Comparison of compost and compost tea effects on Cress (*Lepidium sativum* L.)

Mozhgan Farzamisepehr[1]* and Azade Reza[1, 2]

1. Department of Biology, Faculty of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran
2. Iranian Research Organization for Science and Technology, Tehran, Iran

Abstract

This study was conducted to determine the effect of compost and compost tea on growth and physiological parameters of *Lepidium sativum* L. 30-day-old Cress plants treated with compost and compost tea were harvested and different growth and physiological parameters were measured. Compost tea application increased RGR, SLA, ULR and RL_AGR. The contents of Chlorophyll a and Chlorophyll b in the plants significantly increased at the media supplemented with compost 25% and compost tea 75%. While total soluble sugars in leaves of the treated cress plants decreased total non-soluble sugars, proline and total protein contents increased with an increase in compost and compost tea contents in culture medium.. From among different measured macro elements, K was further observed as compost levels rose in the growing media Finally, the highest contents of micro-elements were measured at medium supplemented with 75% compost and compost tea.

Keywords: Lepidium sativum; compost; compost tea*;* growth; chlorophylls; proline; sugars; macro and micro elements;

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Introduction

Cress (*Lepidium sativum* L.) otherwise known as garden cress, garden cress pepper weed, or garden pepperwort is a fast growing annual herb belonging to the Brassicaceae family. This plant is native to Iran and other parts of Asia but is widely cultivated in temperate climates throughout the world for various culinary and medicinal uses (Gokavi et al., 2004). Compost is commonly defined as the aerobically stabilized or matured organic matter, though anaerobic processes can also lead to the production of stabilized (or mature) organic material. The desired – but not sole – use of compost is its application to crops with the goal to enhance plant growth. High quality compost should be both mature and stable. A thorough understanding of the characteristics of growing media, which greatly affect plant growth, is essential to improve the reuse of biosolids as a peat substitution in container cultivation. Increasing awareness of environment-related issues as well as the need to dispose or reuse ever-increasing amounts of waste and to reduce

*Corresponding author
E-mail address: farzamisepehr@iau-saveh.ac.ir
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the consumption of nonrenewable resources like peat have encouraged the use of composted biosolids in agriculture (Evanylo and Daniels, 1999; Bugbee, 2002; Papafotiou et al., 2004). Many studies based on the morpho-physiological response of plants have focused on factors that limit the use of compost as a substrate (e.g., salinity, pH or physical properties) and on its tolerable quantity in growing media (Bragg et al., 1993; Evanylo and Daniels, 1999; Bugbee, 2002; Atiyeh et al., 2000, 2001; Papafotiou et al., 2004). There are also a number of studies that have assessed the accumulation of nutrients and trace elements in plant tissues following sewage sludge and compost treatments (Smilde, 1981; Liphadzi and Kirkham, 2006). However, very few investigations have addressed the role of the entire element pool. Recent studies have focused on the importance of the nutrient balance in plant tissues for their fitness (Linhart et al., 2001; Alonso and Herrera, 2001, 2003), even though element uptake and concentration in plant tissues can vary depending on many factors, including species, temperature, salinity and salt mix in media (Fritiov et al., 2004; Weis et al., 2004). Recently the use of aerated water extracts or teas from compost to control foliar diseases has been explored (Ryan et al., 2005). The aim of this research is study of production potential of cress by compost and compost tea.

Materials and Methods

Composting procedures

1.5×1.5 m^2 area was designated for the composting and the first compost layer (about 20×25 cm thickness) including green material of new herbs and green material of old herbs such as weeds and cutgrass were spread over the area. The second layer, about 10-15 cm in thickness, which included dry herbaceous material such as dry leaves, was then placed over the first layer. Finally, for the top layer fresh or dry bovine dung was used with a thickness of about 10-15 cm. To provide each layer with the necessary humidity, each layer was sprinkled with some water. Also for better ventilation, a perforated pipe was placed at the center of the mass. To protect the area from rain and animals, a layer comprising mixture of dung and pug was used to cover compost mass. Finally compost was ready when there was no heat in the mass and the volume of mass was halved and had a good smell (Siddiqui et al., 2008).

Preparation of compost tea

Compost tea was prepared from 3-month-old compost according to the method described by Brinton and Droffner (1995) and Siddiqui et al. (2008). Compost and tap water were mixed with the ratio of 1: 5 (w/v), in polyethylene non-degradable containers with covers. The mixtures were supplied with aeration using an aquarium pump and left at ambient temperature for 12 days prior to filtering through double layered cheese cloth. Three 100 ml samples were taken from each container and were either filter-sterilized (micron membrane filter of 0.20 in nylon, low extractable membrane, Sartorius AG, Gottingen, Germany) or heat-sterilized (autoclaving at 121 °C for 20 min).

Cultivation of cress

Cress seeds (*Lepidium sativum* L.) were sown in plastic pots (20 cm in diameter), each containing 3000 g sphagnum peat moss potting material mixed with different concentrations of compost and compost tea in early spring. Mixtures were formed by 25–50–75 % (w/w) compost and 25–50–75 % (v/w) compost tea. A commercial potting medium containing black and white peat moss (2:1 v/v) was used as control. After sowing, the pots were placed in a greenhouse until the seeds germinated. After germination, 1000 cress seedlings of similar size were chosen per pot and the others were removed. Plants were grown under 60% black knitted shade cloth without temperature control. The pots were arranged in a randomized complete block design with three replications. All treatments were drip irrigated with tap water ranging from 1 to 2 dm^3 per plant per day. In the case of compost tea treatments, every pot was irrigated just by compost tea ranging from 1 to 2 dm^3 per plant per day. The growth parameters of treated plants were measured 30 days after starting of treatments. Other physiological

parameters were measured on 30-day-old treated cress plants.

Growth parameters determination

Fresh weight (FW) and dry weight (DW) of treated cress plants were determined after desiccation at 70 °C for 48h. Leaf water content area (LWCA), relative growth rate (RGR), relative leaf growth rate (RLAGR), specific leaf area (SLA) and unit leaf rate (ULR) were measured using the method suggested by Hunt et al. (2002).

Determination of enzymatic activities

The procedure for extraction of Peroxidase (PO) was adapted from Mozzetti et al (1995). Two grams of frozen (liquid nitrogen) cress leaves were homogenized in cold 0.05 M phosphate buffer at pH 7 containing 0.5 g polyvinylpolypyrrolidone (PVP) in a 1:5 tissue to buffer ratio using an ice cold mortar and pestle. The extracts obtained were used for assaying the activities of Peroxidase and Catalase. The homogenates were mixed using a vortex mixer and insoluble materials were separated by centrifugation at 14,000g for 20 min at 4 °C. All extracts were stored at -70 °C until assayed.

1. Peroxidase assay (PO)

Twenty micro-liters of the extract from each sample was added to 3 ml of assay mixture consisting of solution of 0.1 M sodium phosphate buffer (pH 6.0), 1 mM hydrogen peroxide and 0.1 mM O-methoxyphenol (guaiacol). The mixture was blended thoroughly and the increase in absorbance was monitored at 470 nm using a spectrophotometer (Spectronic _ 20 Genesis TM) for 1 min. Peroxidase activity was determined as unit mg-1 protein.

Catalase assay (CAT)

The catalase activity was assayed using Chance and Maehli (1995) method with the following modification: 5 ml of assay mixture for catalase activity contained 300 µM of phosphate buffer (pH 6.8) 100 µM of H2O2 and 1 ml of the twice diluted enzyme was extracted. After incubation at 25 °C for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H2SO4 and the residual H2O2 was titrated against 0.01(N) of KMnO4 until a faint purple color persisted for at least 15 sec. One unit of catalase activity is defined as the amount of enzyme which breaks down 1 µ mol of H2O2/min under the described assay condition.

Determining physiological parameters

Proline assay

The method suggested by Bates et al. (1973) was used to measure the proline content. In brief, 100 mg of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. To make acid ninhydrin, 1.25 g ninhydrin was added to 30 ml glacial acetic acid and warmed until dissolved. 20 ml 6 M phosphoric acid was added to the solution. Then two ml glacial acetic acid and 2 ml acid ninhydrin were added to 100 µl of the extract for 1 h and warmed at 100 °C and the reaction was then completed in an ice bath. The reaction mixture was added 1 ml toluene. The mixture was then warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20 –100 µg.

Protein determination

Protein analysis was carried out using the method described by Lowry et al. (1951). In brief, shoots from different plants were dried in 100 °C oven for 24 hours and 0.02 g of dried samples were used for extraction. Samples were homogenized in 2 ml of Tris-HCl buffer in ice. The homogenized solution was centrifuged for 30 min at 5000 rpm. Afterwards, upper layers of the mixture were collected. 0.5 ml of extracted protein was added to 4.9 ml of Na_2CO_3 (2%), 0.5 ml sodium-potassium tartrate and 0.05 ml copper sulphate (1%). The mixture was kept at room temperature for 10 min. Then 0.5 ml Foline which is a phenol reagent was added into each of the tubes which were kept again in room temperature for another 45 minutes. At the end

of the reaction, the optical density was measured at 700 nm.

Soluble and non-soluble carbohydrate determination

Soluble carbohydrate analysis was performed using the method described by Kochert (1978). Dry weight of shoot and root parts of the collected plants were used for separation of carbohydrate. 40 ml ethanol (80%) was gradually added to about 0.1 g plant material and the mixture was heated up in water bath for 1 hour before it was centrifuged at 2500 rpm for 10 min. About 2 ml of the prepared sample was vigorously shaken with 2 ml of phenol solution (5%) and after addition of 5 ml concentrated sulphuric acid, optical density of the sample was measured at 700 nm.

Measuring chlorophylls contents

Chlorophyll contents were determined using the method suggested by Arnon (1949). According to this method, 0.2 g fresh texture of leaf was weighed and ground in china mortar containing 80% acetone. Then 5 ml acetone was added to it and the solution volume was reached to 15 ml. Three milliliter of this solution was poured into a cuvette and its absorption intensity was read at 663, 647 nm by spectrophotometer. For regulating spectrophotometer, 80% acetone was used as witness. Pigments contents were determined in terms of mg/g leaf fresh weight.

Determination of elements

Cress leaves were dried at 70 °C for 48 h and extracted by dry ashing and use of HCL (2M) at 80 °C for measuring elements. The amounts of Ca, Cu, Fe, K, Mg, Mn and Zn in extractions were measured by an atomic absorptions spectrophotometer (Phoenix 896, England) using relevant standard solutions.

Statistical analysis

Experiments were arranged in a complete randomized design (CRD). Each treatment (soil as control, compost and compost tea at 3 levels) was replicated six times. The obtained data was analyzed with a one-way ANOVA using the Duncan test in SPSS (ver. 14).

Results

Plant growth

Table 1 compares the effect of compost and compost tea on different growth parameters. A glance at the table reveals that contents of RGR, SLA, ULR and RLAGR at medium supplemented by compost tea 50% were higher than other media. High levels of compost and compost tea (75%) decreased growth parameters. Also, leaf water content area in different cress plant grown in different media did not show a regular pattern, and differences with control were not significant at $p<0.05$.

Table 1
The effect of compost and compost tea on growth parameters of cress plants

Culture medium	Relative Growth Rate (RGR) [g/g day]	Specific Leaf Area (SLA) [cm^2g^{-1}]	Unite Leaf Rate(ULR) [g/m^2day]	Relative Leaf Area Growth Rate (RL_AGR) [$cm^2cm^{-2}d^{-1}$]	Leaf Water Content Area(LWCA) [$g(H_2O)m^{-2}$]
Soil (control)	$0.07\pm0.011^{b*}$	303.10 ± 43.82^{ab}	6.17 ± 2.04^{ab}	0.03 ± 0.001^{b}	1525 ± 420.58^{a}
Compost (25%)	0.07 ± 0.005^{b}	166.01 ± 23.81^{a}	8.43 ± 0.9^{ab}	0.05 ± 0.001^{cd}	1385 ± 140.38^{a}
Compost (50%)	0.08 ± 0.011^{b}	473.55 ± 174.39^{b}	7.44 ± 1.23^{ab}	0.03 ± 0.004^{b}	1442 ± 243.52^{a}
Compost (75%)	0.04 ± 0.004^{a}	249.94 ± 29.33^{ab}	3.29 ± 0.93^{a}	0.01 ± 0.0002^{a}	798 ± 280.08^{a}
Compost tea (25%)	0.08 ± 0.005^{b}	271.86 ± 52.69^{ab}	9.80 ± 1.43^{b}	0.03 ± 0.003^{b}	1667.5 ± 304.50^{a}
Compost tea (50%)	0.10 ± 0.004^{c}	350.60 ± 63.27^{ab}	10.63 ± 3.36^{c}	0.08 ± 0.003^{d}	1515 ± 632.09^{a}
Compost tea (75%)	0.07 ± 0.002^{b}	349.27 ± 15.35^{ab}	5.66 ± 0.3^{ab}	0.03 ± 0.003^{b}	1015 ± 169.04^{a}

*The same letter in columns shows no significant difference; mean comparison is carried out by Duncan multiple range test.

Physiological parameters

Table 2 shows the physiological parameters determined in the study. The findings suggest that Chl a and Chl b contents of cress plants were increased significantly at media supplemented with compost 25% and compost tea 75%. Total soluble sugars in leaves of the treated cress plants decreased by increasing compost and compost tea contents in culture medium in comparison with plants grown in soil. On the other hand, total non-soluble sugars increased significantly by adding compost tea to the cultured medium. The highest content of total non-soluble sugars was observed in cress plants in the medium with 25% compost tea, approximately 14 times more than in control). Proline contents in cress plants increased significantly by increasing compost and compost tea. The highest proline content was determined in cress plants grown in compost 75% medium, approximately 3 times more than in control. Total protein contents of cress plants showed an increasing and then decreasing pattern. In fact, protein contents increased at 25% and 50% of compost content in culture medium and then decreased significantly. Although total protein contents of plants increased by compost tea treatment, the differences were not significant compared with control.

Element contents

The contents of 3 macro-elements and 4 micro-elements were measured in cress plants cultured in different concentrations of compost and compost tea media (Table 3). No differences were observed between Ca contents of cress plants at different treatments. Mg contents increased a little by increasing compost and compost tea contents. The highest contents of Mg were observed in 75% compost and compost tea media and the difference with control was significant at $p<0.05$. An increase in K was observed as compost levels rose in the growing media. Also, Cu, Zn, Mn and Fe contents of cress plants increased by increasing compost and compost tea in culture media. The highest contents of these four micro-elements were measured in the media supplemented with 75% compost and compost tea.

Discussion

Table 2
The effect of compost and compost tea on physiological parameters of cress plants

Culture medium	Chl a (mg g^{-1}FW)	Chl b (mg g^{-1}FW)	Total Soluble Sugars (µM g^{-1}FW)	Total Non-soluble Sugars (µM g^{-1}FW)	Proline Contents (mg g^{-1}FW)	Total Proteins (mg/g FW)
Soil (control)	$0.23\pm0.016^{a*}$	0.14 ± 0.0009^{a}	40.95 ± 12.29^{c}	1.45 ± 0.4^{a}	21.3 ± 0.15^{a}	2.98 ± 0.34^{a}
Compost (25%)	0.91 ± 0.061^{d}	0.40 ± 0.045^{ab}	15.67 ± 7.06^{abc}	1.53 ± 0.36^{a}	30.45 ± 0.03^{c}	4.11 ± 0.14^{cd}
Compost (50%)	0.54 ± 0.073^{bc}	0.24 ± 0.025^{a}	7.27 ± 0.69^{ab}	0.37 ± 0.1^{a}	56.31 ± 0.15^{f}	4.71 ± 0.4^{d}
Compost (75%)	0.33 ± 0.031^{ab}	0.17 ± 0.012^{a}	0.76 ± 0.12^{a}	0.39 ± 0.22^{a}	64.33 ± 0.06^{g}	0.04 ± 0.11^{cd}
Compost tea(25%)	1.52 ± 0.137^{e}	0.51 ± 0.043^{bc}	39.51 ± 8.25^{c}	14.73 ± 2.02^{c}	24.8 ± 0.33^{b}	3.54 ± 0.23^{abc}
Compost tea(50%)	2.27 ± 0.017^{f}	0.80 ± 0.010^{d}	36.95 ± 13.7^{c}	6.47 ± 1.55^{b}	35.11 ± 0.32^{d}	3.17 ± 0.005^{ab}
Compost tea(75%)	2.69 ± 0.0151^{g}	1.32 ± 0.0148^{e}	24.7 ± 4.13^{abc}	6.78 ± 0.91^{b}	44.72 ± 0.1^{e}	3.96 ± 0.09^{c}

*The same letter in columns shows no significant difference; mean comparison is carried out by Duncan multiple range test

Table 3. The effect of compost and compost tea on some element contents in cress plants

Culture Medium	Ca %DW	Mg %DW	K %DW	Cu mg Kg^{-1} DW	Mn mg Kg^{-1} DW	Zn mg Kg^{-1} DW	Fe mg Kg^{-1} DW
Soil (control)	19.46±0.44^{d*}	0.2±0.0152^{a}	4.45±0.221^{a}	0.29±0.0057^{a}	50.89±5.17^{a}	165.8±3.22^{a}	534.49±8.74^{a}
Compost (25%)	18.07±2.3^{a}	0.27±0.0057^{b}	4.91±0.0375^{ab}	0.4±0.0057^{b}	61.84±0.42^{b}	178.19±0.98^{ab}	717.06±1.55^{b}
Compost (50%)	18.69±0.08^{abc}	0.28±0.0028^{bc}	6.21±0.0375^{c}	0.47±0.0033^{d}	62.88±0.02^{b}	184.55±0.69^{abc}	798.45±1.62^{d}
Compost (75%)	19.08±0.07^{cd}	0.29±0.0028^{c}	6.41±0.0866^{c}	0.51±0.0028^{e}	68.01±0.26^{bcd}	198.06±0.49^{bc}	827.72±1.25^{ef}
Compost tea (25%)	18.93±0.04^{abc}	0.285±0.0028^{bc}	5.16±0.0783^{b}	0.43±0.0144^{c}	62.02±0.13^{b}	179.24±0.65^{ab}	717.17±0.2^{b}
Compost tea (50%)	19.38±0.23^{cd}	0.285±0.0028^{bc}	5.99±0.0145^{c}	0.47±0.0032^{d}	63.69±0.26^{bc}	186.24±1.03^{abc}	802.99±0.23^{de}
Compos tea (75%)	19.54±0.22^{d}	0.305±0.0028^{cd}	6.46±0.0568^{c}	0.55±0.0057^{f}	69.41±0.14^{cd}	200.77±0.4^{c}	826.01±5.98^{ef}

*The same letter in columns shows no significant difference; mean comparison is carried out by Duncan multiple range test

Plant growth parameters like relative growth rate, specific leaf area, unit leaf rate, relative leaf area growth rate, and leaf water content area were significantly affected in plants receiving compost and compost tea at low and medium level. High rate of compost and compost tea decreased all growth parameter contents. The observed effect of compost and compost tea on plant growth of cress may be attributed to better availability of plant growth regulators and humic acid, which is produced by the increased activity of microbes (Arancon et al., 2004). It is demonstrated that during composting, microbes like fungi, bacteria, yeasts, actinomycetes, algae etc., are capable of producing auxins, gibberellins etc., in appreciable quantity (Brown, 1995; Arancon et al., 2004) and this considerably affects plant growth (Tomati et al., 1987; Arancon et al., 2006). The dissimilar response of plants to different doses of compost and compost tea might be explained by production of growth-promoting substances in lesser quantity with lower doses of compost and compost tea than higher doses (Arancon et al., 2004). In this study, chlorophylls contents increased by increasing compost and compost tea in cultured media. In addition, Mg concentration in plant tissue increased. There was a positive relationship between Mg stored in plant tissue and chlorophylls contents in the compost treated plant (Grigatti et al., 2007). The increasing of total non-soluble sugars in cress plants in compost tea treatments showed an increase in total metabolic activity caused by compost tea. On the other hand, total soluble sugars decreased by increasing of compost and compost tea. It is also possible that when compost is used hexose metabolism increases, leading to water balance in the plants (Prado et al., 2000). The proline contents of cress plants were also increased by increasing compost and compost tea in media. This was further supported by studies with a variety of plants demonstrating that stress or different environmental conditions induced conversion of hexose and other carbohydrates such as sucrose into sugar alcohols (polyols) and proline (Perez-Alfocea and Larher, 1995). Increasing of peroxidase or catalase activity would result in faster removal of H_2O_2 via the ascorbate glutathione cycle helping to alleviate oxidative damage. Enzymes such as poly phenol oxidase (PPO) and peroxidase have been demonstrated to be responsible for the oxidation of phenolic compounds into anti-microbial quinones in plant cells infected by phytopathogens and thus conferring disease resistance during incompatibility reactions (Chittoor et al., 1990). Hence, peroxidase induction, as a result of biological and chemical activities of the compost tea described in this paper, might have resulted in the formation of quinones. PO, PPO and CAT are directly involved in regulation of many physiological processes in plants (Anguelova et al., 1999). The importance of PO and CAT activity in disease resistance might be due to their ability to oxidize phenolic

compounds to quinones, which are more toxic to pathogens than the original phenolics (Kazana et al., 1998). Therefore it is reasonable to assume that an increased activity of PO and CAT will result in higher concentration of toxic products of oxidation and a greater degree of resistance to infection. The increased concentration of PO and CAT activities in plants treated with compost tea indicates that the protective effect of compost tea is due, at least in part, to the induction of resistance in plants (Weltzien, 1991). The micronutrients increased in compost-based mixtures compared to the control. Comparable results were reported by Hemández-Apaolaza et al. (2005) using a blend of pine bark and 15–30% composted sewage sludge. Many researchers have shown the use of sewage sludge generally increases the heavy metal contents in compost (Stabnikova et al., 2005). While contents of Fe were higher, those of Mn, Cu, and Zn were lower. These results demonstrate that the broad chemical heterogeneity of biosolids re-used in pot cultivation is strictly related to the origin (Veeken and Hamelers, 2002; Papafotiou et al., 2004; Casado-Vela et al., 2006). Results also suggest that numerous events occur concurrently in plants cultivated in compost and compost tea based growing media. There are the seemingly positive effects of certain elements which appear in some cases even to be growth-limiting as Cu and Zn. There is also the direct toxic action and the apparently antagonistic effect between some of these elements as explain for K and Mg. These results are in line with the physiological mechanisms proposed by Marschner et al. (1996) whereby mineral nutrient partitioning was dependent on the cycling of mineral nutrients through the source to the leaves. The imbalance of these mineral nutrients disrupts the export of photosynthates and the allocation of nutrients.

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