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Evaluation of Municipal Solid Waste Compost and Agricultural Waste Vermicompost by Growth of Lippia citriodora Under Salinity Stress

Sasan Mohsenzadeh [a,*], Hamideh Zamanpour Shahmansouri [a]

[a] Department of Biology, Faculty of Sciences, University of Shiraz, Shiraz, Iran
[*]Email: mohsenz@shirazu.ac.ir

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A B S T R A C T

The aim of this study was to investigate the effects of compost and vermicompost on growth and physiological characters such as antioxidant, phenol, proline and secondary metabolites of Lippia citriodora in salinity stress. Compost which is prepared from municipal solid waste is the stabilization of organic compounds under aerobic conditions and is produced by the activity of living microorganisms. Vermicompost is prepared from agricultural waste and using various worms. Compost and vermicompost were applied at three levels of 0%, 10% and 30% of pot volume, as well as salinity of 70 mM NaCl to the three month-old plants in a greenhouse. The compost (10% and 30%) and the vermicompost 30% reduced growth and increased proline content, however the vermicompost at 10% improved the growth and phenolic compounds of the plants under salinity stress. These fertilizers at high levels, containing large amounts of organic matters and high salts contents, which increase toxicity and salinity and reduce plant growth. Vermicompost is better fertilizer than compost.

1. Introduction

About 30% to 50% of crop yields are attributed to use of natural or synthetic fertilizers (Gowariker et al. 2009, Stewart et al. 2005). Chemical fertilizers are essential to enhance proper growth and crop yield, but these fertilizers may endanger ecosystems, soil, plants, human and animal lives also (Mishra &Dash 2014). Fertilizers act as catalysts in providing nutrients to the plants for their better growth and yield (Ameta et al. 2015).

Municipal waste compost as an affordable organic fertilizer with a proper and valuable function can be used as a suitable substitute for sustainable agriculture and organic planting from a special site (Summner 2003). The use of compost and vermicompost in agriculture and environment, shows that it have to be compatible with plant growth (Omrani et al. 2005). Compost can be used to increase the amount of soil organic matter, water holding capacity and provide nutrients for plants (Levy &Taylor 2003). Municipal waste compost often contains low amounts of mineral elements compare to chemical fertilizers (Sikora &Enkiri 1999). The contents of municipal waste compost may be introduced into groundwater with irrigation water and also harmful for germination and growth of plants. Therefore, the effects of compost produced from urban waste on sprouts, including early and mature stages of plant growth require further study. It has been shown that several compounds can introduce phytotoxicity in compost including: ammonia, which is especially important when composting materials with low carbon to nitrogen ratio, and ethylene oxide, which is synthesized during the decomposition of the compost after being applied to the soil (Barral &Paradelo 2011). In addition organic acids, which are produced during the decomposition of fresh organic residues, including acetic acid and isobutyric acids (Shiralipour et al. 1997), phenols, which are present in some agricultural wastes and salt, which are present mainly in food wastes, and heavy metals (Prasad 2013). Salinity is one of the most environmental factor that limit plant production (Ashraf &Harris 2004, Hussain et al. 2009). Salinity in water and soil is one of the major abiotic stress on plant growth. (Cirillo et al. 2016, Colla et al. 2010) The toxic effects of salinity can be depend on plant species and soil conditions (Tang et al. 2015). Salt-induced ion toxicity due to nutrient imbalance in the cytosol (Munns 2005). Excessive Cl^- and Na^+ absorption, leading to Ca^{2+} and K^+ deficiency and to other nutrient imbalances. All these responses to salinity can reduce plant growth (Hernández et al. 1993, Mittova et al. 2004). In salinity, plants have to activate different physiological and biochemical mechanisms (Ashraf &Harris 2013). Lemon verbena, scientifically known as Lippia citriodora, is a deciduous shrub of the Verbenaceae family, which grows to a height of 3-5 m. The long light green leaves of this plant are located on the stem in stacks of three. It has small and white flowers. The plant is woody at the bottom and near the soil surface. The genus lippia has over 200 species, among which L. citriodora is especially important. L. citriodora synonym is Aloysia citriodora (Roodbaraky et al. 2017). At present, this species is cultivated on a large scale in Iran. The leaves and vegetative organs of this plant are antipyretic, analgesic, carminative, digestive and calming. It can also be used for colds and headaches. The lemon verbena tea is highly calming and soothing (Valentao et al. 2002). In addition to the essence, its leaf contains alkaloids, flavonoids, mucilage, tannin and phenolic acids (Roodbaraky et al. 2017). The essential oils and leaves are used in the perfume industry and for food production (Pascual et al. 2001). Considering the fact that lemon is a valuable medicinal plant that has many medicinal properties, this study aimed to investigate and evaluate the processed fertilizers of compost and vermicompost on growth, essential oil chemical compounds and some physiological characters such as antioxidant, phenol and proline in various states of salinity stress.

2. Materials and Methods

The Experiments were conducted at the greenhouse of Shiraz University, Iran. This experiment consisted of 18 different treatments including three levels of compost (0%, 10% and 30% of pot volume), three levels of vermicompost (0%, 10% and 30 % of pot volume) and two levels of salinity stress (0 and 70 mM) and with three replications. Therefore, the experiment included a total of 54 pots. Pots were placed in the greenhouse at 28/15˚C, and 16/8 h light/dark periods. Sodium chloride solution was used for salinity stress in plants. Sodium chloride solution was added to the three month-old plants in three steps, each of which were 8 days apart. Compost was prepared from municipal solid wastes and vermicompost from agricultural wastes both produced by Shiraz municipality. Soils and fertilizers were mixed together according to the treatments. Plants were collected after 30 days of treatment. The fresh weight of seedlings were measured and then, the plants were frozen by liquid nitrogen and were kept for further experiments. The antioxidant of the L. citriodora extract was determined by DPPH method (Shimada et al. 1992). At first, 24 mg of DPPH was dissolved in 100 ml of methanol and diluted 10:45 with methanol and stored at 4 C°. Extracts (150 µl) were added to 2850 µl of DPPH. After one hour absorbance was measured at 515nm by the Shimadzu UV-160A (Japan) model spectrophotometer. Using trolox as a standard, total phenolic content of the extract was determined by the Folin-Ciocalteu (Kim et al. 2007) .Free proline content was measured by spectrophotometer following the ninhydrin method (Bates et al. 1973).The essential oil was analyzed by GC/MS in the central laboratory of Shiraz University. In this experiment, leaves of L. citriodora were dried at room temperature in shade for 1 week. Dried leaves with distilled water were poured into the flask and heated for 2 hours. The essential oil was collected from evaporation, cooled with water and then refrigerated at 4ºC for qualitative analysis. Isolation and identification of essential oil compounds was performed using gas chromatography-mass spectrometry (GC-MS). For this purpose, one microliter of each essential oil was diluted in 2 ml dichloromethane and one microliter of it was injected into GC-MS. The essential oil compounds were detected and mass spectra of samples were measured. The volatile components of the leaves were analyzed using an Agilent model 7890-A series gas chromatography and Agilent model 5975-C mass spectrometry (USA). The compounds were identified by comparing the retention indices (RI, HP-5) with those reported in the literature and also by comparing their mass spectra with the Wiley GC-MS Library, Adams Library, Mass Finder 2.1 Library data and published mass spectra data (McLafferty 1989, Sparkman 1997).The data was analyzed using SPSS 16.0 software, and mean comparisons were made with Duncan's multiple range test at 0.05 ($P \leq 0.05$). Data was expressed as mean ± standard error (SE). Some physical and chemical properties of the used soil was analyzed by a commercial laboratory and compost was analyzed by Shiraz municipality laboratory. The data of the physical and chemical properties of the soil and compost are shown respectively in table 1 and table 2.

Table 1. Physical and chemical properties of the soil

TDS (mg/L)	TH (mg/L)	TA (mg/L)	pH	EC ($mScm^{-1}$)	K^+ (mE/L)	Mg^{2+} (mE/L)	Na^+ (mE/L)	Ca^{2+} (mE/L)	Anion (mE/L)	N (%)	OC (%)	OM (%)
780.53	525.00	162.50	7.98	1.18	0.04	1.25	4.17	9.25	10.16	0.004	0.40	0.06

TDS **total dissolved solids,** ***TH*** **total hardness,** ***TA*** **total alkaline,** ***OC*** **organic carbon,** ***OM*** **organic matter**

Table 2. Physical and chemical properties of the compost

C/N	Specific gravity	pH	Ammonium (%)	Salt (dS/m)	Moisture (%)	N (%)	OC (%)	OM (%)
18	0.5	8	0.009	6	32	1.5	30	50

OC **organic carbon,** ***OM*** **organic matter**

3. Results & Discussion

The results of the fresh weight of the shoots showed that those treated with vermicompost 10% alone and control plants had the highest growth. Four out of 18 experiments were dried in the first week of treatment, including: compost 30% with vermicompost 10% under salt stress, compost 30% with vermicompost 10% without salt stress, compost 30% without vermicompost with salt stress, compost 30% with vermicompost 30% without salt stress. The two remaining with compost 30% had the lowest growth (Fig 1). Figure 2 shows that the total antioxidant level released by of L. citriodora decreases under salt stress in comparison to the control when combined with compost and or vermicompost 30%. Compost at 10% and 30% levels significantly ($P \leq 0.05$) reduced the antioxidant level compared to the control. Figure 3 shows that total phenolic content decreased in plants treated by compost fertilizer. However in salinity stress alone, and when treated with vermicompost 10% alone increased the phenolic content ($P \leq 0.05$) in comprison to the control.Phenolic content decreased with the increase of compost (10% and 30%) and vermicompost 30% fertilizers, compared to the control. The lowest phenol is from the combination of compost 30%, vermicompost 30% and salinity.The proline increased under salt stress and in different levels and combinations of compost and vermicompost. However when treated with vermicompost 10% alone, the phenolic content did not change in comprison to the control (P≤ 0.05). The highest level of proline was in the combination of compost 10%, vermicompost 10% and salinity.

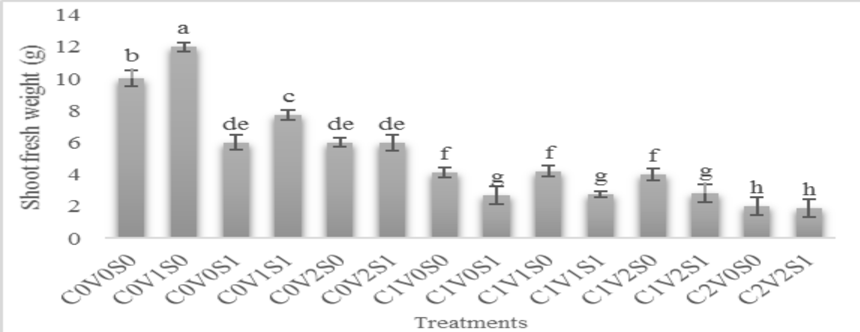

Figure1. Effects of the interaction of salinity, compost and vermicompost against the growth of *L. citriodora* . S_0 : salinity stress 0, S_1: salinity stress 70 mM, C_0: compost 0%, C_1: compost 10%, C_2: compost 30%, V_0: vermicompost 0%, V_1: vermicompost 10%, V_2: vermicompost 30%. Means with the same letters have no significant difference (P≤ 0.05).

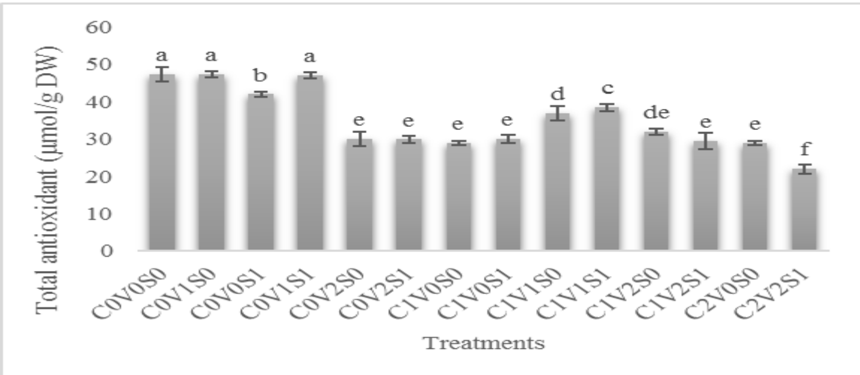

Figure 2. Effects of interaction of salinity, compost and vermicompost against the total antioxidant level released from *L. citriodora* . S_0 : salinity stress 0, S_1: salinity stress 70 mM, C_0: compost 0%, C_1: compost 10%, C_2: compost 30%, V_0: vermicompost 0%, V_1: vermicompost 10%, V_2: vermicompost 30%. Means with same letters have no significant difference (P≤ 0.05).

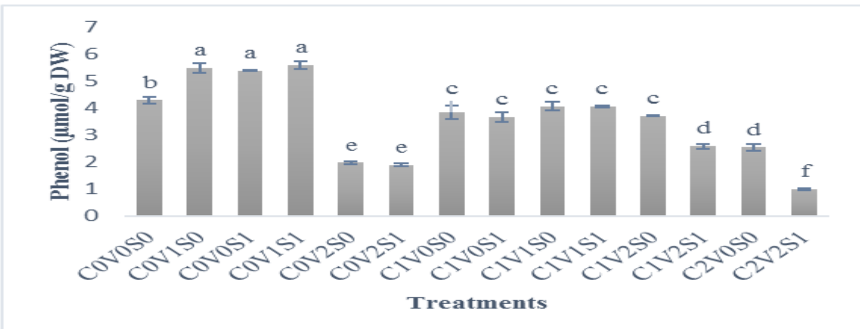

Figure 3. Effects of interaction of salinity, compost and vermicompost against the phenol content of *L. citriodora* . S_0 : salinity stress 0, S_1: salinity stress 70 m M, C_0: compost 0%, C_1: compost 10%, C_2: compost 30%, V_0: vermicompost 0%, V_1: vermicompost 10%, V_2: vermicompost 30%.Means with same letters have no significant difference (P≤ 0.05).

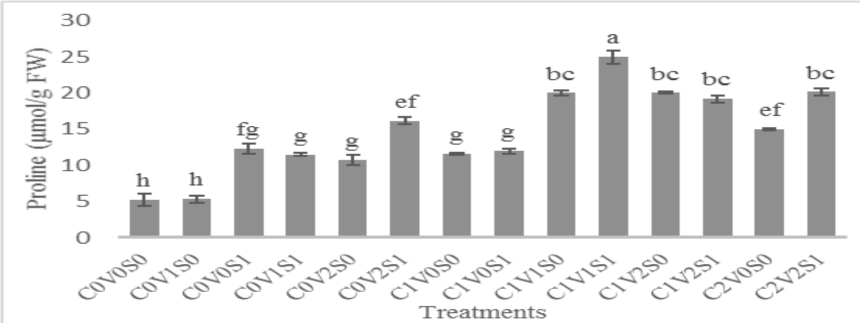

Figure 4. Effects of interaction of salinity, compost and vermicompost against the proline of *L. citriodora* . S_0 : salinity stress 0, S_1: salinity stress 70 mM, C_0: compost 0%, C_1: compost 10%, C_2: compost 30%, V_0: vermicompost 0%, V_1: vermicompost 10%, V_2: vermicompost 30%.Means with same letters have no significant difference (P≤ 0.05).

Table 3 shows that essential oil compounds change with compost, vermicompost and salinity in different directions. The results showed that the fertilizers sometimes increased and sometimes decreased the secondary metabolite, but application of salinity alone increased the essential oil composition of L. citriodora (Table 3).

Table 3: Effect of compost, vermicompost and salt stress on essential oil compounds percentage

Compounds	$C_0V_0S_0$	$C_0V_{1,2}S_0$	$C_{1,2}V_0S_0$	$C_0V_{1,2}S_1$	$C_{1,2}V_0S_1$	$C_{1,2}V_{1,2}S_0$	$C_{1,2}V_{1,2}S_1$	$C_0V_0S_1$
dl-Limonene	6.30	6.72	6.34	5.33	4.17	5.51	-	7.74
1,3,6-Octatriene, 3,7-dimethyl-, (E)	-	-	2.691	2.84	2.39	3.97	-	1.61
1,8-Cineole	3.82	3.82	3.10	2.42	1.12	2.49	-	4.04
6-Methyl-5-hepten-2-one	-	0.46	0.69	1.00	0.41	0.63	-	0.34
2,6-Octadienal, 3,7-dimethyl-, (Z)	3.85	2.68	2.69	2.84	-	0.17	-	3.96
Cyclohexane, ethenyl	-	1.91	2.71	2.01	1.80	2.06	-	1.78
Trans-Caryophyllene	2.18	-	4.11	3.96	4.52	4.50	-	2.33
Z-Citral	21.98	23.12	23.63	23.63	24.46	24.66	2.11	22.56
Benzene	-	0.37	0.89	0.47	0.46	0.49	-	0.48
Geranyl acetate	-	-	0.28	6.23	-	0.26	-	2.04
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	18.56	33.73	31.05	29.29	20.08	31.90	-	32.43
2,6- Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)	3.66	2.03	2.17	-	-	-	-	3.86
Decane	-	-	-	-	0.57	2.19	9.00	-
Citral	-	1.49	-	34.26	38.21	-	5.14	-
Isopropyl Myristate	-	-	0.33	1.096	2.52	0.69	1.63	-
Di-(2-ethylhexyl)phthalate	-	-	-	-	-	-	22.52	-
1,2-Benzenedicarboxylic acid, 3-nitro	-	-	-	-	-	-	45.08	-
Octadecane	-	-	-	-	0.88	-	2.29	-

S_0 : salinity stress 0, S_1: salinity stress 70 m M, C_0: compost 0%, C_1: compost 10%, C_2: compost 30%, V_0: vermicompost 0%, V_1: vermicompost 10%, V_2: vermicompost 30%

In this study, the salinity reduced the fresh weight of the shoots, which is consistent with some of the results previously (Najafi &Khavari-Nejad 2010). The results also reveal that the fresh and dry-weights of leaves, stems and roots were significantly decreased by increasing salinity level (Dunlap &Binzel 1996). Plant growth reduce under salinity and high sodium uptake reduces water movement through the root. The salinity influence turgor and photosynthesis (Munns 1992). Pre-treatment with some molecules can increase tolerance to salt stress (Hossain et al. 2015). The application of vermicompost as processed organic fertilizer sometimes can induce salt tolerance in *L. citriodora* and sometimes increase the growth of the plant (Paul &Metzger 2005) and sometimes growth did not increase (Bachman &Metzger 2007). In our study the vermicompost 10% without compost increased the shoot's fresh weight in salinity and without salinity (Fig.1). Plants use enzymes and non-enzymatic antioxidant defense systems to counteract the adverse effects of active oxygen species (Wu et al. 2012). The interaction of vermicompost humic acid with the plant root system activates the antioxidant enzymes and thus controls the amount of free radicals (García et al. 2012). According to this study, the compost fertilizer shoot decreased in growth, which could be due to its toxicity. Phytotoxic effects of compost is the result of several factors, including: ammonia, ethylene oxide, organic acids and salt (Rawat &Suthar 2014). The data shows that the vermicompost 10% alone helped the plant to increase antioxidant levels in salt stress but compost did not. This plant is sensitive to salt stress and the total antioxidant decrease in salinity alone compared to the control the lowest total antioxidant combination of compost 30% vermicompost 30% and salinity (Fig.1).The high levels of organic compounds and toxicity of fertilizer can change the activity of the enzyme phenylalanine ammonilase that is a key enzyme in the phenol biosynthesis compounds (Asami et al. 2003, Estiarte et al. 1994). Phenolic compounds are non-enzymatic antioxidants. So differences between total antioxidant and total phenol (Fig 2 and 3) can be explained by this difference.At high salinity, plants need to synthesize some organic solutes such as proline as a free amino acid (Gharsallah et al. 2016). Proline has a significant role in reactive oxygen species scavenging and membrane stability (Ahanger et al. 2014). By mixing the compost, vermicompost and salinity in the treatment, proline content was increased (Fig.4). The highest proline content was in the mixed compost 10%, vermicompost 10% and salinity. Proline was accumulated in the leaf of plants in response to stress. According to the results of this experiment, salinity stress increased the amount of proline. Proline protects metabolic processes in stress by replacing water and maintenance of cellular structures (Zhifang &Loescher 2003). It has been reported that high levels of leaf proline can protect plants against severe stress and increase stress tolerance (Cvikrová et al. 2013, Ruiz-Lozano 2003). In salinity plant balances its water potential by adjustment of osmolytes (Hasegawa et al. 2000), and accumulation of osmolytes reduces osmotic potential (Munns 2002). Fertilizer treated plants show higher proline content compared to control plants, even under stressful environments (Salehi et al. 2016).The production of secondary metabolites in medicinal plants is not always at the same level, and there are several factors that affect the production of these compounds. Individual genotype variations, developmental stages, access to minerals and stress conditions are among these factors and in response to salinity or drought stress, the production of secondary compounds may increase or decrease (Aghai et al. 2014).Since the presence of phosphorus and other nutrients and their availability are essential for the formation of essential oils, it can be discussed that the use of fertilizers can increase the essential oil percentage. Due to the sensitivity of the *L. citriodora* to salinity and its sensitivity to the compost and vermicompost fertilizers, changes in the secondary metabolites and removal of some compounds and synthesis of others occur. Therefore, use of fertilizers in medicinal plants should be optimized. The unusual changes of essential oil (%) were in compost 10% and 30 % and vermicompost 10% and 30%, under salt stress.The absence of data for some compounds may be related to their biosynthesis or not being detected by GC/MS. These results are in agreement with those observed on Melissa officinalis (Santos et al. 2009) and on Thymus vulgaris (Hendawy et al. 2010) and on rosemary (Valiki et al. 2015).

4- Conclusion

Optimum non-toxic levels of fertilizers can improve growth plant in combination with salinity. Vermicompost is a better fertilizer than compost for growth and reducing stress. Hence, it could be recommended to apply vermicompost at low level in regions of salted soils or irrigated with saline water, to help the plants. Salt stress, compost and high level of vermicompost all reduced growth of *L. citriodora*. Salt stress, compost and vermicompost increase the proline content of *L. citriodora*. None of the treatments increased total antioxidants but salinity alone and vermicompost 10% increased phenolic compounds. The results showed that compost reduced growth and increased proline content but vermicompost at 10% level improved the growth of plants under salinity stress. These fertilizers at high levels, containing large amounts of organic matters and high salts which increase the salinity of the soil and reduce plant growth.

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