plants than the controls. Autoclaved TH1 produced slightly larger or plants similar to the controls.

Fig. 2. Comparison of shoot growth of 5 lettuce plants per pot grown from seed in peat/sand compost uninoculated and inoculated with WT 1% produced from 6, 12, 24 and 48 day fermentation times after 31 days.

Table 3. Effect of time of *Trichoderma* inoculation on lettuce seedling growth

Strain	Shoot fresh weight per pot[a] (g)			Shoot dry weight per pot[a] (g)		
	Seeds sown into fresh inoc. compost	5-Day-old seedlings transferred to		Seeds sown into fresh inoc. compost	5-Day-old seedlings transferred to	
		Fresh inoc. compost	5-Day-old inoc. compost		Fresh inoc. compost	5-Day-old inoc. compost
Control (no *Trichoderma*)	18.4	20.0	17.7	1.23	1.25	1.19
WT NA[b]	26.4	29.2	29.6	1.29	1.56	1.70
WT A[b]	22.2	23.6	23.9	1.21	1.38	1.56
TH1 NA	15.4	15.5	0.5	0.70	0.70	0.01
TH1 A	21.3	19.5	23.7	1.15	1.21	1.50
SED[d]		1.07			0.117	
DF[e] = 67						

[a] Values are means of 6 replicate pots containing 5 plants, 39 days after sowing
[b] NA = nonautoclaved; A = Autoclaved at 1% w/w compost
[c] TH1 results excluded from statistical analyses
[d] Standard error of difference between means
[e] Degrees of freedom

Discussion

Some *Trichoderma* strains can produce substantial plant growth promotion when autoclaved. For example, TH1 and 8MF2, which have biocontrol potential [6, 9], were previously poor growth promoters when used nonautoclaved at 1% [11, 14], yet became good growth promoters when autoclaved, although TH1 improved yield only slightly in trial 3. T35, a good growth promoter when incorporated at 1% NA [14], produced a 70% increase in shoot fresh weight in trial 1, but when incorporated autoclaved resulted in shoot fresh weights which were 65% less than

the uninoculated control. This suggests that seedling growth promotion by *Trichoderma* could be a balance between growth inhibition and growth promotion properties, with the balance altered in some strains by autoclaving.

Autoclaved *Trichoderma* inocula do not appear to be more favorable to germinating seeds than nonautoclaved inocula, as both autoclaved and nonautoclaved *Trichoderma* inocula reduced the rate of germination. In addition, the effect of *Trichoderma* on the rate of seed germination appears to be strain dependent. Different rates of production of a variety of secondary metabolites may be involved here. For example, the phytotoxin viridiol produced by some *Trichoderma* spp. [13] has been shown [7] to delay germination in lettuce, with viridiol production reaching a peak after 5 days. In contrast, an oil that consisted of fatty acids and glycerol was demonstrated to be involved in *Trichoderma* growth stimulation of wheat [5]. Further work is required to clarify the role, if any, of secondary metabolite products in growth promotion and inhibition by the strains of *Trichoderma* used here.

We have shown that the NPK content of the inocula does not relate to differences in strain performance (not reported). However, the inconsistencies in some of our results could be due to microbial action on the autoclaved inocula, as nonsterile compost was used in all of the trials. The nonautoclaved *Trichoderma* inocula as used in trials 1, 2, and 3 consists of approximately 90% available substrate, with *Trichoderma* chlamydospores distributed within it [8]. This suggests that the introduced *Trichoderma* would have a distinct advantage over the indigenous microbial population in colonizing the substrate. However, the microbial action on introduced autoclaved inoculum could produce positive and negative effects on plant growth, as there is no control of the microbial population that develops. Even so, the pots with autoclaved inocula incorporated into the compost always had *Mucor* growing on the compost surface, but *Mucor* inocula, produced by the same method as the *Trichoderma* inocula, did not affect the growth of lettuce when incorporated into compost (not reported). Similarly, the growth of tomato seedlings grown in steamed soil inoculated separately with microorganisms including *Mucor,* isolated from naturally infested autoclaved soil, was also not enhanced [18]. In contrast, the presence of soil microflora on peat-bran has been suggested to result in increased dry weight of radish leaves [2]. Interestingly, some *Trichoderma* strains gained growth promotion activity when autoclaved, whereas others lost it, suggesting that some chemical(s) with biological activity are affected by heat but, with different strains, affected by varying degrees. For example, in lettuce biocontrol trials, autoclaved TH1 grown on peat-bran has produced similar results to nonautoclaved TH1, suggesting that this strain may produce a biologically active heat stable chemical [12]. In contrast, growth of petunia was not increased by using autoclaved *Trichoderma* inocula produced by the peat-bran method in the absence of pathogens [3], but small, significant increases in leaf dry weight of radish were observed when high concentrations of autoclaved inocula were used [2].

Trichoderma inocula produced by molasses/yeast fermentation culture, incorporated into compost at low concentrations (0.1% w/w), produced small, usually nonsignificant increases in growth, although other workers have shown that low concentrations of inocula grown on peat bran reduced yield [2]. Inocula added at 0.1% w/w autoclaved either reduced or produced no increase in yield, suggesting that microbial action on the inoculum may be occurring, perhaps without enough *Trichoderma* propagules to restrict it.

The time taken for lettuce seeds to germinate was increased and the fresh weight of 31-day-old seedlings was lower when compost was inoculated with *Trichoderma* inocula produced in fermentation culture over 24 and 48 days than that grown up over shorter periods of time. The cultures were in stationary-autolysis phase by this time, the yield of biomass was markedly reduced, and secondary metabolite production was likely to be occurring.

It is clear from these results that plant growth promotion in unsterile compost by *Trichoderma* is a highly variable property. It depends upon strain, age of culture, and inoculum application rate, and the effects may be stable or unstable if the inoculum is autoclaved. These findings may help to explain the inconsistencies previously reported with *Trichoderma* strains for both growth promotion and biocontrol studies. They further emphasize the need to standardize the application method and obtain stable strains for this type of work if practical application is to be made in the future.

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