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## ORIGINAL RESEARCH

### **Effect of vermicomposting plant residues on phytochemical properties of Zarrin-giah (*Dracocephalum kotschy* Boiss.)**

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## Abstract

**Purpose:** This study was conducted to evaluate whether vermicompost production from various plant residuals could improve the biomass and essential oil properties of Zarrin-giah (*Dracocephalum kotschy* Boiss.).

**Method:** In this experiment, different types of vermicompost were prepared from five residual biomasses of chamomile, madder, licorice, valerian, and pistachio with cow dung and *Eisenia fetida* worms. Subsequently, Zarrin-giah plants were treated with 0, 10%, and, 20% of vermicompost. After 176 days, plants were harvested, and the influence of treatments on Zarrin-giah biomass and phytochemical properties was evaluated.

**Results:** The use of vermicompost improved plant biomass and phytochemical traits. The highest Zarrin-giah essential oil contents were noted with 20% vermicompost derived from madder and pistachio waste. Neral,  $\alpha$ -pinene, geranial, geranyl acetate, and limonene were identified as the main chemical compounds, with the best results observed at 10% vermicompost amendment. Samples amended with 10% and 20% vermicompost from pistachio waste and 10% vermicompost from valerian waste had the highest antioxidant activity (displaying the lowest IC<sub>50</sub> values of 19.29, 19.26, and 20.25  $\mu$ g/mL, respectively). Vermicompost derived from licorice waste improved Zarrin-giah biomass, with yields of 29.05 and 27.93 g/plant at 20% and 10%, respectively. Moreover, significant differences in substrate nutrient recycling and electrical conductivity were observed among various treatments.

**Conclusion:** High electrical conductivity levels indicate a salinity issue in vermicomposts derived from pistachio and madder waste. Nonetheless, these treatments positively affected substrates, enhancing the antioxidant activity and essential oil content of the medicinal plant Zarrin-giah, which were the primary objectives of this research.

**Keywords:** Essential oil, Medicinal plant, Macro-element, Salinity, Secondary metabolites.

## Introduction

Zarrin-giah (*Dracocephalum kotschy* Boiss.) is a perennial plant belonging to the Lamiaceae family with branched stems that can grow up to about 33 cm in length. Its flowers are typically white or slightly yellowish, arranged in terminal clusters (Foroozandeh and Asadi-Gharneh 2021). Zarrin-giah is identified as an endangered endemic medicinal plant species in Iran, possessing numerous beneficial properties such as antioxidant, anti-inflammatory, anti-tumor (Kamali et al. 2016), anti-spasmodic, pain-relieving effects, and the ability to reduce high fat levels. Methoxylated flavonoids present in this plant are reported to inhibit the proliferation of malignant cells. Notable chemical compounds found in the essential oil of this medicinal plant include limonene, carvacrol (Golparvar et al. 2016), linalool, geraniol, geranyl acetate (Ashrafi et al. 2017), myrtenol, and neral (Mafakheri et al. 2018).

Over the last century, the world's population has increased to 7.9 billion in 2021 (Sarkar et al., 2021). To meet the food needs of millions of people, agricultural production has been escalated annually. However, post-harvest disposal of plant residues in farm fields poses a significant environmental challenge (Koul et al. 2022). These huge volumes of residual biomasses are not mere waste; rather, these are organic materials that can be recycled into enriched nutritional fertilizers (Huntley et al. 2021). Vermicompost is a biological technology aimed at enhancing soil properties without generating odor or causing environmental pollution. This process, which transforms raw materials into suitable manure by the activity of *Eisenia fetida*, is termed vermicomposting (Esmaeili et al. 2020; Syarifinnur et al. 2023). *Eisenia fetida* is a type of earthworm known for its high reproductive capacity, commonly

serves as a crucial agent in vermicomposting research (Esmaeili et al. 2020; Mago et al. 2021). In 2021, global production of pistachio was about 0.9 million tons. The United States and Iran emerged as leading producers, jointly contributing to 72% of the total output. Turkey, China, and Syria followed as secondary producers (Fereidooni et al. 2024). A single pistachio nut typically yields 1 kg of kernel and 2 kg of waste from each 3 kg fruit, resulting in production waste averaging about 132-165 tons for every 250 tons of crop harvested (Esmaeili et al. 2020).

The utilization of various medicinal plants is pivotal in the cosmetics and pharmaceuticals industry. Essential oil (EO) extraction from small herbal parts plants like chamomile and valerian results in significant biomass residues, which are often wasted or incinerated (Fardad et al. 2018). The reported amount of these residues stands at 3000 kg per 1000 kg of flowers (Saha and Basak 2020) and 9.03 tons per 5.37 tons of roots (Morteza et al. 2010) respectively. Also, madder and licorice roots are employed for powder production, with waste materials amounting to 9% of madder production (Dehghani Bidgoli 2019) and 14400 kg per 11400 kg of licorice roots (Khaitov et al. 2022). In recent years, farmers have been encouraged to expand the cultivation of medicinal plants to protect the environment. Meanwhile, numerous studies have explored the transformation of medicinal plant waste post-industrial processes; however, none have highlighted a value-added method for utilizing this herbal residual biomass directly in the field. This research serves as a guideline for future scholars aiming to select suitable organic manure materials to enhance the quantity and quality of Zarrin-giah essential oil. Considering these factors, the present research aims to:

- I) Cultivate the medicinal plant Zarrin-giah, known for its valuable essential oil properties crucial in cancer treatment studies. Cultivation serves as a means of protection against the endangered status of this plant.
- II) Recycle nutrients from residual biomasses by converting them into vermicompost, a rich source of antioxidant activities often disregarded and left unused. In the current study, vermicompost was prepared using five different types of aromatic raw materials derived from waste chamomile (VCW), madder (VMW), licorice (VLW), valerian (VWV) and pistachio (VPW), along with cow dung and *Eisenia fetida* worms.

## Materials and methods

### Preparation and cultivation of plant matter

Zarrin-giah seeds were applied from the Natural Resources Organization of Isfahan Province. The seeds were sown in a seed tray containing a mixture of 80% (v/v) coco peat and 20% (v/v) perlite. After two months, 126 seedlings were transplanted into 63 pots in a greenhouse by the end of October. The greenhouse conditions were maintained with a temperature range of 25-28°C, humidity at 75%, and light intensity kept below 3000 lux.

### Vermicompost processing method

Pistachio dehulling waste and plant residues from chamomile, licorice, madder and valerian were sourced from the Gol Daro Pharmaceutical Company. Earthworms and cow dung were purchased from a local market. The herbal materials were air-dried for about 50 days at 25°C and then cut into pieces measuring 1-2 cm. Boxes measuring 52×33×26 cm were prepared for the vermicomposting process. Each treatment consisted of 8 kg of plant residue, 1.5 kg of cow dung and 250 g ± 10 earthworms. The worms constituted 5% of the substrate volume in each box, equating to around 1000 to 1200 active worms per box. The samples were kept in a shaded place where

temperature was maintained at 20-25°C, humidity at 70%, and pH in the range of 6.8-7.2 were controlled throughout the process. After 60-120 days, mature vermicomposts were harvested for further analysis and use.

### Soil and vermicompost physicochemical characteristics

The soil texture was determined using hydrometer testing based on Stokes' law (Gholami et al. 2018). pH and EC were measured in 1:2 and 1:5 (W/V) soil and vermicompost-water suspensions, respectively, using a digital pH meter (Model METROHM\_827) and an EC meter (Model T-712) (Deka et al. 2011). Total nitrogen (TN) was analyzed using the automatic Kjeldahl digestion (Model KJD20), where concentrated sulfuric acid digested the sample and converted total organic nitrogen to ammonium sulfate (Bremner and Mulvaney, 1982). Total phosphorus (TP) was assessed spectrophotometrically (Model UV-2100) at 840 nm following digestion with perchloric acid and determination of extracted orthophosphate using the ascorbic acid method (Kamegam et al. 2021). Total potassium (TK) was determined according to Abasi et al. (2018); ash samples were treated with hydrochloric acid, filtered, and measured using a flame photometer (Model 410C). Total organic carbon (TOC) in soil and vermicompost was estimated using the wet dichromate oxidation method and a dry combustion carbon analyzer, respectively (Cucina et al. 2018).

### Greenhouse experiment

The soil was obtained from Zefreh village in Isfahan Province. Treatments involved mixing 10% and 20% (v/v) vermicompost with soil in pots (14×16 cm), alongside a control (without vermicompost). Irrigation was applied based on field capacity. Flowering commenced in certain treatments 176 days after transplanting seedlings into the greenhouse. Aerial parts of Zarrin-giah were harvested in June after reaching 50% flowering.

### Preparation of herbal extract

Fresh leaf tissue (0.2 g) from each treatment was weighed, powdered in a mortar, and mixed with water and ethanol (1:10 ratio). The mixture was filtered, and 10 mg was stored at 4°C for experiments (Ebadi et al. 2022).

### Biomass measure

After harvesting the areal part of Zarrin-giah during the flowering stage, plants were shade-dried at 25°C for a week. Dry biomass was calculated using a digital scale.

### Essential oil content and yield determination

The amount of EO was determined following the method outlined in the British Pharmacopoeia (Brondz 1988). Specifically, 100 g of each dried sample was subjected to hydro-distillation for 3 hours using a Clevenger apparatus. The resulting essential oil was then dehydrated with sodium sulfate. The EO obtained from different treatments was stored in vials and kept at 4 °C. The content of EO (%) and its yield (g/plant) were calculated using the following equations:

$$\text{Essential oil content (\%)} = \frac{\text{Mass of oil obtained (g)}}{\text{Zarrin - giah mass of dry matter (g)}} \times 100 \quad (1)$$

$$\text{Essential oil yield (g/plant)} = \frac{(\text{Zarrin - giah biomass } \frac{\text{g}}{\text{plant}} \times \text{essential oil content \%})}{100} \quad (2)$$

## Essential oil chemical compounds analysis

To analyze EO compounds, Gas Chromatography (GC) was performed using a Thermo-UFM instrument equipped with an HP-5MS column measuring 30m in length and 0.25mm in diameter, with a stationary phase thickness of 0.25  $\mu\text{m}$ . The EO sample was injected into the GC/FID system. Helium gas served as the carrier gas. The injector and FID detector were maintained at 280°C, while the oven temperature was programmed to increase from 60 to 210 °C at a rate of 3 °C/min, then raised to 240 °C at a rate of 20 °C/min. The final temperature was held for 8.5 min. Chemical compounds of Zarrin-giah EO were identified by comparing mass spectrum and Retention Indices (RI) were calculated using the retention times of non-alkane standards.

## Antioxidant activity

The antioxidant activity was measured using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) inhibitory activity method. 3.9 ml of DPPH stock (0.004 g DPPH in 100 ml methanol) was poured into a falcon tube, followed by the addition of 0.1 ml of each herbal extract. The mixture was then placed in darkness for 30 min. The absorbance spectrum was visible at 517 nm. According to equation (3), the inhibition percentage was calculated using equation (3) as per (Mazaiei and Fahmideh 2018):

$$\text{Antioxidant activity (\%)} = \frac{(AB - AA)}{AB} \times 100 \quad (3)$$

AA and AB are the DPPH radical absorbance amounts in the herbal extract sample and the blank, respectively.

## Total phenolic content

The total phenolic content was measured using the Folin–Ciocalteu reagent method. 4 ml of 0.2 normal Folin–Ciocalteu reagent was added to 0.1 ml of each herbal extract. Five minutes later, 2 ml of a 75 g/l sodium carbonate solution was added to the mixture. The resulting solution was allowed to stand for 2 hours, and the absorbance was measured at 762 nm using a spectrophotometer. Gallic acid was used as a standard to draw the calibration curve graph (Mazaiei and Fahmideh 2018).

## Statistical analysis

The study was based on a completely randomized design as mean  $\pm$  SEM of 3 replications. To do the statistical analysis, SPSS version 22.0 was applied. Data were analyzed via analysis of variance (ANOVA) at a significance level of  $P \leq 0.05$ . Additionally, Duncan's Multiple Range test was utilized to assess treatment differentiation. Charts were generated using Excel version 2013 to visualize the results.

## Results and discussion

### Biomass measure

The analysis of variance showed a statistically significant difference ( $P \leq 0.01$ ) between the treatments (Table 1). According to Fig. 1, the highest biomass of Zarrin-giah was observed in VLW 20% treatment with a value of 29.05 (g/plant). The results of VMW 10%, VMW 20%, and VPW 10% were 20.33, 20.66, and 20.83 (g/plant), respectively, and fall within a similar range. Also, no significant difference was found between VVW 10% and C, with the lowest biomass recorded in the 20% VPW treatment at 19.60 g/plant.

**Table 1.** Variance analysis (mean square) for biomass, essential oil content, essential oil yield, antioxidant activity, and total phenol.

Source of variation	df	Bio-mass	Essential oil content	Essential oil yield	Total phenol	Antioxidant activity
Treatments	10	33.56**	0.072**	54.16**	2.93*	1411.58**
Error	22	4.60	0.003	0.35	1.44	2.38
CV	-	0.15	0.13	0.23	0.01	0.54

\* and \*\* significant at  $P \leq 0.05$  and  $P \leq 0.01$  respectively.

Various fertilization had a significant effect on Zarrin-giah biomass due to the relationship between mineral absorption from the soil solution by roots and plant functions. Huang et al. (2019) remarked that carbohydrates produced through photosynthesis are converted into energy (ATP), which serves as a vital cofactor in plant life cycle processes. In this instance, leaf area development is an essential factor for plant growth, respiration and crop quality, closely related to the wet and dry biomass of plants (Liu et al. 2020).

Plant residues consist of carbonic polymers such as cellulose, lignin, pectin, and chitin, which are broken down into monomers during vermicompost processing. This breakdown happens with the help of earthworms hydrolyzing enzymes, leading to a decrease in the C/N ratio due to an increase in nitrogen content in substrates (Enebe and Erasmus 2023). According to Table 2, it seems that during the vermicompost process, some enzymes are produced that increase the amount of TN, TP, and TK by acting on the vermicompost substrate, creating a suitable nutritional environment to improve Zarrin-giah growth and development.

**Table 2.** Soil and vermicompost physicochemical properties.

Parameters	Soil	VCW	VLW	VMW	VPW	VVW
Texture	Sandy loam	-	-	-	-	-
EC (dS/m)*	1.28	6.25	5.72	8.78	7.08	5.61
pH*	7.70	7.41	8.06	7.91	7.85	8.12
TN (g/kg)	1.80	11.66	11.91	11.66	22.04	22.07
TP (g/kg)	2.31	7.73	8.10	12.19	8.14	6.71
TK (g/kg)	9.05	14.01	20.64	19.92	24.50	36.34
TOC (%)	1.47	24.25	25.75	21.45	44.95	44.31

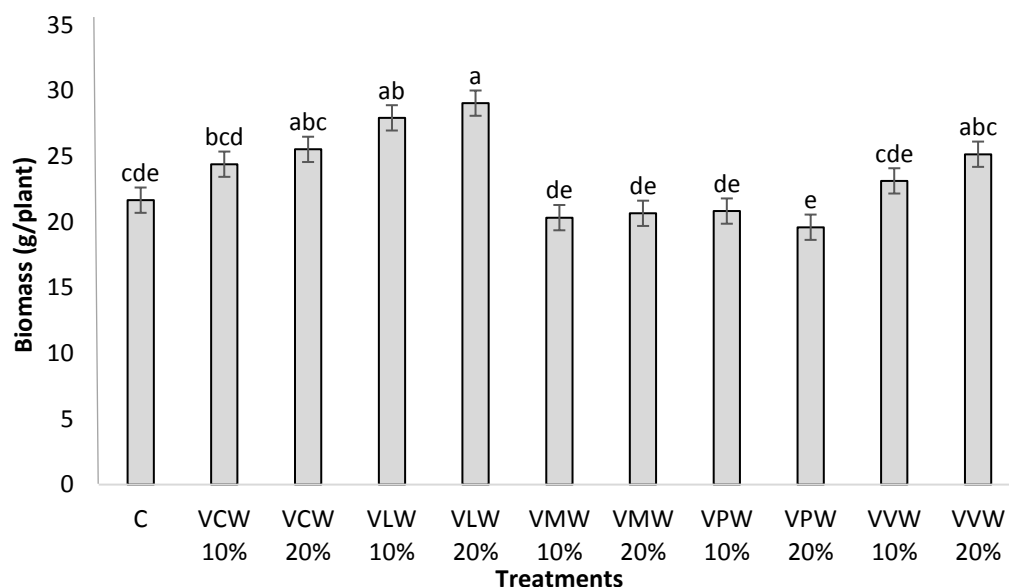
VCW, VLW, VMW, VPW and VVW are vermicompost from the waste of chamomile, licorice, madder, pistachio and valerian. EC is electrical conductivity and TN, TP, TK and TOC are total of (nitrogen, phosphorus, potassium and organic carbon), respectively.

\*Soil and vermicompost saturation extract-water were measured in 1:2 and 1:5(W/V), respectively.

The optimal concentration of macro-elements increased the Zarrin-giah biomass treated with vermicompost (Fig. 1). The results indicate that the biomass of plant samples treated with both levels of VLW exceeded that of other treatments and the control.

The humic substances in vermicompost increase cation exchange and stimulate growth-regulating hormones. Also, soil micro-organisms activities have improved the accumulation of nitrogen in the plant, resulting in profuse plant biomass (Ghimire et al. 2023). Li et al. (2016) reported that the application of vermicompost increases the

vegetative growth potential and biomass in *Mentha haplocalyx* × *Briq*. Moreover bio-fertilizers improved Greek oregano dry biomass in nutrient-poor soil by increasing mineral availability (Ninue et al. 2021). Emami Bistgani et al. (2018) described that vermicompost use (10 t ha<sup>-1</sup>) greatly expanded the photosynthetic surface area in *Thymus daenensis*. In another study, juxtaposing, the chemical and organic manures was shown the highest peppermint dry biomass in vermicompost (Asadi et al. 2023).

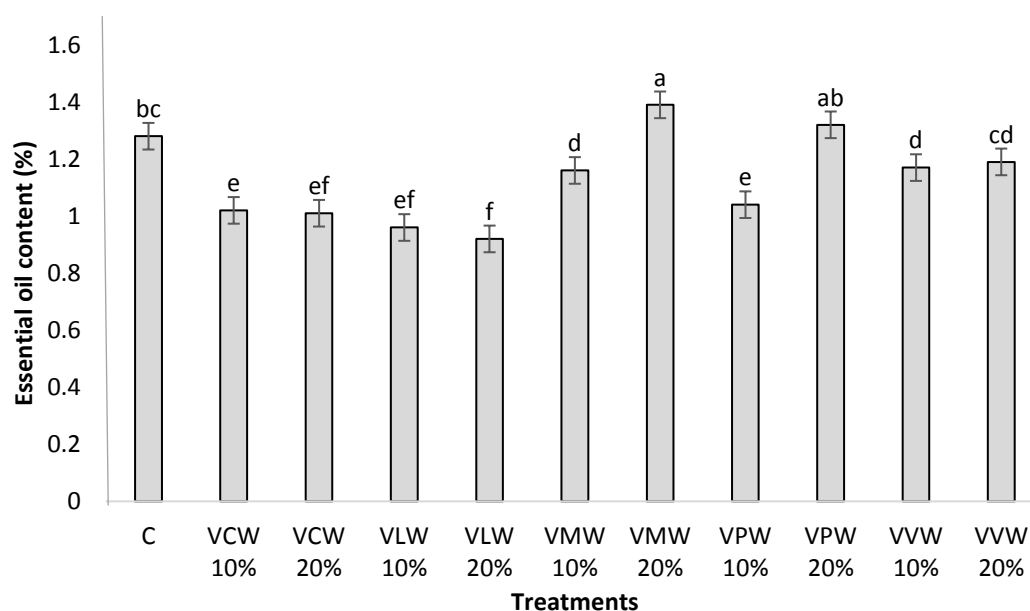


**Fig. 1** Biomass of Zarrin-giah results under different organic fertilizers using VCW, VLW, VMW, VPW and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio and valerian, respectively. The amount of 10% (V/V) and 20% (V/V) of vermicompost added in soil  $\pm$  SEM = 3 and Duncan's test at  $P \leq 0.05$ .

### Essential oil content

As presented in Table 2, the EO content in different treatments was statistically significant ( $P \leq 0.01$ ). Fig. 2 shows using 10% of vermicompost increased the EO content compared to the 20% and control treatments. According to the result, the vermicompost of chamomile and licorice waste in all levels have no significant differences in Zarrin-giah EO content analyzed.

Fallah et al. (2020) found a direct relationship between Zarrin-giah content and biomass. They observed that the organic manure substrate (broiler and sheep manure) supplies important nutrients, especially nitrogen, and phosphorus, which enhance EO content by improving biomass performance and leaf surface development. In the present study, the plant samples treated with VMW 20% and VPW 20% had the highest EO content. EC of some raw materials was excessively high and could influence the prepared vermicompost EC substrate. Thus, a higher EC in the vermicompost substrate, acting as mild stress, could contribute to increased Zarrin-giah EO content. The results obtained from this study were consistent with the results from previous reports about organic manure effects on EO content of *Lavandula officinalis* (Abbaszadeh et al. 2016); *Thymus vulgaris* (Amooaghaei and Gholmohammadi, 2017); *Satureja hortensis* (Heidarpour et al. 2019) and *Coriandrum sativum* (Rasouli et al. 2022).



**Fig. 2** The essential oil content of Zarrin-giah results under different organic fertilizers using VCW, VLW, VMW, VPW and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio and valerian, respectively. The amount of 10% (V/V) and 20% (V/V) of vermicompost added in soil  $\pm$  SEM = 3 and Duncan's test at  $P \leq 0.05$ .

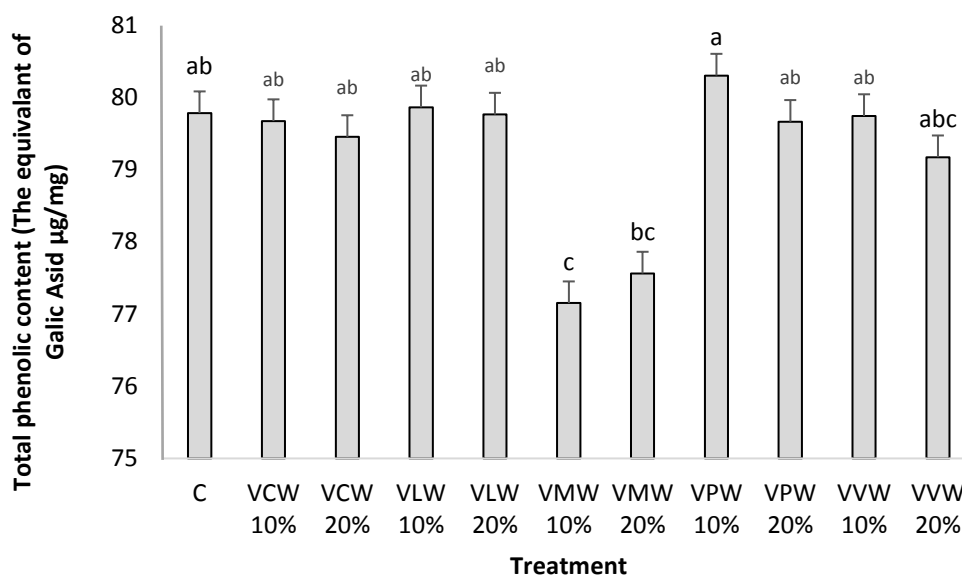
#### Total phenolic content assay

Table 2 displayed that the use of vermicompost made a significant difference ( $P \leq 0.05$ ) in the results of Zarrin-giah total phenol content. As illustrated in Fig. 3, Zarrin-giah treated with 10% VPW with 80.30 ( $\mu\text{g}/\text{mg}$ ) showed the highest phenol content. Other treatments did not significantly differ from the control, except for VVW 20%, VMW 10%, and 20%. The lowest phenol content was observed with VMW 10% at 77.15  $\mu\text{g}/\text{mg}$ .

Medicinal plants and pistachio are known for their bioactive agents such as phenolic compounds and flavonoids, which act as important antioxidants (Tungmunnithum et al. 2018; Ozey et al., 2021). Several studies have proven a relationship between phenolic compounds and antioxidant activity (Miliauskas et al. 2004; Chan et al. 2010; Kamali 2014), although some others have reported differing results (Bahramikia and Yazdanparast 2008; Moradi et al. 2020).

Genetics, cultivation conditions, and harvest time generally the phenolic compound composition in plant tissues (Kalaivanan et al. 2016). Jafari et al. (2015) illustrated that stress factors (UV rays, salinity, dryness, and high temperatures) can affect the content and percentage of secondary metabolites. The increase in EC observed in vermicompost is attributed to the digestive tract of *E. foetida* facilitating the exchange and decomposition of compounds containing calcium, magnesium, and potassium during vermicomposting (Kalaivanan et al. 2016).

Vermicompost-induced salinity enhances plant enzyme activities involved in the phenolic production pathway, thereby reducing oxidative damage and negative effects (Firoozeh et al. 2019). Secondary metabolites such as polyphenols are synthesized as secondary compounds, often stimulated by enzymes involved in starch and protein biosynthesis. Our results are consistent in line with Narimani et al. (2020).



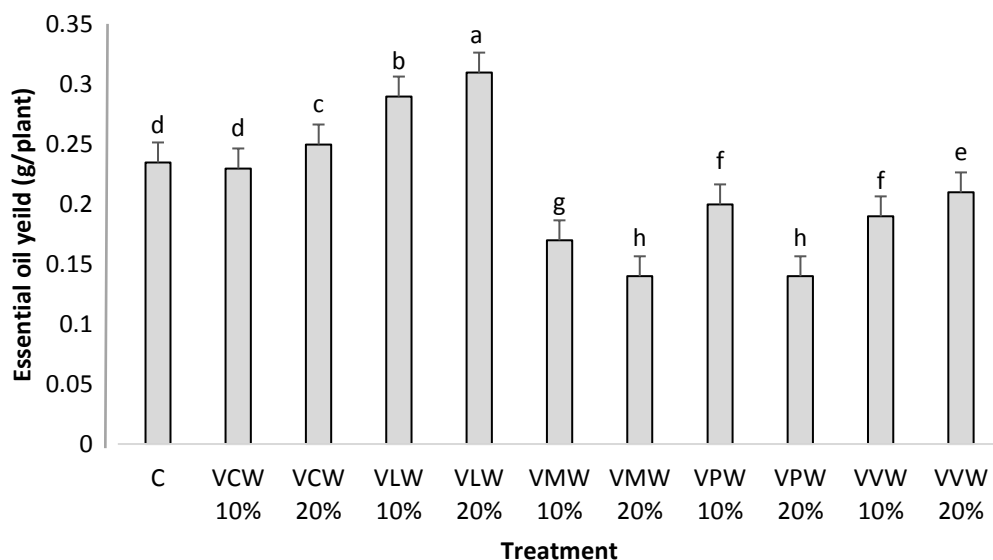
**Fig. 3** Zarrin-giah total phenolic content cultivated in different vermicompost treatments.

VCW, VLW, VMW, VPW, and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio, and valerian, respectively. The amount of 10% (V/V) and 20% (V/V) of vermicompost added in soil  $\pm$  SEM = 3 and Duncan's test at  $P \leq 0.05$ .

### Essential oil yield

The analyzed results indicate that there is a significant difference between the treated Zarrin-giah samples ( $P \leq 0.01$ ). The EO yield was lowest in VMW 20% and VPW 20% (0.14 g/plant) and highest in VLW 20% (0.31 g/plant). VCW 10% (0.23 g/plant) showed no significant difference compared to the control (0.24 g/plant). It seems that the high range of EO yield in VLW 20% is likely attributed to biomass enhancement (Fig. 4).

Numerous studies found a positive correlation between EO yield and biomass (Li et al. 2016; Ghimire et al. 2023). Although VMW and VPW mostly had a significant effect on Zarrin-giah glandular trichomes, EO yield increased slowly, potentially due to inhibited potassium (K) absorption by the plants. K plays a critical role in activating CO<sub>2</sub> reduction enzymes, and its deficiency can lead to a reduction in leaf area. Studies have demonstrated that cultivation location, harvest timing, type of fertilizer used, and soil properties significantly influence EO yield (Malaka et al. 2022). The improvement of nutrient availability in VLW 20% is well evident that adequate absorption of nutrients from the substrate increased the effectiveness of EO yield in Zarrin-giah. The EO yield of the samples treated with vermicompost produced from madder, pistachio, and valerian waste was lower than the control. Although vermicompost facilitates longer access to macronutrients, the high EC in the soil solution decreases the absorption of these elements by the roots (Ninou et al. 2021). Organic fertilizer increased the yield of basil EO compared to chemical fertilizer (Rostaei et al. 2018). Also, vermicompost increased the number of secondary metabolites in dill EO (Falah et al. 2018). The research of Malka et al. (2022) showed that the use of natural manure affects both the quantity and quality of nitrogen availability and increases the efficiency of EO in Marjoram by about 0.09%.



**Fig.4** The essential oil yield of Zarrin-giah results under different organic fertilizers using VCW, VLW, VMW, VPW, and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio, and valerian, respectively. The amount of 10% (V/V) and 20% (V/V) of vermicompost added in soil  $\pm$  SEM = 3 and Duncan's test at  $P \leq 0.05$ .

#### Chemical compositions of essential oil

The analysis of GC results in Tables 3 and 4 display that the application of 10% of vermicompost increased the percentage of EO compounds in Zarrin-giah, but these values decreased at higher vermicompost levels except for VMW 20% and VPW 20%.

The compounds extracted from the aerial parts of Zarrin-giah are listed in Table 3, where 34-36 compounds were identified at the 10% treatment level and 33 compounds were indicated in C. Also, 23-30 compounds were found at the level of 20% (Table 4). In all samples,  $\alpha$ -pinene (17.42-24.15%), limonene (5.51-13.09%), neral (19.17-24.18%), geranial (29.58-20.165%), and geranyl acetate (3.13-6.99%) were identified as the main compounds.

Limonene has been recognized as an essential compound for inhibiting cancer cell pathways in animal models (Yu et al. 2018). Also,  $\alpha$ -pinene is one of the unique components in many essential oils with bioactive anti-viral properties. Because of the fight against COVID-19, there was a focus on propagating plants rich in  $\alpha$ -pinene compounds in 2020-2021 (Panikar et al. 2021). Laboratory synthesis of these chemical compositions from plants is often challenging or impossible.

Increased salinity in vermicompost depends on mineral salt concentration. With the help of biological agents, the insoluble mineral salts are dissolved in organic manure (Gupta and Garg, 2008). Salinity increases the percentage of essential oil in most medicinal plants. Secondary metabolites are produced due to salt stress to prevent cellular oxidation (Amiri and Ghasemi 2018).

The amount of essential oil in VMW and VPW with EC values of 8.78 and 7.08 dS/m, respectively, was recorded to be higher than in other treatments. This could be attributed to the initial vermicompost materials (madder and pistachio) being resistant to saline soil and capable of regulating osmosis. In these plants, the absorption of potassium by the roots and its transfer to the aerial parts is more than that of sodium. Accumulation of salt in vacuoles occurs due to increased sodium around the roots (Alipour et al. 2017; Banakar et al. 2018)

Previous studies have shown that the application of vermicompost modifies soil physicochemical properties, enhancing nutrient absorption and consequently increasing essential oil production in medicinal plants (Al-Amri 2021). Various organic materials such as broilers, cow manure, and sheep manure as some different organic materials have a significant effect on Zarrin-giah essential oil content and compound percentage (neral, geranium, and  $\alpha$ -pinene) increase compared to the control (Fallah et al. 2020).

According to Amiri and Ghasemi's (2018) research, vermicompost with salinity feedstock had no significant effect on the vegetative growth of *Satureja rechingeri* but it could improve the biochemical properties and essential oil percentage in this plant (Amiri and Ghasemi 2018). Also, their result showed that the increasing trend in salt levels (from 150 to 300  $\mu$ S) decreased the main components of the essential oil namely linalool, thymol, and 4-terpineol in *S. rechingeri* (Amiri and Ghasemi 2018). Pandey et al. (2016) explained that vermicompost use increased chavicol and linalool in basil EO but resulted in lower levels of other compounds like  $\beta$ -caryophyllene,  $\alpha$ -cadinene, (E)- $\beta$ -farnesene, and  $\gamma$ -moverolene.

### **Antioxidant activity**

There was a significant difference ( $P \leq 0.01$ ) in the studied samples of antioxidant activities (Table 2). According to Fig. 5, the concentration of antioxidant compounds ranged between 19.26 and 84.28  $\mu$ g/ml. As a result, the lowest amount of antioxidant activity was in C ( $IC_{50} = 84.28 \mu$ g/ml), while the highest antioxidant was obtained with VPW 10%, VPW 20%, and VVW 10% ( $IC_{50} = 19.26, 19.29, 20.35 \mu$ g/ml), respectively. VLW 10%, VMW 20%, and VVW 20% were not significantly different in exhibiting considerable DPPH radical scavenging, although antioxidant activity in all treatments increased compared to the control.

The structure of the phenolic group is responsible for antioxidant functions (Firoozeh et al. 2019). The presence of variable polyphenolic compounds such as ascorbate, anthocyanin, carotenoids, and flavonoids affects the plant's antioxidant activity under different fertilizer treatments (Rostaei et al. 2018; Falah et al. 2020a). Although the oxygenated monoterpenes increase the plant extracts antioxidant properties, the research results of Fallah et al. (2018) discovered that the use of organic manure prepared from broilers had less oxygenated monoterpenes in Zarrin-giah compared to the other treatments, but it had a higher antioxidant capacity. The increase in antioxidant activity may be due to the vermicompost's ability to supply essential elements (N, P, and S) in the amino acids pathway synthesis. In medicinal plants, the basic structure of chemical compounds are hydroxyl and carboxyl groups and because of that, they show strong antioxidant activity. Vermicompost application rates have beneficial effects on medicinal plants' antioxidant activity (Saha and Basak 2020). It was found that humic acid is an important substance in vermicompost that has a positive correlation with involved antioxidant compounds enzymes in the root, thereby enhancing antioxidant capacity, increasing plant tolerance to biotic and abiotic stresses, and improving plant growth (Abbaszadeh et al. 2016).

**Table 3.** Zarrin-giah chemical compositions of essential oil results under 10% (V/V) treatments and control

Compound (%)	RI	VCW	VLW	VMW	VPW	VVW	C
		10%	10%	10%	10%	10%	
$\alpha$ -Pinene	934	17.49 $\pm$ 1.04	19.89 $\pm$ 2.01	17.71 $\pm$ 2.56	20.85 $\pm$ 3.10	17.42 $\pm$ 3.11	23.8 $\pm$ 4.12
Camphene	946	0.09 $\pm$ 0.11	0.08 $\pm$ 0.52	0.07 $\pm$ 0.18	0.08 $\pm$ 0.30	0.07 $\pm$ 0.01	0.1 $\pm$ 0.08
Sabinene	971	0.39 $\pm$ 0.28	0.43 $\pm$ 0.09	0.35 $\pm$ 0.90	0.49 $\pm$ 0.64	0.45 $\pm$ 0.31	0.41 $\pm$ 0.16
$\beta$ -Pinene	975	0.26 $\pm$ 0.14	0.31 $\pm$ 0.16	0.27 $\pm$ 0.11	0.31 $\pm$ 0.08	0.28 $\pm$ 0.45	1.11 $\pm$ 0.21
6-methyl-5-Hepten-2-one	984	1.59 $\pm$ 0.23	0.01 $\pm$ 0.46	-	-	-	0.09 $\pm$ 0.01
Myrcene	989	0.22 $\pm$ 0.04	1.99 $\pm$ 0.15	1.52 $\pm$ 0.85	1.98 $\pm$ 0.45	1.47 $\pm$ 0.01	2.12 $\pm$ 0.09
$\alpha$ -Phellandrene	1004	0.05 $\pm$ 0.17	0.12 $\pm$ 0.68	0.13 $\pm$ 0.25	0.15 $\pm$ 0.31	0.30 $\pm$ 0.43	0.54 $\pm$ 0.56
$\alpha$ -Terpinene	1015	0.29 $\pm$ 0.36	0.03 $\pm$ 0.41	0.03 $\pm$ 0.38	0.03 $\pm$ 0.52	0.09 $\pm$ 0.01	0.28 $\pm$ 0.31
p-Cymene	1022	0.44 $\pm$ 0.06	0.17 $\pm$ 0.10	0.23 $\pm$ 0.36	0.22 $\pm$ 0.09	0.39 $\pm$ 0.42	3.43 $\pm$ 0.76
Limonene	1028	7.14 $\pm$ 0.41	5.51 $\pm$ 0.09	11.52 $\pm$ 0.41	9.01 $\pm$ 0.28	10.1 $\pm$ 0.75	13.09 $\pm$ 0.25
1,8-Cineole	1029	0.01 $\pm$ 0.64	1.16 $\pm$ 0.31	1.25 $\pm$ 0.72	1.53 $\pm$ 0.76	1.48 $\pm$ 0.72	0.05 $\pm$ 0.09
(Z)- $\beta$ -Ocimene	1034	0.10 $\pm$ 0.09	0.01 $\pm$ 0.32	-	-	-	0.080.28
(E)- $\beta$ -Ocimene	1044	0.10 $\pm$ 0.43	0.06 $\pm$ 0.38	0.07 $\pm$ 0.69	0.08 $\pm$ 0.71	0.10 $\pm$ 0.05	0.15 $\pm$ 0.38
$\gamma$ -Terpinene	1055	0.07 $\pm$ 0.61	0.06 $\pm$ 0.73	0.08 $\pm$ 0.65	0.06 $\pm$ 0.17	0.09 $\pm$ 0.34	-
cis-Sabinene hydrate	1064	0.16 $\pm$ 0.53	0.11 $\pm$ 0.62	0.09 $\pm$ 0.10	0.12 $\pm$ 0.04	0.08 $\pm$ 0.16	-
Terpinolene	1086	0.82 $\pm$ 0.18	0.20 $\pm$ 0.01	0.14 $\pm$ 0.38	0.17 $\pm$ 0.41	0.16 $\pm$ 0.37	0.28 $\pm$ 0.46
Linalool	1098	0.47 $\pm$ 0.22	0.39 $\pm$ 0.15	0.45 $\pm$ 0.76	0.40 $\pm$ 0.36	0.31 $\pm$ 0.44	1.160.09
$\alpha$ -Campholenal	1123	1.12 $\pm$ 0.31	0.93 $\pm$ 0.65	1.14 $\pm$ 0.44	1.00 $\pm$ 0.09	1.43 $\pm$ 0.22	2.940.30
cis-Limonene oxide	1134	0.26 $\pm$ 0.15	0.09 $\pm$ 0.64	0.15 $\pm$ 0.07	0.12 $\pm$ 0.08	0.22 $\pm$ 0.58	-
cis-Verbenol	1141	0.46 $\pm$ 0.01	0.19 $\pm$ 0.58	0.26 $\pm$ 0.06	0.24 $\pm$ 0.63	0.40 $\pm$ 0.63	0.52 $\pm$ 0.92
Borneol	1171	1.42 $\pm$ 0.33	0.88 $\pm$ 0.48	0.67 $\pm$ 0.62	0.70 $\pm$ 0.31	0.67 $\pm$ 0.04	-
Terpinen-4-ol	1179	0.35 $\pm$ 0.01	1.40 $\pm$ 0.20	1.13 $\pm$ 0.54	1.06 $\pm$ 0.09	0.13 $\pm$ 0.28	1.16 $\pm$ 0.23
$\alpha$ -Terpineol	1188	0.15 $\pm$ 0.18	0.34 $\pm$ 0.48	0.28 $\pm$ 0.76	0.35 $\pm$ 0.22	0.40 $\pm$ 0.01	0.41 $\pm$ 0.65
Myrtenal	1192	0.44 $\pm$ 0.22	0.20 $\pm$ 0.14	0.24 $\pm$ 0.54	0.38 $\pm$ 0.01	0.17 $\pm$ 0.76	0.32 $\pm$ 0.14
trans-Carveol	1218	0.17 $\pm$ 0.04	0.11 $\pm$ 0.75	0.53 $\pm$ 0.01	0.09 $\pm$ 0.21	0.50 $\pm$ 0.11	0.35 $\pm$ 0.39
Nerol	1230	0.74 $\pm$ 0.08	0.33 $\pm$ 0.18	0.21 $\pm$ 0.08	0.41 $\pm$ 0.31	0.22 $\pm$ 0.55	1.63 $\pm$ 0.48
Neral	1238	20.99 $\pm$ 3.33	21.15 $\pm$ 2.36	21.6 $\pm$ 3.61	19.33 $\pm$ 4.28	19.17 $\pm$ 2.34	22.13 $\pm$ 3.29
Geraniol	1255	0.14 $\pm$ 0.27	0.95 $\pm$ 0.22	-	0.74 $\pm$ 0.31	0.55 $\pm$ 0.01	0.27 $\pm$ 0.20
Geranial	1268	29.58 $\pm$ 2.57	28.65 $\pm$ 3.24	26.11 $\pm$ 2.18	26.67 $\pm$ 1.11	28.55 $\pm$ 3.22	20.16 $\pm$ 0.49
Isobornyl acetate	1285	0.08 $\pm$ 0.35	0.08 $\pm$ 0.13	0.16 $\pm$ 0.22	0.10 $\pm$ 0.18	0.22 $\pm$ 0.09	-
Methyl geranate	1324	4.71 $\pm$ 0.26	5.34 $\pm$ 0.33	4.23 $\pm$ 0.61	4.30 $\pm$ 0.28	4.80 $\pm$ 0.16	5.25 $\pm$ 0.22
Eugenol	1354	0.01 $\pm$ 0.05	0.03 $\pm$ 0.21	-	-	0.04 $\pm$ 0.08	0.09 $\pm$ 0.08
Neryl acetate	1362	0.10 $\pm$ 0.15	0.15 $\pm$ 0.38	0.08 $\pm$ 0.43	0.10 $\pm$ 0.61	0.09 $\pm$ 0.73	0.12 $\pm$ 0.15
$\alpha$ -Copaene	1372	0.07 $\pm$ 0.33	0.07 $\pm$ 0.11	0.08 $\pm$ 0.27	0.06 $\pm$ 0.56	0.04 $\pm$ 0.12	5.78 $\pm$ 0.55
Geranyl acetate	1384	6.27 $\pm$ 0.13	5.25 $\pm$ 0.95	5.41 $\pm$ 0.04	6.37 $\pm$ 0.22	6.99 $\pm$ 0.47	3.13 $\pm$ 0.87
Germacrene D	1481	0.17 $\pm$ 0.79	0.11 $\pm$ 0.45	0.12 $\pm$ 3.37	0.15 $\pm$ 0.11	0.14 $\pm$ 0.06	1.16 $\pm$ 1.12
Total	-	96.92	96.80	96.31	96.65	97.52	92.11

$\pm$  SEM = 3. RI = Retention index

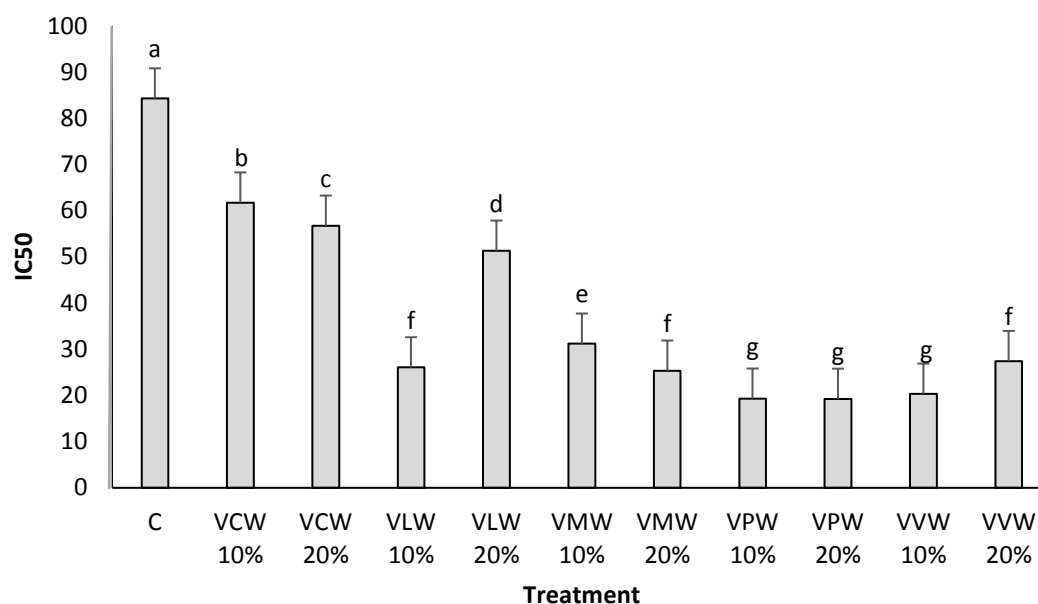
VCW, VLW, VMW, VPW, VVW, and C are vermicompost from waste of chamomile, licorice, madder, pistachio, valerian, and control, respectively.

**Table 4.** Zarrin-giah chemical compositions of essential oil results under 20% (V/V) treatments

Compound (%)	RI	VCW	VLW	VMW	VPW	VVW
		20%	20%	20%	20%	20%
$\alpha$ -Pinene	934	19.6 $\pm$ 2.30	20.78 $\pm$ 1.18	21.09 $\pm$ 1.16	24.15 $\pm$ 1.48	24.05 $\pm$ 3.22
Camphene	946	0.07 $\pm$ 0.12	-	-	-	-
Sabinene	971	0.09 $\pm$ 0.27	0.16 $\pm$ 0.22	0.45 $\pm$ 0.01	0.20 $\pm$ 0.12	0.09 $\pm$ 0.54
$\beta$ -Pinene	975	-	-	0.20 $\pm$ 0.01	0.12 $\pm$ 0.05	0.86 $\pm$ 0.63
6-methyl-5-Hepten-2-one	984	-	-	-	-	-
Myrcene	989	0.20 $\pm$ 0.11	1.25 $\pm$ 0.02	1.50 $\pm$ 0.62	1.83 $\pm$ 0.76	1.8 $\pm$ 0.63
$\alpha$ -Phellandrene	1004	-	0.18 $\pm$ 0.09	-	-	-
$\alpha$ -Terpinene	1015	0.11 $\pm$ 0.01	0.01 $\pm$ 0.76	-	-	-
p-Cymene	1022	0.47 $\pm$ 0.15	0.45 $\pm$ 0.87	0.27 $\pm$ 0.04	0.29 $\pm$ 0.11	0.23 $\pm$ 0.01
Limonene	1028	9.08 $\pm$ 0.22	10.45 $\pm$ 0.60	14.15 $\pm$ 0.34	12.00 $\pm$ 0.01	6.70 $\pm$ 0.45
1,8-Cineole	1029	-	-	-	0.25 $\pm$ 0.48	-
(Z)- $\beta$ -Ocimene	1034	0.05 $\pm$ 0.48	-	-	-	-
(E)- $\beta$ -Ocimene	1044	0.07 $\pm$ 0.87	0.06 $\pm$ 0.47	0.05 $\pm$ 0.55	0.06 $\pm$ 0.68	-
$\gamma$ -Terpinene	1055	-	-	-	-	-
cis-Sabinene hydrate	1064	-	0.21 $\pm$ 0.01	-	-	-
Terpinolene	1086	0.26 $\pm$ 0.23	-	-	-	-
Linalool	1098	0.33 $\pm$ 0.57	0.28 $\pm$ 0.75	0.41 $\pm$ 0.22	0.35 $\pm$ 0.14	0.43 $\pm$ 0.07
$\alpha$ -Campholenal	1123	0.91 $\pm$ 0.54	1.21 $\pm$ 0.03	1.07 $\pm$ 0.01	0.05 $\pm$ 0.23	-
cis-Limonene oxide	1134	0.12 $\pm$ 0.66	0.15 $\pm$ 0.72	1.31 $\pm$ 0.11	0.03 $\pm$ 0.12	-
cis-Verbenol	1141	0.41 $\pm$ 0.25	0.37 $\pm$ 0.01	0.21 $\pm$ 0.24	0.19 $\pm$ 0.18	0.17 $\pm$ 0.01
Borneol	1171	1.35 $\pm$ 0.95	0.60 $\pm$ 0.22	0.64 $\pm$ 0.33	0.65 $\pm$ 0.76	0.78 $\pm$ 0.21
Terpinen-4-ol	1179	-	-	1.07 $\pm$ 0.41	0.06 $\pm$ 0.28	1.22 $\pm$ 0.05
$\alpha$ -Terpineol	1188	0.11 $\pm$ 0.87	0.36 $\pm$ 0.74	0.34 $\pm$ 0.32	0.30 $\pm$ 0.45	0.26 $\pm$ 0.01
Myrtenal	1192	0.46 $\pm$ 0.53	0.22 $\pm$ 0.37	0.31 $\pm$ 0.26	0.45 $\pm$ 0.88	0.26 $\pm$ 0.26
trans-Carveol	1218	0.21 $\pm$ 0.32	0.55 $\pm$ 0.95	0.61 $\pm$ 0.32	0.14 $\pm$ 0.76	0.17 $\pm$ 0.22
Nerol	1230	0.70 $\pm$ 0.31	0.19 $\pm$ 0.74	0.19 $\pm$ 0.64	0.39 $\pm$ 0.48	0.29 $\pm$ 0.09
Neral	1238	22.09 $\pm$ 1.87	21.34 $\pm$ 2.54	23.08 $\pm$ 1.80	24.18 $\pm$ 2.15	20.42 $\pm$ 1.11
Geraniol	1255	0.04 $\pm$ 1.31	-	-	0.56 $\pm$ 0.45	0.76 $\pm$ 0.65
Geranial	1268	28.06 $\pm$ 1.85	27.50 $\pm$ 2.12	25.09 $\pm$ 1.76	25.01 $\pm$ 4.12	26.81 $\pm$ 2.28
Isobornyl acetate	1285	0.11 $\pm$ 0.48	0.24 $\pm$ 0.58	0.18 $\pm$ 0.74	0.12 $\pm$ 0.91	0.10 $\pm$ 0.21
Methyl geranate	1324	4.31 $\pm$ 0.40	3.36 $\pm$ 0.53	-	-	-
Eugenol	1354	-	0.01 $\pm$ 0.12	0.07 $\pm$ 0.11	-	-
Neryl acetate	1362	0.08 $\pm$ 0.45	0.07 $\pm$ 0.13	0.60 $\pm$ 0.11	0.08 $\pm$ 0.22	0.09 $\pm$ 0.01
$\alpha$ -Copaene	1372	0.06 $\pm$ 2.25	-	-	-	-
Geranyl acetate	1384	4.00 $\pm$ 0.15	4.75 $\pm$ 0.09	5.35 $\pm$ 0.10	4.05 $\pm$ 0.11	6.00 $\pm$ 0.01
Germacrene D	1481	0.13 $\pm$ 0.22	0.10 $\pm$ 0.51	0.90 $\pm$ 0.69	0.09 $\pm$ 0.67	1.30 $\pm$ 0.20
Total	-	96.30	96.55	99.14	95.60	96.79

$\pm$  SEM = 3. RI = Retention index

VCW, VLW, VMW, VPW and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio, and valerian, respectively.



**Fig. 5** Antioxidant activity of Zarrin-giah results under different organic fertilizers using VCW, VLW, VMW, VPW and VVW are vermicompost from waste of chamomile, licorice, madder, pistachio and valerian, respectively. The amount of 10% (V/V) and 20% (V/V) of vermicompost added in soil. According to mean  $\pm$  SEM of 3 replications and Duncan's test at  $P \leq 0.05$ .

## Conclusion

Agricultural producers and industrial owners are seeking solutions to manage the substantial amounts of residual biomass generated from pistachio and medicinal plants to avoid environmental harm. This biomass can be effectively recycled into value-added manure such as vermicompost. The results of this research show that using 20% of VMW and VPW treatments increased the EO content of Zarrin-giah essential by 1.39 and 1.32%, respectively. The best results in antioxidant activity were observed in plant samples treated with 10% vermicompost. Also, neral,  $\alpha$ -pinene, geranial, geranyl acetate, and limonene were identified as the main chemical compounds in all treatments. Herbal primary sources with high phenol compounds and EC have the potential to be converted into vermicompost. Although high EC in the raw material and final vermicompost production did not have a positive effect on Zarrin-giah biomass, it increased the essential oil content. Therefore, it is suggested to utilize these materials as safe and suitable fertilizers to enhance the extraction of Zarrin-giah medicinal plant components.

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