Vermicompost suppresses Rhizoctonia solani Kühn in cucumber seedlings

Article *in* Journal of Plant Diseases and Protection · August 2009

DOI: 10.1007/BF03356308

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Journal of Plant Diseases and Protection, **116** (4), 182–188, 2009, ISSN 1861-3829.

Vermicompost suppresses *Rhizoctonia solani* Kühn in cucumber seedlings

Wurmkompost unterdrückt die Entwicklung von *Rhizoctonia solani* Kühn an Gurkenkeimlingen

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Received 5 December 2008; accepted 30 April 2009

Abstract

Disease suppressiveness of vermicompost produced from agricultural wastes consisting of cattle manure, tree bark (*Salix* spp.), potato culls, and apples was assayed on damping-off of two days-old cucumber (*Cucumis sativus* cv. Cevher) seedlings infected by *Rhizoctonia solani* Kühn (AG-4). Suppression effect was assessed at the rates of 0, 10, 20 and 30% (v/v) vermicompost, either blended with *Trichoderma harzianum* Rifai (KRL-AG2), amended with potting mixtures consisting of sand and garden soil (1:1, v/v). Effect of water extracts of vermicompost on growth of *R. solani* mycelium in Petri dishes was also analyzed. Disease suppression effect increased in proportion to the pot amendment rate of vermicompost. Vermicomposts not blended with *T. harzianum* effectively controlled damping-off of cucumber by *R. solani* (AG-4) at the rate of 20% and 30%. Vermicompost not blended with *T. harzianum* improved plant growth as well as that blended with *T. harzianum*. Analysis of the effect of water extracts of vermicompost on growth of *R. solani* mycelium in Petri dishes revealed antagonistic activity of a putative bacterium. Heat sterilization eliminated the suppressive and antagonistic effect by vermicompost and its water extracts, respectively. Activity of an antagonistic bacterium, which expressed a strong inhibition of growth of the pathogen mycelium, indicated that the type of suppressiveness against *Rhizoctonia* disease by the vermicompost is specific.

Key words: biological control, damping-off, seedling rot, soil-borne pathogens, *Trichoderma harzianum*, vermicomposting

Zusammenfassung

Die suppressive Wirkung von Wurmkompost aus Rinderdung, Baumrinde (*Salix* spp.) und nicht mehr verkaufsfähigen Kartoffeln und Äpfeln wurde gegenüber der durch *Rhizoctonia solani* Kühn (AG-4) verursachten Umfallkrankheit an infizierten, zwei Tage alten Gurkenkeimlingen (*Cucumis sativus* cv. Cevher) untersucht. Zu diesem Zweck wurde eine 1:1-Mischung von Sand und Gartenerde in Töpfen mit 0, 10, 20 oder 30% Wurmkompost gemischt und mit oder ohne Zugabe des Antagonisten *Trichoderma harzianum* Rifai (KRL-AG2) zur Anzucht der Keimlinge verwendet. Darüber hinaus wurde die Wirkung wässriger Kompostextrakte auf das Myzelwachstum von *R. solani* in Petrischalen untersucht. Die suppressive Wirkung des Komposts stieg mit zunehmendem Substratanteil. Kompost ohne Zugabe von *T. harzianum* besaß eine Wirkungseffizienz gegenüber der Umfallkrankheit an Gurkenkeimlingen von 20 bis 30%. Er förderte das Pflanzenwachstum genauso wie Kompost mit Zugabe von *T. harzianum*. Die wässrigen Kompostextrakte zeigten eine möglicherweise bakteriell bedingte antagonistische Wirkung auf das Myzelwachstum von *R. solani*. Eine Hitzesterilisation hob die suppressive und antagonistische Wirkung des Komposts bzw. seines wässrigen Extrakts vollständig auf. Eine deutliche antagonistische Wirkung eines Bakteriums auf das Myzelwachstum von *R. solani* bedeutete, dass die suppressive Wirkung des Komposts auf den Erreger spezifisch wäre.

Stichwörter: biologische Bekämpfung, bodenbürtige Pathogene, Keimlingsfäule, *Trichoderma harzianum*, Umfallkrankheit, Wurmkompost

1 Introduction

Along with the increasing awareness on environmental issues regarding pollution and food safety which resulted from intensive use of agro-chemicals as pesticides and fertilizers, application of compost products utilized as potting/soil amendments has received tremendous attention for their plant growth effect and biological control efficiency on soil-borne diseases since suggested by Hoitink et al. (1975, 1997). Vermicomposting as an environmentally sound technique of organic waste management with earthworms carrying out the composting process produces an economically high valuable end product named "vermicast", mostly called vermicompost. Over the last two decades, studies on application of vermicompost products as soil conditioners promoting plant growth (Kale et al. 1992; Atiyeh et al. 2000) and biological control agents on plant roots (Szczech et al. 1993; Szczech 1999) and foliar diseases (Scheurell and Mahaffee 2002; Zaller 2006) have proved to have greater potential than their counter outputs produced by conventional thermophilic composting methods (Dominguez and Edwards 1997).Disease suppression effects of vermicompost on some soil-borne plant pathogens such as *Pythium* (damping-off), *Rhizoctonia* (root rot), *Verticillium* (wilt) (Chaoui et al. 2002; Edwards and Arancon 2004), *Fusarium* (wilt) (Szczech 1999) and *Phytophthora* (Szczech and Smolinska 2001) have been reported. The suppression effect was suggested to have a biological nature rather than chemical since heat-sterilized vermicompost lost its suppressive effect (Szczech 1999). This effect has been ascribed to the unique microbial environment rich in both diversity and biomass. Mucus, excreted through the earthworm`s digestive canal, stimulates antagonism and competition between diverse microbial populations that results in production of some antibiotics and hormone-like biochemicals, boosting plant growth (Edwards and Bohlen 1996). In addition, the mucus accelerates and enhances decomposition of organic matter composing stabilized humic substances which embody mostly water-soluble phytohormonal elements (Edwards and Arancon 2004) and plant-available nutrients at high levels (Atiyeh et al. 2000).The disease suppression properties of compost products are influenced by the chemistry (Hoitink and Boehm 1999) and type (Szczech and Smolinska 2001) of parent waste material used for composting process as well as the method of composting process (Kanangara et al. 2000). Among various parent waste materials used for composting, lignocellulosic residuals have provided the most promising results with respect to efficacy of suppression against soil-borne plant pathogens (Hoitink and Boehm 1999).

Rhizoctonia solani Kühn, an exceedingly common soil-borne pathogen worldwide, causes diseases on many important crop plants including almost all vegetables and ornamentals, annual field crops, and perennial plants. Its extremely wide host range with a high degree of specificity, its enduring sclerotia and its ability of saprophytic activity render control of *Rhizoctonia* diseases troublesome (Agrios 1988). There has been an increasing intense need for efficient alternative strategies for control of soil-borne diseases, especially in organic farming. The objective of this study was to investigate the potential suppressive effect of the vermicompost produced from the mixture of willow bark (*Salix* spp.), cow manure, and vegetable wastes against the damping-off of *R. solani* AG-4 in cucumber seedlings. Pot amendments mixed at different ratios of vermicompost partially blended with *Trichoderma harzianum*, an effective antagonistic fungus widely used for the control of soil-borne diseases (Paulitz and Belanger 2001), were assessed for disease suppression and plant growth promotion of the vermicompost produced.

2 Materials and methods

2.1 Vermicompost production process

Agricultural wastes consisting of cow manure, apple, and potato were used for worm feeding. In addition, wood dust was added to vermicomposting mixture as a carbon source to maintain a well-balanced carbon:nitrogen ratio in the mixture and a sound material for inducement of the humification process. Separated cow manure was obtained from a local dairy operation farm (Tasliciftlik, Tokat, Turkey) where the barn is cleaned regularly by shoveling 3–4 times per week. Manure had been rested inside the barn at least two months before its use for vermicomposting. Shreds of willow tree bark (*Salix* spp.) obtained from a local carpenter were ground to wood dust by a grinding machine at Soil Science Department (Agricultural Faculty in Gazıosmanpasa University, Tokat, Turkey). Un-sold potato culls and apples provided by a local producer were ground before they were offered to the worms. Corrugated card board was shredded and used as the bedding material for worms. The mixture was inoculated with vermicompost which was harvested during propagation of earthworm culture. Vermicomposting materials were mixed thoroughly by hand then earthworms (*Eisenia foetida* Savingy) were allowed to feed on the mixture as the top layer consisted of wetted shreds of card board. No fertilization during or at the end of the vermicomposting activities was carried out.

The starting carbon to nitrogen (C: N) ratio of composting materials offered to worms throughout all vermicomposting activities was maintained between 20:1 to 25:1 (Larsen and Cartney 2000). This C: N ratio was calculated according to Rynk (1992). The total N and C values of manure, apple, and potato used for vermicomposting were determined by Kjeldahl analysis and combustion method, respectively. The C: N ratios for wood dust and card board given by Rynk (1992) were accepted as stated. Six months prior to vermicompost production process to start, the earthworms were propagated from a stock population (Rose Wood, Columbia, SC, USA) of which fresh weight was about 20 g.

Vermicomposting was carried out in a 1-m long by 1-m wide worm bed on a concrete floor at temperatures varied between 20 and 30 °C. Initial stocking density for each batch – an individual vermicomposting activity usually lasted around 3–4 months – was maintained between 2 to 5 kg m^{-2}. Initial organic material amount for each batch was determined as three times of the total fresh weight of the worms to be employed in the batch. Each worm bed was covered by a thick black coloured polyethylene cover to protect worms from light infiltration and prevent the moisture loss. The worm bed was mixed manually two times per week throughout vermicomposting processes and watered with tap water as needed. Weekly feeding rate of earthworms was 3 kg organic wastes-mixture per kg worms. The moisture content of the vermicomposting material was kept at field capacity to prevent nutrient loses through leaching as a result of excessive watering. After 14 to 16 weeks of vermicomposting, worms were allowed to migrate toward fresh feed material. The remaining vermicompost was left partially open at room temperature to dry it enough to be sieved using a chrome plated metal screen with 3 mm openings to separate vermicast from un-digested material.Sieved (harvested) vermicompost from each of the batches was collected and split into two parts; one part was blended with *Trichoderma harzianum* Rifai *KRL-AG2*, the powder marketed as T22 Planter Box (BioWorks Inc., Geneva, NY, USA) at a ratio of 10 g of the powder per 1 l of vermicompost. The vermicomposts either blended with *T. harzianum* or not were kept separately at room temperature inside a cupboard in the laboratory and watered as needed to prevent drying. Nine months after blending with *Trichoderma*, vermicomposts were used in pot experiments repeated twice.

2.2 Characterizing the vermicompost

Some physical, chemical, and biological characteristics of the vermicompost were determined. First, the vermicompost, rested for 3 months after harvest, was sieved through a 2-mm screen and then dried at room temperature before all analyses to be performed. Organic matter content was determined in a combustion oven (550 °C) as described by Kacar (2003). Water content of vermicompost was determined by drying 5 g of fresh vermicompost at 65 °C constant oven temperature. EC and pH were determined using an EC and pH meter in a 1:5 (v:v) and (1:2.5) vermicompost to 1 N KCl mixtures, respectively, after continuous shaking for 1 h. Total N content was determined by the Kjeldahl method as described by Kacar (2003). To determine the C: N ratio of the vermicompost, the combustion method (Kacar 2003) was carried out at 550 °C. Cation exchange capacity of vermicompost was measured using the ammonium acetate procedure as described by Kacar (2003). Five g of dried vermicompost was saturated with 1 N sodium acetate (pH 7) and then 1 M ammonium acetate was added to cast out sodium. The filtrates obtained from three repeats of the procedure described above were accumulated and used in flame photometer reading for Na. Micronutrient analysis of water extract of the vermicompost in distilled water (1:2, v/v) was carried out with an atomic absorption spectrophotometer (model Varian Vista). The number of bacteria, actinomycetes, and fungi in vermicompost were determined by plate count technique using selective media as described by Szczech (1999). The results were expressed as number of colony-forming units (cfu) per 1 g of vermicompost dried at 105 °C.

2.3 Phytotoxicity test

To determine possible phototoxic effect of vermicompost on cucumber seedlings, a total of 20 cucumber seeds (2 seeds per pot) were sown in pots, each consisting of 100% vermicompost (Szczech and Smolinska 2001). The germination rate was recorded at the end of the fourth week.

2.4 Disease suppression assay

The pathogen fungus, *R. solani* AG-4, was isolated from diseased cucumber plants (*Cucumis sativus* L.). The anastomosis group of the isolate was verified by pairing them with the isolates of *R. solani* AG-4. Inocula reproduction was carried out as described by Sneh and Ichielevich-Auster (1998), and stock liquid inocula were kept at 4 °C in 1-l bottles (Bradley et al. 2001).

Sand and garden soil were sieved through a 2-mm screen, then used as the base pot mixture at the ratio of 1:1 (v:v) and then autoclaved for 30 min at 121°C at 1.5 bar during three consecutive days. 'Cevher' (*Cucumis sativus* L.), a local cucumber cultivar, was preferred because of its high susceptibility to *R. solani* AG-4. Cucumber seedlings, germinated elsewhere with hypocotyls 5 to 8 cm in length, were transplanted into pots approximately 1 cm above the inocula. Inocula ratio per pot was applied as 3% (w/w) pathogen culture, grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) in 8-cm diameter Petri plates. Each pot assay was carried out in complete randomized design as three seedlings per half-liter pot, each containing only one of the vermicompost rates as 0%, 10%, 20%, and 30% either blended with *T. harzianum* or not, and either inoculated or un-inoculated with the pathogen as five replicates per treatment. The pots were kept at 18–20°C for the first two weeks and then at room temperature under day light. This procedure was followed for pot assays using autoclaved vermicompost as well.

Seedlings inoculated with the pathogen were harvested at the end of the fourth week to assess the disease development using the index: 0, no lesions; 1, lesions smaller than 2.5 mm; 2, lesions between 2.5 to 5 mm; 3, lesions greater than 5 mm; 4, lesions girdling the plant and leaves wilting; and 5, seedling damped-off or dead (Cardoso and Echandi 1987). The pathogen re-isolated from the diseased seedlings was also diagnosed. Those seedlings not inoculated with the pathogen were harvested at the end of the eighth week to evaluate the plant growth effect of the vermicompost. During the harvest, the content of each pot as a whole was placed inside a water filled bucket, and left for a while to release the soil attached to collateral roots. Then soil-free plants were cut at soil line to obtain root and shoot measurements as fresh and dry weights, and lengths. Fresh weights were recorded just after cutting the plant within two parts. Dry weights were determined after drying the plant parts at 55°C until their weights became constant.

2.5 Chlorophyll content

Chlorophyll was extracted from fresh seedling leaves (0.5 g) into 80% (v/v) acetone and centrifuged at 3000 g (5 min). Chlorophyll content of the extract was analyzed spectrophotometrically at 645 and 663 nm (Arnon 1949). Measurement of chlorophyll contents was performed twice in five replicates.

2.6 Effect of vermicompost water extracts on mycelium growth of R. solani in Petri dishes

Vermicompost mixed with distilled sterile water at the ratio of 1:2 (v/v) was stirred for 5 min then incubated at room temperature for 1 h. This mixture was filtered with a Whatman filter to obtain vermicast extract. One ml of this extract was pipetted into two opposite holes on PDA and 1 ml of sterile distilled water as control treatment was pipetted into the other two opposite holes. Then a disc (1-cm) of 3-day old mycelium of *R. solani* AG-4 was placed at each plate periphery. This procedure was carried out using tryptic soy agar (TSA; Merck, Darmstadt, Germany), yeast peptone glucose agar (YPGA; Fluka, Buchs, Switzerland) and malt extract agar (MEA; Merck). The plates in ten replicates per treatment were incubated at 20 Cº for 2 weeks, and then evaluated. This procedure was followed for an evaluation of the effect of heat-sterilized (20 min at 121 Cº) vermicomposts extracts on mycelium growth of *R. solani* AG-4 (Szczech 1999). Then, isolation of bacterial cultures from plates exhibiting clear zones of growth inhibition of the pathogen mycelium was also carried out using TSA.

2.7 Statistical analysis

The data of all variables measured through pot experiments repeated twice were averaged (Table 3), and the means were analyzed by ANOVA ($P < 0.05$). When the "null hypothesis" was rejected, means for treatments were grouped according to LSD (Least Significant Difference) procedure. The same procedure for statistical analysis was carried out to group mean values obtained from chlorophyll content measurements.

3 Results

3.1 Characteristics of the vermicompost

Some physical, chemical, and biological characteristics of the vermicompost and its micro- element contents are presented in Table 1 and Table 2, respectively.

3.2 Phytotoxicity test

When the cucumber seeds cv. 'Cevher' were germinated in the heat-sterilized potting medium consisting of sand and garden soil (1:1, v:v), the germination rate was 98%. However, the germination rate of cucumber seeds in pots with 100% of vermicompost was 32%. The high phytotoxicity level in 100% vermicompost was attributed to high EC level that accounts for high osmotic pressure in soil solution.

3.3 Disease suppression assays

Infection of cucumber seedlings by *R. solani* AG-4 was consistently inhibited in pots amended with 20% of vermicompost

Table 1: Some physical, chemical, and biological characteristics of the vermicompost used in pot experiments

Organic matter content (g kg^{-1})	4.60
Total N content (g kg^{-1})	15.2
EC (dS cm^{-1})	6.07
pH	7.61
CEC (cmol kg^{-1})	52.35
Moisture content (%) at harvest	95.0
Total fungal colony (CFU g^{-1} dwt)	1.9×10^5
Total bacterial colony (CFU g^{-1} dwt)	1.0×10^7
Total actinomycetes colony (CFU g^{-1} dwt)	0.7×10^7

CFU g^{-1} dwt: colony-forming units per gram dry weight.

Table 2: Vermicompost concentrations of micro-elements and heavy metals

Macroelements (g kg^{-1})	K	S	Ca	Mg	P	
	25.336	6.8855	0.31883	0.76476	106.8	
Microelements (ppm)	Fe	Mn	B	Cu	Zn	
	12.20	0.46	2.318	2.40	7.76	
Heavy metals (ppm)	Pb	Cd	Co	Ni	Cr	Al
	0.50	0.0094	0.57	1.08	0.125	3.46

Table 3: The average values for growth parameters of cucumber seedlings, either inoculated with *Rhizoctonia solani* AG-4 or not, in pot experiments

	Dose (%)[&]	Growth parameters					
		Fresh weight (g)		Dry weight (g)		Length (cm)	
		Root*	Shoot**	Root**	Shoot**	Root**	Shoot*
With pathogen, no *Trichoderma*	0	[a]0.037 ± 0.054[#]	[a]0.766 ± 0.305	[a]0.004 ± 0.004	[a]0.066 ± 0.076	[a]6.33 ± 4.72	[a]14.33 ± 3.78
	10	[a]0.103 ± 0.077	[ac]0.970 ± 0.121	[a]0.005 ± 0.004	[a]0.030 ± 0.006	[a]7.75 ± 2.21	[a]16.00 ± 1.82
	20	[b]0.1500 ± 0.054	[b]1.883 ± 0.556	[b]0.009 ± 0.003	[a] .190 ± 0.280	[b]12.00 ± 2.28	[b]24.33 ± 1.63
	30	[b]0.122 ± 0.092	[c]1.350 ± 0.234	[ab]0.006 ± 0.001	[a]0.039 ± 0.018	[b]11.50 ± 2.88	[c]20.66 ± 1.51
With pathogen, and *Trichoderma*	0	[a] 0.037 ± 0.026	[a] 0.590 ± 0.245	[a]0.002 ± 0.000	[a]0.066 ± 0.076	[a]3.25 ± 1.19	[a]8.25 ± 3.40
	10	[ba]0.090 ± 0.085	[a] 0.740 ± 0.351	[a]0.002 ± 0.001	[a]0.030 ± 0.006	[a]4.08 ± 1.80	[a]8.66 ± 1.86
	20	[b] 0.207 ± 0.175	[a] 0.812 ± 0.535	[b]0.004 ± 0.002	[a]0.190 ± 0.280	[b]6.50 ± 3.41	[a]11.25 ± 4.35
	30	[ab]0.117 ± 0.047	[a] 0.835 ± 0.399	[b]0.005 ± 0.002	[a]0.039 ± 0.018	[a]5.25 ± 1.25	[a]12.25 ± 4.03
Without pathogen, with *Trichoderma*	0	[a]0.491 ± 0.178	[a]2.348 ± 0.634	[a]0.033 ± 0.013	[a]0.176 ± 0.022	[a]19.66 ± 2.58	[a]22.66 ± 3.72
	10	[b]1.492 ± 0.293	[a]3.550 ± 0.335	[b]0.061 ± 0.004	[b]0.254 ± 0.023	[b]16.66 ± 2.25	[bc]30.16 ± 4.44
	20	[bc]1.308 ± 0.376	[b]9.815 ± 1.933	[b]0.067 ± 0.018	[c]0.671 ± 0.038	[b]15.00 ± 2.82	[c]33.00 ± 5.25
	30	[c]1.083 ± 0.363	[b]8.837 ± 2.034	[b]0.063 ± 0.007	[c]0.65 ± 0.083	[b]14.66 ± 1.86	[ab]26.50 ± 1.97
With no pathogen, no *Trichoderma*	0	[a]0.388 ± 0.158	[a]4.510 ± 1.403	[a]0.028 ± 0.009	[a]0.329 ± 0.124	[a]19.33 ± 2.33	[a]25.83 ± 3.31
	10	[a]1.177 ± 1.193	[a]7.670 ± 3.887	[a]0.045 ± 0.046	[ab]0.530 ± 0.252	[a]15.75 ± 4.92	[a]30.75 ± 7.18
	20	[a]0.665 ± 0.478	[a]8.128 ± 4.205	[a]0.040 ± 0.028	[b]0.634 ± 0.301	[a]16.16 ± 5.84	[a]33.16 ± 12.85
	30	[a]0.886 ± 0.641	[a]5.627 ± 2.946	[a]0.036 ± 0.021	[ab]0.425 ± 0.240	[a]18.50 ± 4.68	[a]27.33 ± 6.97

[&] As the percent of the vermicompost with respect to total pot volume.
[#] Figures following ± are standard deviations of the means.
*, ** Means with different letters are different at the 0.05 and 0.01 level of significance, respectively.

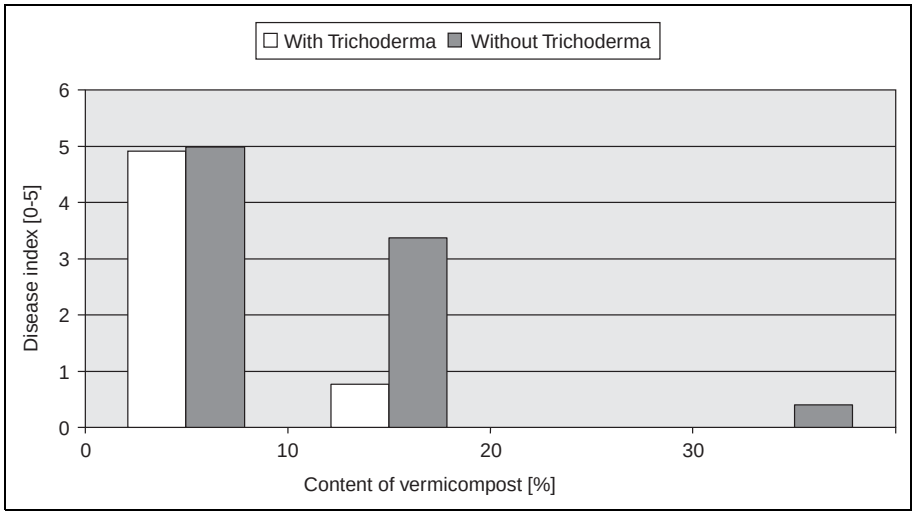

Fig. 1: Effect of vermicompost, either blended with *Trichoderma harzianum* or not, on damping-off of 4 weeks-old cucumber seedlings inoculated with *Rhizoctonia solani* (Ag-4).

not blended with *T. harzianum* (Fig. 1). Also, 30% of vermicompost not blended with *T. harzianum* effectively controlled damping-off of cucumber seedlings caused by *R. solani*. Disease index of seedlings in 30% of vermicompost, not blended with *T. harzianum*, was 0.4 out of 5.0 and only faint disease symptoms on a few seedling roots were observed. The high EC that accounts for high osmotic pressure in soil solution in 30% of vermicompost would cause improper growth conditions, rendering seedling roots to be more vulnerable to pathogen infestation. Compared to that not blended with *T. harzianum*, blended vermicompost sustained an effective disease control (0.74) even at low amendment ratio (10%). In general, the decrease in disease incidence in pots with vermicompost either blended with *T. harzianum* or not was strongly correlated with the increase in the amount of vermicompost (Fig. 1). Expectedly, the most severe symptoms that occurred in the seedlings grown in 0% and 10% of vermicompost not blended with *T. harzianum* were observed as severe growth retardation, growth inhibition, and death of the seedlings.

The t-test statistics on the average values for root length, root fresh weight, root dry weight, shoot fresh weight, and shoot length of the seedlings not inoculated with the pathogen revealed that *T. harzianum* had no significant effect ($P > 0.05$) on root and shoot dry weight (Table 3). In general, the vermicompost not blended with *Trichoderma* stimulated plant growth just as blended vermicompost in all experiments (Fig. 2).

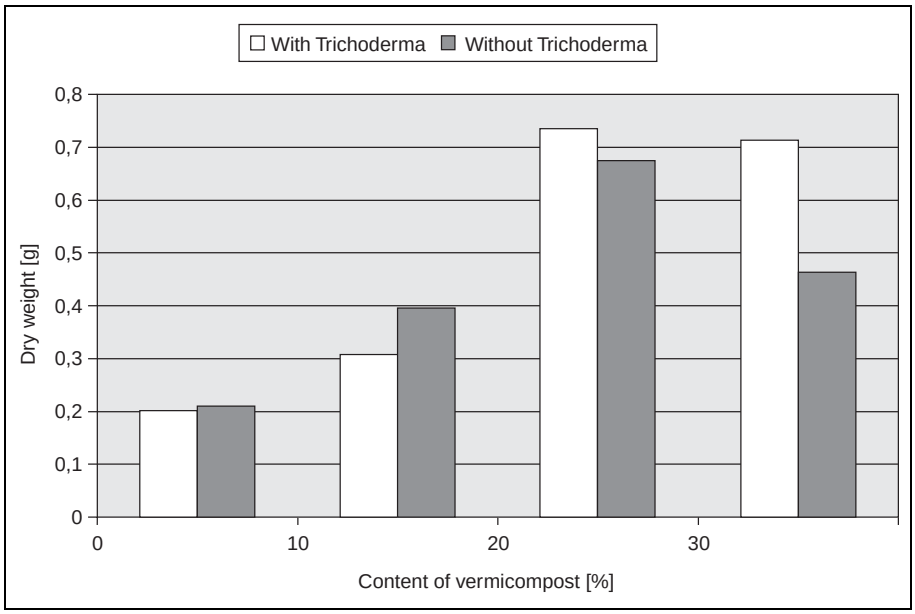

Fig. 2: Effect of vermicompost, either blended with *Trichoderma harzianum* or not, on dry weight of 8 weeks-old cucumber seedlings not inoculated with *Rhizoctonia solani* (Ag-4).

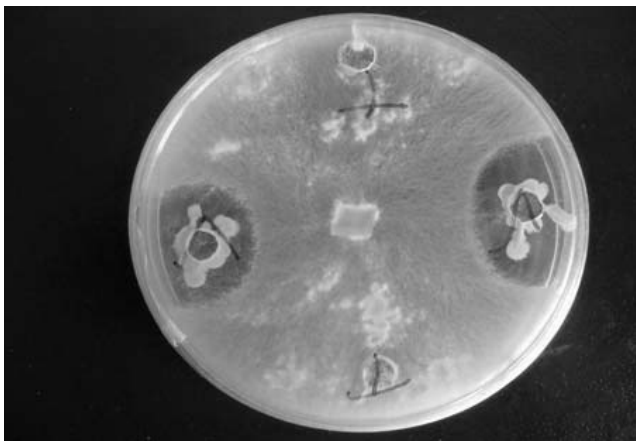

Fig. 3: Antagonism of water extract of the vermicompost not blended with *Trichoderma harzianum* against *Rhizoctonia solani* (AG-4) on malt agar medium. Un-sterilized vermicompost's water extract was injected into the right and left holes and sterile water into the top and bottom holes, and then a mycelium disc of *Rhizoctonia solani* (AG-4) was placed in the center of the plate.

Although seedlings in pots with 30% of vermicompost (V30) blended with *Trichoderma* appeared to have tolerated side effects of high EC, exhibiting high values of fresh and dry weights, seedlings in pots with 10% of vermicompost not blended with *Trichoderma* had greater fresh and dry weight values (Table 3). Plant growth effect was maintained at the greatest level at 20% of with vermicomposts (V20) either blended with *Trichoderma* or not. Observations throughout experiments showed that seedlings in 30% of vermicompost, with and without *Trichoderma*, had reduced leaf size, leaf number, lateral root formations, and plant height (Table 3). In addition, the leaf colour of seedlings of V30 was slightly lighter than those of V20.

3.4 *Effect of vermicompost water extracts on mycelium growth of* R. solani *in Petri dishes*

The un-sterilized water extracts of the vermicompost inhibited mycelium growth of *R. solani* AG-4 on all types of agar media (Fig. 3). This effect was observed as clear inhibition zones of at least 1 cm for all types of the media except PDA (0.5 cm). In addition, overgrowing of a/some bacterial formation/s derived from the vermicompost water extract toward the pathogen mycelium was also observed on all media, except PDA. Those bacterial formations were isolated onto TSA, continued to express a strong inhibition of growth of the pathogen mycelium. The isolated bacteria on TSA induced inhibition zones greater than 1.5 cm.

3.5 *Evaluation of heat-sterilized vermicompost extracts*

The heat sterilized water extracts of the vermicompost completely lost their inhibition effect on mycelium growth of *R. solani* AG-4 in all media plates. This outcome was expected since heat sterilization of the vermicompost during pot experiments eliminated the disease suppression effect of the vermicompost.

4 Discussion

In the present study, the vermicompost, not blended with *T. harzianum* Rifai KRL-AG2, sustained effective control of damping-off of cucumber seedlings caused by *R. solani* AG-4 in the pots amended at the rates of 20 and 30% of vermicompost. Our results correspond to previous studies on application of the vermicompost products for control of root diseases, revealing some consequential factors constituting the disease suppression effect, such as the biotic nature of the impact (Szczech 1999; Edwards and Arancon 2004), the kind of parent material from which vermicompost had been derived (Szczech and Smolinska 2001), and proportion of the vermicompost applied (Szczech 1999). On the other hand, a possible indirect influence by physical and chemical characteristics of the vermicompost on expression of the suppressiveness effect was out of the scope of this study, although this needs to be explored as well.

Suppressiveness of vermicompost produced from animal manure (Szczech 1999; Szczech and Smolinska 2001) and aerobic compost from bark (Chef et al. 1983; Kwok et al. 1987; Boehm et al. 1993; Erhart et al. 1999) on fungal soil-borne root diseases have been previously reported. Suppressiveness of mature tree bark composts (Boehm and Hoitink 1992; De Custer and Hoitink 1999) was attributed to the possibility that tree barks are more likely to harbour a diverse microbial

Table 4: Average total chlorophyll amounts of leaves of 4 weeks-old cucumber seedlings, either inoculated or not with *Rhizoctonia solani* AG-4, in pots amended with vermicompost not blended with *Trichoderma harzianum*

	Chlorophyll amount (mg ml^{-1})	
Vermicompost ratio (%)[&]	Seedlings inoculated with *R. solani*[*]	Seedlings not inoculated[*]
0	17.0 ± 0.56[#] A,c	16.2 ± 0.14 A, c
10	16.0 ± 0.28 A,c	17.7 ± 0.14 A, c
20	18.0 ± 0.35 B,b	28.4 ± 0.26 A, b
30	23.5 ± 0.15 B,a	74.9 ± 0.07 A, a

[&] As percent of the vermicompost with respect to total pot volume.
[#] Numbers following ± are standard deviations of the means.
[*] All means are different at 0.05 level of significance.
abc indicate differences ($P < 0.05$) among rows.
ABC indicate differences ($P < 0.05$) among columns.

variety of biological control agents derived from the environments such as forests or open areas close to forests. In addition, mucus excreted by worm digestive system amplifies both diversity and biomass of bacteria, fungi (Szczech 1999), and actinomycetes (Szczech and Smolinska 2001) in vermicompost up to a much greater level than the parent material that promotes microbial competition (Edwards and Arancon 2004). In addition, the decomposition level of the organic material was stated to have a substantial effect on both composition and activities of the biological control agents (Hoitink and Boehm 1999). The low level of readily available energy sources in highly decomposed organic materials was pointed out to stimulate micro-biostatis (Krause et al. 2001), inducing activities of biological control agents. Since the humification process continuous after vermicast had been ejected, the additional curing period of vermicompost, 9 months in our case, would intensify the activities of naturally occurring biological control agents. It is likely that those agents, antagonistic against *R. solani* AG-4 in vermicompost, would be derived from either tree bark or manure or the both.

Inhibition of mycelium growth of *Rhizoctonia* in Petri dishes by putative bacterial formation/s, antagonistic against *R. solani* AG-4 derived from un-sterilized water extracts of the vermicompost, supported the descriptions made by Hoitink et al. (1997) on the nature of suppressiveness toward *Rhizoctonia* diseases by organic materials being specific rather than general. The effective suppression of the pathogen in pots amended with 20 and 30% of vermicompost not blended *T. harzianum* was thought to be the outcome of antagonistic activity of the prospective biocontrol agent/s observed in Petri dishes. The suppression impact was lost both in pot experiments and in Petri dishes upon heat sterilization. This signifies the microbiological nature of the suppression effect by the vermicompost as stated by (Krause et al. 2001).

In this study, the disease suppression effect of the vermicompost was defined to be correlated with the proportion of the vermicompost amount used in the pot amendments, reaching absolute suppressive level at 20% (v/v) which agrees to the results reported by Szczech (1999). In addition, Noble and Coventry (2005) reported that compost amendment rates of at least 20% (v/v) were mostly needed to maintain consistent disease suppressive effect, particularly in container-based media. The amendment ratio of 10% vermicompost not blended with *T. harzianum* was thought to lack sufficient biomass of specific biocontrol agent/s, contrary to that the same amount of vermicompost blended with *T. harzianum* maintained effective disease control. This result agrees to the idea that application of combined biological control agents with antagonistic activity toward *R. solani* increases the likeliness of maintaining a consistent and efficient suppressiveness against the pathogen (Krause et al. 2001).

In general, observations throughout the pot trials such as retardation on the leaf numbers and area, root and shoot length, and lateral root formation on plants grown in 30% (v/v) of vermicompost either blended with *T. harzianum* or not suggest the presence of salinity stress derived from excess amount of vermicompost, leading to damages in macro- and micro-nutrient taking mechanisms as suggested by Grattan and Grieve (1999). In addition, salt stress observed at 30% of vermicompost would create a hostile environment, a limiting factor for the activities of prospected bacterial biocontrol agent/s antagonistic against *R. solani* AG-4. Fresh and dry weights and lengths of cucumber seedlings were affected negatively by high amount of vermicompost (30%), indicating salt stress that resulted in growth retardation as indicated by Abd-Allah et al. (1992). We observed a decrease in both plant height and unit leaf area similar to that reported by others (Folagatti and Blanco 2000; Wang and Nil 2000). On the other hand, the leap in total chlorophyll content at 30% of vermicompost (Table 4), in spite of high Na content of the vermicompost, particularly deserves attention since Kaya et al. (2003) reported that salinity stress induced lower biomass production, chlorophyll concentration, and fruit yield in cucumber. Chartzoulakis (1994) also pointed out reduction in photosynthesis and photosynthesis area in cucumber under salt stress. Expected growth retardation and decrease in chlorophyll content at 30% of vermicompost might be alleviated by the presence of some plant growth promoting compounds or biochemicals with phytohormonal effects in vermicast as previously stated by Edwards and Arancon (2004).

In conclusion, the inhibition of infection of cucumber seedlings by *R. solani* AG-4 in pots with 20 and 30% (v/v) of vermicompost not blended with *T. harzianum*, is suggested to be the outcome of the activities of prospective biocontrol agent/s. The loss of suppression effect upon heat sterilization of the vermicompost and its water extracts supported the microbiological nature of the suppressive effect as mostly stated in the literature. In future studies, utilization of this putative biocontrol agent/s antagonistic toward *R. solani* AG-4 will be investigated in detail. Potential chemical factors affecting suppressiveness by vermicompost should be assayed as well.

Acknowledgements

This study was supported by Gaziosmanpaşa University, Tokat, Turkey (2006/16). Authors thank Dres. Izzet Kadioglu, Sonay Sozudogru OK, and Yener Okatan for their comments and critics during the study.

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