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# Growth of *Rosmarinus officinalis* under Drought Stress Can Evaluate Municipal Solid Waste Compost and Agricultural Waste Vermicompost

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**A B S T R A C T**

Evaluation of compost and vermicompost by growth of plants under stress is a bioassay analysis to know their activity in the environment. Compost prepared from municipal solid waste under aerobic condition and produce by the activity of microorganisms. Vermicompost prepared from agricultural waste by composting process using various worms. Drought stress was applied at levels of 0, 5 and 9 days of no irrigation and fertilizers at levels of 0, 10 and 30% of pot volume, each with three replications in a greenhouse. Drought stress reduced the growth of the plants. Vermicompost at 30% level increased the growth of shoot but compost in both levels reduce the growth of shoot and root. Drought stress increased antioxidant, phenolic compounds and proline. Compost and vermicompost increased the antioxidant and proline. Compost and vermicompost contain a lot of phosphorus, nitrogen, organic matters and salts so can increase the amount of some essential oil compounds. It sometimes increase toxicity and reduce plant growth, especially if prepared from raw materials containing toxic organic compounds and heavy metals and not completely processed. It is recommended that agricultural waste can be used in the preparation of compost and urban wastage can be consumed as little as possible.

## 1. Introduction

Evaluation of compost and vermicompost by growth of plants especially medicinal plants under abiotic stress is a bioassay analysis. Knowing activity of these fertilizers in the field for production of organic medicinal crop with high concentration of secondary metabolite is essential [1]. Rosemary (*Rosmarinus officinalis* L.) belongs to Lamiaceae family. It is a bushy, evergreen, with 2m height and elate or sometimes crawling on the ground which is green and aromatic. The leaves and the buds of the branches are the medicinal parts of rosemary plants which were harvested in spring and summer [2]. It is used for treating headache, rheumatoid arthritis, blood circulatory disruptions, peripheral artery diseases, hair fall, neurological disorders, and urinary tract disorders, enhancing eyesight, chronic weakness, and disability in folk medicine [3]. The major volatile oils compounds in the leaves and inflorescence of rosemary plants are cineole, borneol, bornyl acetate, camphor, alpha and beta-pinene [4]. The most important physiological factors for resistance against drought stress are osmotic adjustment, antioxidant production, and ROS scavenger. Proline is one of the active amino acids in osmotic regulation, that have a significant role in creating and maintaining the osmotic pressure within plant cells [5]. In the field, compost produced from municipal solid waste enriched with chemical fertilizers, increase accessibility of macro-elements and result in higher fertility and productivity [6]. The positive effects of compost on physical properties of the soil including saturate and unsaturated hydraulic conductance, water conservation capacity, volumetric weight, porosity, dispersal, and the size of pores, point to permeability and condensation of the soil [7]. Vermicompost is the result of aerobic process that produced as the result of decayed organic materials by worm and soil microorganisms [8]. Soils with vermicompost have higher nitrogen, phosphor, and potassium than soil without it and is rich in growth hormones and vitamins and acts as a strong bio-pesticide [9]. Also, vermicompost increase water conservation capacity of the soil and prevent washing of nutritional elements. It enhances physical structure of the soil and improves root growth [10]. In this research the growth of *Rosmarinus officinalis* under drought stress that can evaluate municipal solid waste compost and agricultural waste vermicompost was studied.

## 2. Materials and Methods

This investigation were performed as a completely randomized design with 27 treatments and three replications in pod and in a greenhouse of Shiraz University. The treatments include three levels of 0, 5 and 9 days of drought stress and volumetric ratio of 0, 10 and 30 percent of organic fertilizers including compost and vermicompost. Cultivation in pots was performed with a mixture of sand and clay and compost and vermicompost fertilizer which were purchased from Shiraz municipality. The pots were irrigated with 50 ml water for two weeks on alternative days. Then, the drought stress were initiated. At the end, the samples were harvested and the weight of shoots and roots of rosemary was measured.

The amount of proline was showed with ninhydrin indicator based on the Bates method (Bates, 1937). Total phenol content was evaluated based on Kim and co-workers with Folin-Ciocalteu indicator and using Gallic acid as the standard compound [11]. In order to determine antioxidant potential using stable DPPH standard based on the Shimada and co-workers method, the Trolox were used as standard compound [12]. In order to evaluate relative water content, the samples from all treated leaves was prepared and their fresh, saturated and dry weight were measured.

The volatile components of the leaves were analysed using an Agilent model 7890-A series gas chromatography and Agilent model 5975-C mass spectrometry (USA). The HP-5 MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm i.d × 25 μm) was used with helium gas at the rate of 1mL/min as carrier. The GC oven temperature was programmed from 60°C to 210°C at the rate of 3°C /min and then was increased from 210°C to 240°C at the rate of 20°C /min and kept constant at 240°C for 8.5 min. The split ratio was adjusted to 1:50 and the injection volume was 1000 μl. The injector temperature was 280°C. The quadruple mass spectrometer was scanned over 40-550 amu with an ionizing voltage of 70 eV. Retention indices were determined using retention times of n-alkanes (C8-C25) that were injected after the essential oils under the same chromatographic conditions. The retention indices for all components were determined using n-alkanes as standard. 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 [13].

The data was analyzed using SPSS 16.0 software, and mean comparisons were made with Duncan's multiple range test at 0.05 (P≤

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.

## 3. Results & Discussion

### 3.1. Shoot and root growth

Figure 1 and 2 show the effect of compost and vermicompost on growth of Rosemary plant under drought stress. Only vermicompost 30% with no drought increased the shoot fresh weight significantly compare to control, but when it combined with compost no increases were shown. Under severe drought stress, the shoot fresh weight significantly decreased only with high level of compost (30%) but the root fresh weight significantly decreased with high level of compost under control and severe drought stress.

**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 (%) | TNV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 46 |

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

**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**

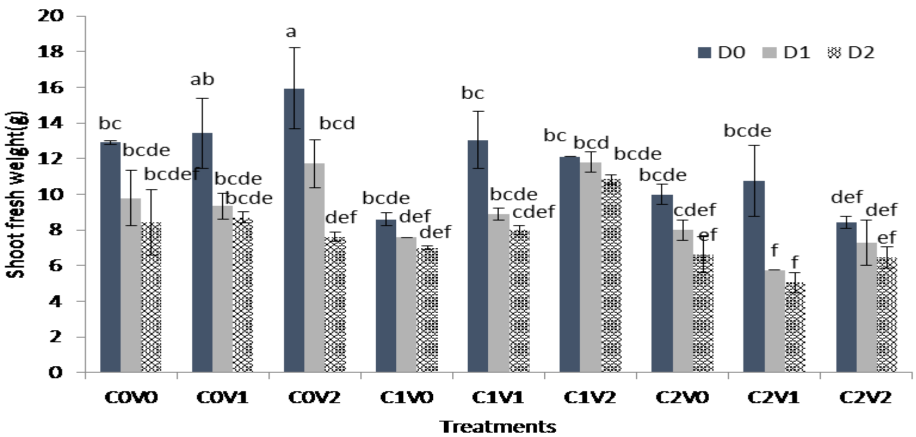


**Figure 1. Effect of compost and vermicompost on shoot fresh weight of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference (P≤ 0.05) with Duncan test.**

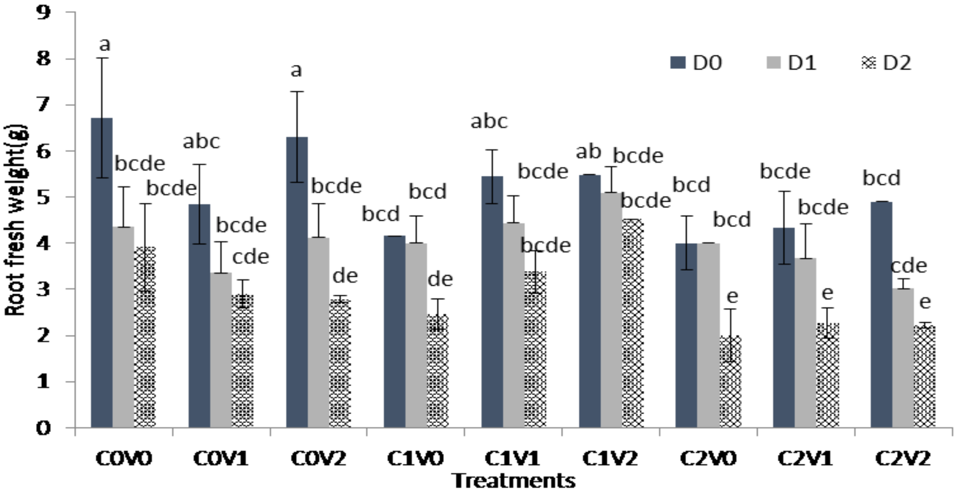


**Figure 2. Effect of compost and vermicompost on root fresh weight of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference (P≤ 0.05) with Duncan test.**

Since the compost was made by municipal solid wastes, therefore it had high mineral and organic which are toxic and its negative effect on the shoot and root growth is acceptable. In treatments which used a combination of both compost and vermicompost fertilizers, the vermicompost reduced the negative effects of compost (figures 1 and 2) because vermicompost is made using agricultural waste. So, proper amounts of vermicompost will be necessary to prevent this problem [14]. In drought stress due to the closure of stomata, the concentration of $CO_2$ in leaf tissue reduced therefore, the products of photosynthesis reactions, including NADPH, is not consumed, since NADPH is not oxidized. Thus, the amount $NADP^+$ decreases in order to receive electrons.

## 3.2. Relative water content

As the figure 3 shows, 5 and 9 days drought stress in control, 10 percent vermicompost, 10 and 30 percent compost, 10 percent of compost and vermicompost, mix of 30 percent of compost and 10 percent of vermicompost and mix of 30 percent of compost and vermicompost treatments reduced RWC percentage in drought stress and without drought stress, compare to the control in well watered condition.

So it can be said that the drought stress and compost will result in RWC reduction and the 30 percent vermicompost fertilizer can alleviate the negative effects of drought stress and compost. The other researchers reported that drought stress will result in the reduction of water content in plant and consequently limit growth [15, 16] The RWC increased with 30 percent vermicompost maybe for its high nitrogen. Mohammadzadeh and co-workers reported that consuming the nitrogen from biofertilizer lead to higher water content in the leaves and the highest RWC was observed in the 150 Kg nitrogen treatment per hectare [17].

## 3.3. Antioxidant capacity

According to the figure 4 it can be said that the drought stress and processed organic fertilizer (compost and vermicompost), and also the mix of them showed higher antioxidant capacity than control.

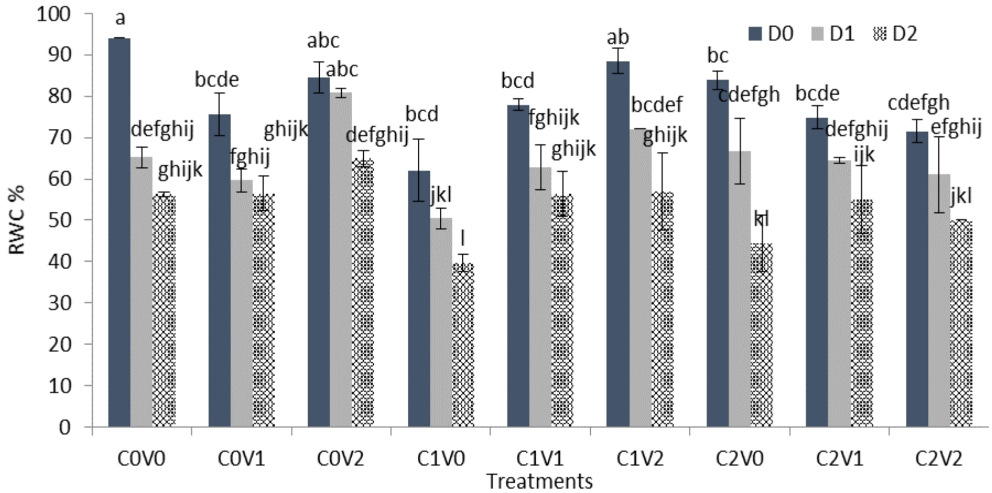


**Figure 3. Effect of compost and vermicompost on relative water content of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference ($P \leq 0.05$) with Duncan test.**

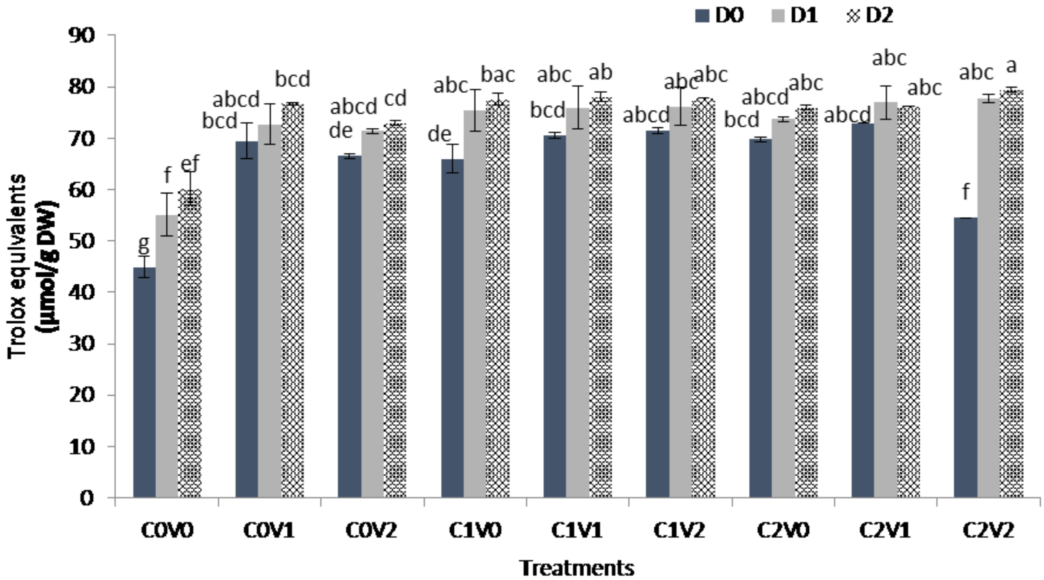


**Figure 4. Effect of compost and vermicompost on antioxidant of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference ($P \leq 0.05$) with Duncan test.**

The researchers reported that catalase activity as an antioxidant was increased significantly in drought stress levels [18, 19] and also vermicompost application showed a significant increase in the activity of the antioxidant enzymes [20]. The activity of antioxidant enzymes plays an important role in plant survival under drought stress condition. The interaction between humic acid from vermicompost and root system of the plant will lead to activation of the antioxidant enzymes. Consequently, the levels of free radicals will be controlled. It seems that the role of humic acid is identified with anti-stress defence system in plants [21]. In drought stress, the CO2 concentration in the leaf decrease due to stomata closure, therefore the products of light reactions of the photosynthesis including NADPH will not be used and the NADP+ concentration used as the electron receiver will be decrease. Thus, the oxygen will act as the receiver of the electron from electron transfer chain in the photosynthesis, and reactive oxygen species (ROS) like superoxide radical ($O^{-2}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^-$) will be produce and increased antioxidants under drought stress and with compost and vermicompost can help plant to tolerance [18].

3.4. Phenolic compounds

Vermicompost fertilizer at the two levels of 10 and 30 percent showed higher phenolic compounds. Drought stress, compost fertilizer and mix of two fertilizers had no effect on the phenolic compounds. Phenolic compounds are important for the quality of plant based foods. They are responsible for the color of red fruits, juices and wines and substrates for enzymatic browning, and are also involved in flavor properties. The vermicompost fertilizer produced from agricultural waste like fruit and vegetable, so has more phenolic compounds.

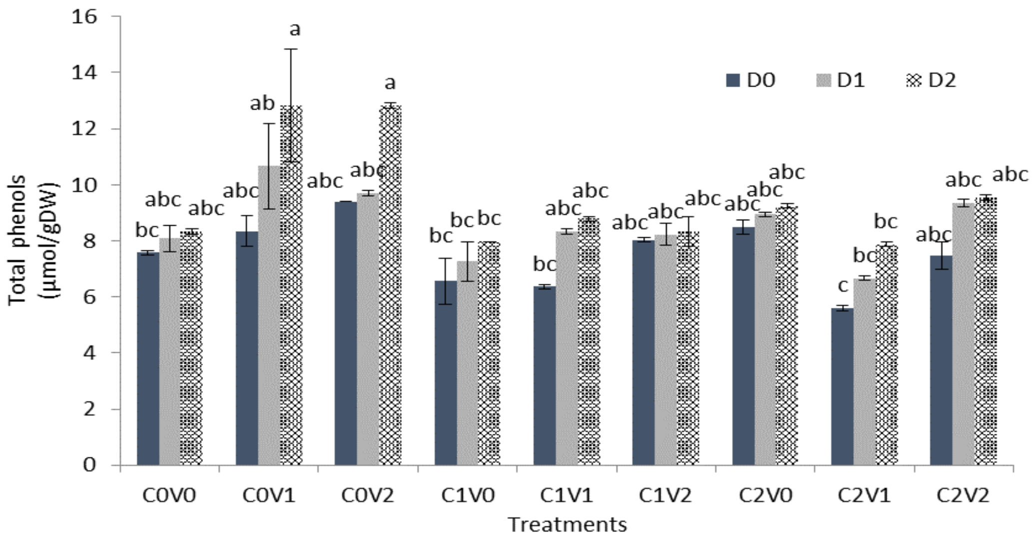


**Figure 5. Effect of compost and vermicompost on phenolic compounds of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference ($P \leq 0.05$) with Duncan test.**

3.5. Proline levels

The figure 6 shows that compost and vermicompost fertilizers and their mix enhanced proline level under drought stress.

During drought stress incident, the accumulation levels of organic compounds like proline will increase in all parts of plant. Proline is the stored amino acid in the cytoplasm, and probably have an effective role in protecting intracellular macromolecules [22]. Accumulation of compounds like proline and other amino acids in green tissues of rapeseed plant under drought stress could relatively provide requirements to continue water absorption through roots system, but relying on these compounds is very expensive for plants and will lead to lower production [23].

Processed organic fertilizer like compost and vermicompost increased proline content. Proline is an amino acid with nitrogen structure, and nitrogenous fertilizers could help its higher production [24].

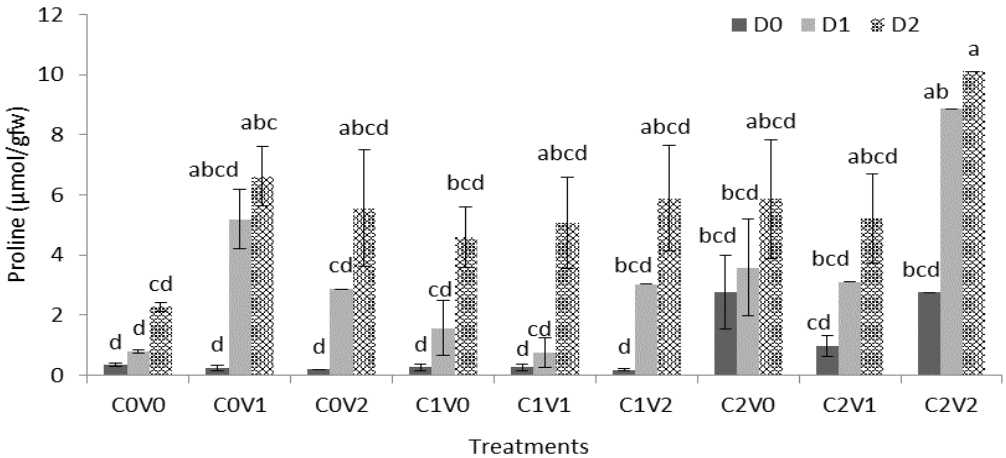


**Figure 6. Effect of compost and vermicompost on proline amount of Rosemary plant under drought stress. $C_0$ (Compost zero), $V_0$ (Vermicompost zero), $D_0$ (Without drought stress), $C_1$ (Compost 10%), $V_2$ (Vermicompost 10%), $D_1$ (5 day drought stress), $C_2$ (Compost 30%), $V_2$ (Vermicompost 30%), $D_2$ (9 day drought stress). Means with the same letters have no significant difference (P≤ 0.05) with Duncan test.**

3.6. Essential oil compounds

The highest amount of compounds in the essential oil were observed in alpha pinene, camphene, camphor, cineole, borneol, limonene, beta myrcene (table 3). The amount of compounds like alpha pinene, limonene, camphene, gamma-terpinene, bicycle 3,1-1-heptan, camphor, 3-cyclohexen-1-ol, were not changed among different treatments. The level of compounds like trans-caryophyllene, alpha-terpinolene, beta-myrcene, and pinene in control was higher than other treatments. The content of compounds like cineole and linalool were increased in drought stress and with compost and vermicompost treatments. The amount of octanone was higher in drought stress and compost treatment.

Generally, it can be said that compost and vermicompost fertilizers will increase the amount of some compounds in essential oil. The effect of the nitrogen on essence yield is due to the active role that nitrogen plays in development of essence-containing new cells. Also, nitrogen increase carbohydrate, auxin and gibberellin hormones and consequently the essential oil yield will increase due to higher leaf yield [25, 26]. Since the terpenoid compounds and their building block strongly need NADH and ATP, and since elements like nitrogen and phosphor are necessary for their biosynthesis, higher amount of compost and vermicompost provide higher nitrogen and phosphor absorption which in turn accommodate in the secondary compounds [25].

The severe stress will not always resulted to higher essence percentage, because during sever stresses plant use much of its photosynthetic product to synthesize osmoregulatory compounds like proline, sugar compounds, so that the plant could maintain required situation in order to continue its life [27]. These fertilizers at high levels, contain large amounts of organic materials and high salts contents (table 2), which increase toxicity and reduce plant growth. Vermicompost which is prepare from agricultural waste is better fertilizer than compost which is prepare from municipal solid waste. Jalili and co-workers reported that the compost extract, causes toxicity and limitation in germination and growth of seedlings, if prepared from raw materials containing toxic organic compounds and heavy metals and not completely processed [28].

## 4- Conclusion

Evaluation of compost and vermicompost by growth of plants under stress is a bioassay analysis for knowledge to their activity in the natural environment and instruction to producers. Compost prepared from municipal solid waste stabilize organic compounds under aerobic condition and produce by the activity of microorganisms. Vermicompost which is prepared from agricultural waste by composting process using various worms. Compost and vermicompost contain a lot of phosphorus and nitrogen so can increase the amount of some essential oil compounds. These fertilizers at high levels, contain large amounts of organic matters and high salts contents, which increase toxicity and reduce plant growth, especially if prepared from raw materials containing toxic organic compounds and heavy metals and not completely processed. Vermicompost which is prepare from agricultural waste is better fertilizer than compost which is prepare from municipal solid waste and it is recommended that agricultural waste can be used in the preparation of compost and urban wastage can be consumed as little as possible.


## Acknowledgment

We would like to thank the Shiraz University Research Council for financial support of this research.


**Table 3. Effect of compost, vermicompost and drought stress on percentage of essential oil compounds**

| Compounds | $C_0V_0D_0$ | $C_0V_0D_{1,2}$ | $C_0V_{1,2}D_0$ | $C_0V_{1,2}D_{1,2}$ | $C_{1,2}V_0D_0$ | $C_{1,2}V_0D_{1,2}$ | $C_{1,2}V_{1,2}D_0$ | $C_{1,2}V_{1,2}D_{1,2}$ |
|---|---|---|---|---|---|---|---|---|
| **Alpha Pinene** | 22.133 | 237.327 | 23.672 | 23.835 | 25.334 | 24.34 | 24.794 | 23.162 |
| **Camphene** | 8.711 | 6.576 | 5.959 | 5.495 | 5.567 | 6.449 | 6.147 | 6.354 |
| **Pinene** | 3.654 | 1.930 | 2.883 | 2.249 | 2.158 | 2.177 | 2.205 | 2.111 |
| **Beta-myrcene** | 4.129 | 2.468 | 2.478 | 2.006 | 2.154 | 2.421 | 2.240 | 2.143 |
| **Dl-limonene** | 5.308 | 4.236 | 4.041 | 3.775 | 4.041 | 3.997 | 4.127 | 3.659 |
| **Gamma –terpinene** | 1.658 | 0.764 | 1.421 | 0.178 | 1.421 | 0.973 | 1.056 | 0.885 |
| **1,8Cineole** | 4.067 | 19.013 | 21.331 | 21.641 | 21.841 | 16.196 | 19.314 | 20.888 |
| **Alpha-terpinolene** | 1.854 | 0.779 | 1.081 | 0.718 | 0.902 | 0.920 | 0.932 | 0.773 |
| **3-Octanone (CAS)** | 0.545 | 1.397 | 0.643 | 0.875 | 0.609 | 1.960 | 1.102 | 0.091 |
| **Linalol** | 1.406 | 2.662 | 2.759 | 2.509 | 2.481 | 2.167 | 2.396 | 2.439 |
| **3-Cyclohexen-1-ol,** | 0.853 | 0.975 | 1.320 | 1.131 | 1.173 | 1.173 | 1.077 | 1.228 |
| **Camphor** | 5.984 | 7.247 | 4.412 | 5.736 | 5.316 | 7.542 | 6.340 | 8.420 |
| **Bicyclo[3.1.1]heptan-3-one** | 1.622 | 1.495 | 1.403 | 7.625 | 1.354 | 1.473 | 1.355 | 1.502 |
| **Borneol** | 5.647 | 7.144 | 6.547 | 7.34 | 6.852 | 7.22 | 6.900 | 7.582 |
| **Bicyclo[2.2.1]heptan-2-ol,** | 3.788 | 1.495 | 6.175 | 7.625 | 6.075 | 6.629 | 7.111 | 6.897 |
| **Trans-caryophyllene** | 2.802 | 1.138 | 1.293 | 1.236 | 1.240 | 1.085 | 1.383 | 0.784 |

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