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Organic vs. Inorganic Fertilizers: Effects on Growth Parameters and Phenol Content in Cultivated and Wild Safflower

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ABSTRACT

Aims: The objective of this study was to compare growth parameters and phenol content of different safflower (*Carthamus tinctorius*) cultivars and one wild species of safflower (*Carthamus oxyacantha)* under urea fertilizer and vermicompost treatments to distinguish can we use vermicompost instead of urea fertilizer.

Materials & Methods: The experiment was carried out under greenhouse conditions. A factorial randomized complete block design with seven replications was used. Experimental treatments included three vermicompost rates, three nitrogen fertilizer levels, and three safflower types.

Findings: The number of leaves, leaf area, and fresh and dry biomass weights significantly increased with vermicompost application. Urea fertilizer only increased the plant's height. The interaction between plant cultivars and vermicompost was significant for phenol content. All growth parameters of safflower except dry biomass weight significantly differed between cultivars.

Conclusions: Wild safflower responded more than other cultivars to treatments so that it can be used in plant breeding programs. Vermicompost treatment acts better than urea fertilizer for growing safflower so it can be used instead of chemical fertilizer.

Keywords: Growth Parameters; Safflower; Secondary Metabolites; Urea Fertilizer; Vermicompost.

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Introduction

Vermicompost is a stabilized organic matter produced through interaction between earthworms and microorganisms in an aerobic and mesophilic process [1, 2]. Vermicompost as a plant growth media and soil amendment is used considerably in agriculture. It provides nutrients such as available N, soluble K, exchangeable Ca, Mg, and P with a balanced release pattern so plants can take them up easily [1, 3]. Organic manure like vermicompost improves the physical properties of soil [4], such as making high porosity, aeration, drainage, and water holding, by increasing humus content in the soil [5]. In addition, microbiota, especially fungi, bacteria, and actinomycetes in vermicompost, help to convert unavailable nutrients in the soil into available forms [6]. Humic acid in vermicompost can operate as a plant growth regulator or hormonal plant growth regulators may have absorbed by the humates that are produced by microorganisms [7,10].

Many scientific studies demonstrate that adding low amounts of vermicompost into plant growth media in a greenhouse or as amendments to field soils significantly improves plant growth and yield of various plant species [7, 11-21]. For example, in a study, the effects of different vermicompost rates on Brassica oleracea growth parameters were investigated, and it was observed that plant stand height, cabbage head, and cabbage leaves were significantly increased by vermicompost application. Consequently, vermicompost significantly affected cabbage growth promotion [12].

Phenolic compounds are known as secondary metabolites in all plant species and are synthesized in response to different environmental factors, such as nutrient stress or attack by pests and diseases [22-24]. Phenolic substances exist in fruits and vegetables and provide natural pigmentation, taste, and aroma to fruit and a wide range of antioxidant protection and therapeutic benefits for human health [25-27], so it seems necessary for food production for more marketability [28]. The total amount of phenolic compounds in plants grown in organic conditions like vermicompost is higher than those grown in inorganic conditions [29,31], so plants grown in vermicompost have more resistance against diseases and arthropod pests that could be attributed to the higher amounts of phenolic compounds in such plants [2, 7, 12, 32,37].

Nitrogen is required for the metabolic processes during plant growth because it is a component of proteins that plays an essential role in photosynthesis, cell division and differentiation, growth and somatic embryogenesis, chlorophyll content, electron transport rate, photosynthetic rate and anthocyanin production [38,42]. Adequate amounts of nitrogen fertilizer are required for all crop plants [43].

A common form of nitrogen used in agriculture is urea fertilizer, which generally increases the growth of plants and, specifically, the leaf area index that, causes an increase in light absorption and, consequently, more dry matter and yield [44, 45]. Using urea fertilizer is one of crop plants' most crucial crop management strategies. Hence, many studies have different results about nitrogen fertilizer amounts [46-48]. For example, in a study where three rates of nitrogen (0, 100, and 200 kg ha^{-1}) and two hybrids of safflower were used, N fertilizer increased the growth of safflower. It increased dry matter and seed yields under rain-fed conditions [46]. In another study, 75 kg ha^{-1} nitrogen was adequate for safflower's optimum seed, oil, and protein yield, but higher N rates (up to 600 kg ha^{-1}) did not increase seed yield or any yield components [49]. In contrast, in another study on safflower, N fertilization yielded no yield increase [50]. In California, it is recommended to apply 110 –170 kg ha^{-1}

for irrigated safflower [47].
Safflower (*Carthamus tinctorius* L., Asteraceae) is an oil seed crop mainly grown in semi-arid regions [51]. Safflower is a strongly tap-rooted annual plant well adapted to dry and salty lands, resists saline conditions and drought stress, and can reach the deep–lying water [51]. Safflower is mainly cultivated for edible oil with a higher percentage of essential unsaturated fatty acids like oleic and linoleic acid. However, it uses in many aspects like margarine, liqueur, candle, drying oil in paints, linoleum varnishes, wax cloths, birdseed food, and medicines [52-57]. These days the importance of oil seed crops like safflower increased remarkably due to biofuel production [51-58]. Some safflower varieties have different responses to fertilizer treatment, but wild species can be necessary because of their tolerance to saline conditions and drought stress.
Careful attention is required to apply adequate quality of nutrients through proper sources. Appropriate fertilizer with proper amounts and application time can significantly affect crop yield [59]. The addition of chemical fertilizers guarantees a high yield to farmers. However, it harms the physical, chemical, and biological properties of soil and other organisms in an ecosystem. Thus, any step in reducing chemical fertilizer use can be a good management strategy for crop production [60].
The objectives of this study were: i) to compare growth parameters and phenol content of safflower (agricultural and wild species) under different rates of urea fertilizer and vermicompost, ii) to see if there is any interaction between urea fertilizer and vermicompost that affect plant growth parameters iii) to see if there is a possibility of replacing urea fertilizer with vermicompost in crop management of safflower to exclude the disadvantages of urea fertilizer usage, iv) to distinguish how the wild species responses to these treatments and compare it with agricultural varieties.

Materials & Methods

Experimental Design

The experiment was conducted under greenhouse conditions at 24˚C in the day and 20˚C at night with a relative humidity of 50-60% and a light period of 16:8 (Light: Darkness). A factorial randomized complete block design with seven replications was used. Experimental treatments were three vermicompost rates (V_0=0% (control), V_{20}=20%, V_{40}=40% of growth media); three levels of nitrogen fertilizer (N_0=0 (control), N_{50}=50, N_{100}=100 Kg.ha^{-1}) in the form of urea; and three types of a safflower plant (two agricultural varieties and one wild species). The two agricultural cultivars were Goldasht and Mahali-Isfahan, and the wild safflower species was Carthamus oxyacantha.

Experimental Procedure

The growth media used in the experiment consisted of one-half clay and one-half sand. Depending on vermicompost treatment, it was mixed with the growth media. Seeds were sown into pots, and after germination, two plants in each pot were kept, and the others were removed. Plants were irrigated as needed. Depending on the N treatment level, urea fertilizer was applied in two stages: one-half in the four leaves stage and another in the eight leaves stage. After 35 days (10 leaves stage), all plants were cut from the soil surface, and parameters such as plant height, number of leaves, plant fourth leaf area, and fresh plant weight were measured. Four replicates were used to measure plant dry weight, and the rest were used to measure the relative water and phenol content. Plants were placed in an oven at 70˚C for 72 hours to obtain dry weight and then weighed. The plant's fourth leaf was cut and weighed to measure the relative water content. Then emerged in deionized water for five hours

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and then weighed again. Afterward, leaves were placed for 72 hours in an oven at 70°C, then the dry weight was measured. The leaf's relative water content was calculated using the corresponding equation [61]. Total phenolic compounds were measured using Roland & Laima's [62] method. According to the method, Folin-Ciocalteu was used as a reagent, and results were expressed as milligrams of gallic acid equivalent per gram of fresh weight. Solution absorbance was read at 760 nm by a micro-plate reader, model BioTek ELx 808.

Statistical Analysis

Data analyses were performed using IBM SPSS software version 22. Among plant parameters that measured only plant height, leaf area and phenol content had normal distributions (Shapiro-Wilk test). The other data were transformed as fresh plant weight by $\log_{10}$, number of leaves, dry weight, and relative water content by square root. The data were analyzed statistically by analysis of variance (ANOVA) followed by LSD (the Least Significant Difference) test to compare means at a probability level of <0.05. Pearson correlation coefficients among the studied parameters were also calculated.

Findings

Data analysis revealed that the effects of both treatments (Vermicompost and Urea fertilizer) were statistically significant for some of the parameters compared to the control (Table 1). In addition, some parameters were also significantly different between plant varieties.

Plant Height

Plant height was significantly affected by urea fertilizer, and it was different between

Table 1) Analysis of variance of growth parameters and phenol content of safflower under three levels of vermicompost treatments, three amounts of urea fertilizer, and their interactions (α= 0.05) in different plant cultivars.

Source of Variation	Plant Height		Number of Leaves		Plant Fresh Biomass		Plant Dry Biomass		Plant Leaf Area		Relative Water Content		Plant Phenol Content	
	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df
Vermicompost	55.67^{ns}	2	3.28^{**}	2	1.79^{**}	2	0.63^{**}	2	197.72^{**}	2	0.22^{ns}	2	17.79^{ns}	2
Urea Fertilizer	369.09^{*}	2	0.54^{ns}	2	0.01^{ns}	2	0.06^{ns}	2	11.27^{ns}	2	0.69^{ns}	2	85.58^{ns}	2
Plant Cultivar	3819.79^{**}	2	1.72^{**}	2	0.76^{**}	2	0.08^{ns}	2	157.7^{**}	2	6.70^{**}	2	20.17^{ns}	2
Vermicompost * Plant Cultivar	123.57^{ns}	4	0.11^{ns}	4	0.03^{ns}	4	0.01^{ns}	4	7.38^{ns}	4	0.97^{ns}	4	66.41^{*}	4
Urea fertilizer * Plant Cultivar	51.48^{ns}	4	0.25^{ns}	4	0.08^{ns}	4	0.05^{ns}	4	34.95^{**}	4	0.74^{ns}	4	33.16^{ns}	4
Vermicompost * Urea Fertilizer	153.18^{ns}	4	0.16^{ns}	4	0.02^{ns}	4	0.02^{ns}	4	1.11^{ns}	4	1.14^{ns}	4	47.54^{ns}	4
Vermicompost * Urea Fertilizer * Plant Cultivar	65.65^{ns}	8	0.22^{ns}	8	0.06^{ns}	8	0.02^{ns}	8	7.97^{ns}	8	0.58^{ns}	8	16.84^{ns}	8
Error	108.18	137	0.36	157	0.11	157	0.05	78	10.47	157	1.00	54	22.70	54

*, **: significant at the 5 and 1 % levels of probability, respectively.

plant varieties (Table 1). Urea fertilizer significantly increased plant height, especially in the Goldasht and Mahali-Isfahan cvs. There was no difference between N_{50} and N_{100}, but the difference was significant compared to control (N_0) (Figure 1). Our data indicated that plant height differs in plant cultivars, so the highest mean value of plant height was observed in Mahali-Isfahan cv. While the lowest value belonged to wild species (Figure 1). The Vermicompost application did not significantly affect this parameter (Table 1). Plant height negatively and significantly correlated with plant leaf area, RWC, and fresh biomass (Table 2).

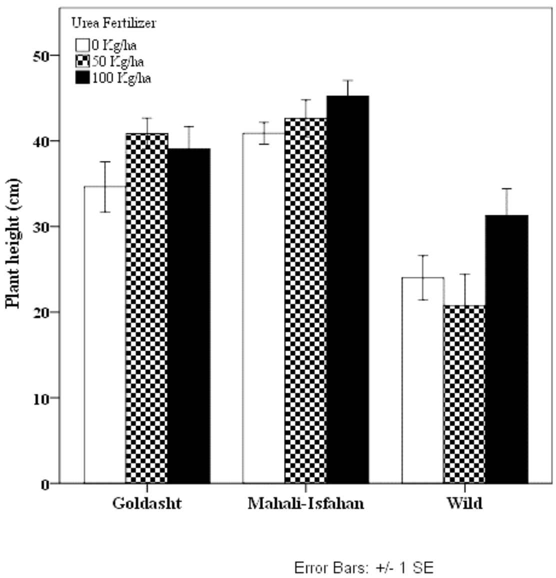

Figure 1) Effect of different rates of urea fertilizer (0, 20 & 40 $Kg.ha^{-1}$) on mean plant height of safflower (Goldasht, Mahali-Isfahan, and Wild species).

Number of Leaves

A comparison of mean leaves numbers showed that this growth parameter is significantly affected by vermicompost treatments (Table 1). The maximum number of leaves was recorded in V_{20} and V_{40} treatments so that it differed from the control (V_0). There was no difference between V_{20} and V_{40} (Figure 2). The number of leaves differed between the plant cultivars (Table 1). Results revealed that the most considerable value of leaves number was in the wild species and Goldasht cv. The lowest was observed in Mahali-Isfahan cv. (Figure 2). There was no significant effect of urea fertilizer on plant leaves number (Table 1). The number of leaves positively and significantly correlated with fresh and dry biomass and leaf area (Table 2).

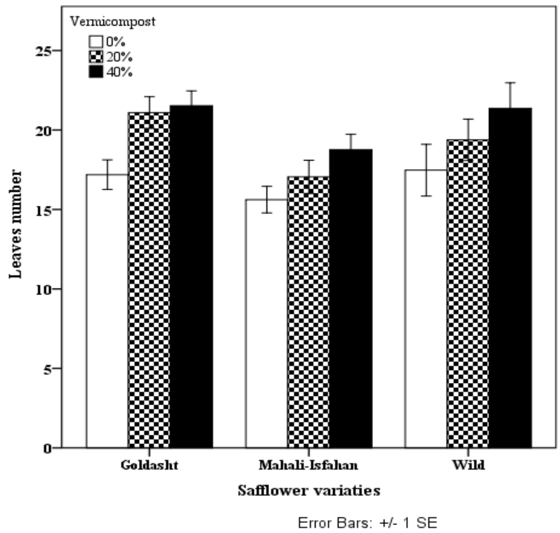

Figure 2) Effect of different rates of vermicompost treatment (0%, 20% & 40%) on mean plant number of the leaf of safflower (Goldasht, Mahali-Isfahan, and Wild species).

Plant Fresh Biomass

Vermicompost treatments affected fresh plant biomass (Table 1), significantly increasing with the vermicompost application rate. The highest and lowest weights were V_{40} and V_0, respectively (Figure 3). There were apparent differences between all plant cultivars (Table 1), so the most plant fresh biomass was measured in the wild species, and the least was recorded in Mahali-Isfahan cv. (Figure 3). Urea fertilizer application did not cause any significant differences in this parameter (Table 1). This parameter had a high positive correlation (r=0.89) with leaf area, dry plant biomass, and RWC (Table 2).

Table 2) Pearson correlation coefficient among studied growth parameters of safflower.

	Plant Height	Number of Leaves	Fresh Biomass	Leaf Area	TPC	RWC	Dry Biomass
Plant Height	1						
Number of Leaves	-0.20^{ns}	1					
Fresh Biomass	**-0.57****	**0.71****	1				
Leaf Area	**-0.51****	**0.71****	**0.89****	1			
TPC	0.09^{ns}	-0.04^{ns}	-0.13^{ns}	-0.16^{ns}	1		
RWC	**-0.60****	0.11^{ns}	**0.42****	**0.41****	-0.22^{ns}	1	
Dry Biomass	0.06^{ns}	**0.77****	**0.58****	**0.65****	-0.13^{ns}	-0.09^{ns}	1

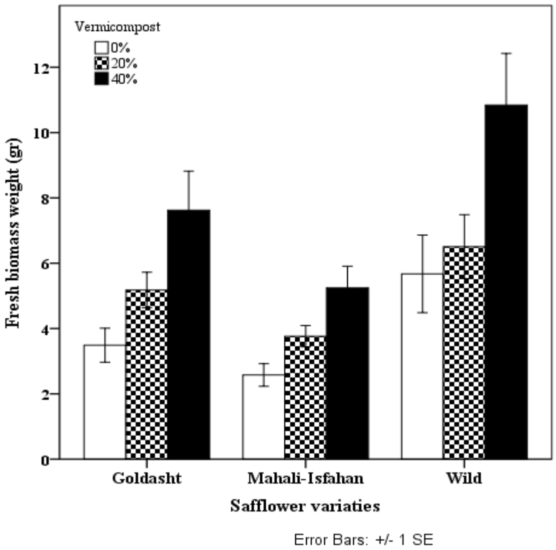

Figure 3) Effect of different rates of vermicompost treatment (0%, 20% & 40%) on mean plant fresh biomass weight of safflower (Goldasht, Mahali-Isfahan, and Wild species).

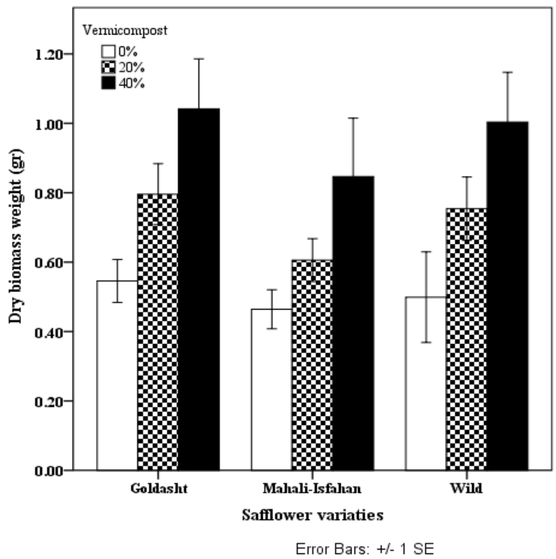

Figure 4) Effect of different rates of vermicompost treatment (0%, 20% & 40%) on mean dry plant biomass weight of safflower (Goldasht, Mahali-Isfahan, and Wild species).

Plant Dry Biomass

This study had a significant effect of vermicompost on plant dry biomass (Table 1), so maximum and minimum plant dry biomass weight was observed in V_{40} and V_0, respectively (Figure 4). Statistical analyses showed no significant effect of urea fertilizer on dry plant biomass. The dry biomass of different plant cultivars was statistically the same (Table 1). Plant dry biomass positively and significantly correlated with the number of leaves, fresh biomass, and leaf area (Table 2).

Plant Leaf Area

Our results indicated that the application of vermicompost significantly increased the plant leaf area (Table 1), so the maximum plant leaf area was achieved at V_{40}, and the minimum was in V_0 (Figure 5). Plant leaf area was significantly different between plant cultivars (Table 1). This parameter's highest and lowest values were recorded in wild species and Mahali-Isfahan cv., respectively (Figure 5). There was no significant effect of urea fertilizer levels on plant leaf area as a growth parameter (Table 1), but the

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interaction between plant cultivars and urea fertilizer was significant (Table 1). Plant leaf area positively and significantly correlated with RWC and dry biomass (Table 2).

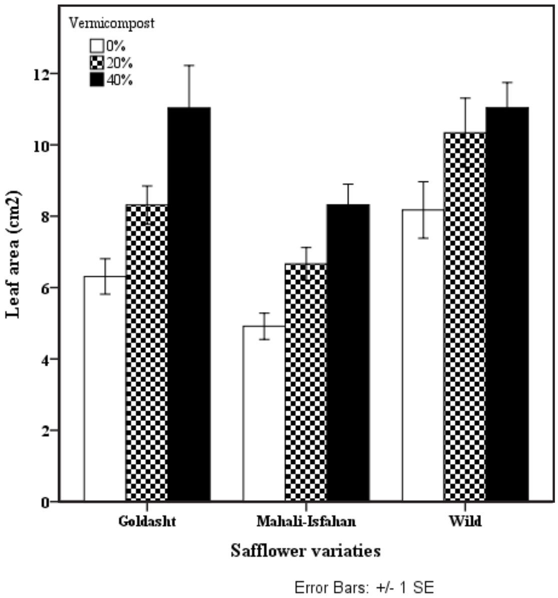

Figure 5) Effect of different rates of vermicompost treatment (0%, 20% & 40%) on mean plant leaf area of safflower (Goldasht, Mahali-Isfahan, and Wild species).

Plant Relative Water Content (RWC)

Applying urea fertilizer and vermicompost did not affect RWC (Table 1). RWC significantly differed between plant cultivars (Table 1), so the maximum amount of RWC was observed in the wild species than in other plant cvs. (Figure 6). RWC had a positive and significant correlation with fresh plant biomass and leaf area and a negative correlation with plant height (Table 2).

Plant Phenol Content

Data analysis showed no significant effect of vermicompost and urea fertilizers solely on plant phenol content. There was also no difference between plant cultivars under these treatments compared to the control. However, the interaction between plant cultivars and vermicompost treatment apparently affected plant phenol content (Table 1).

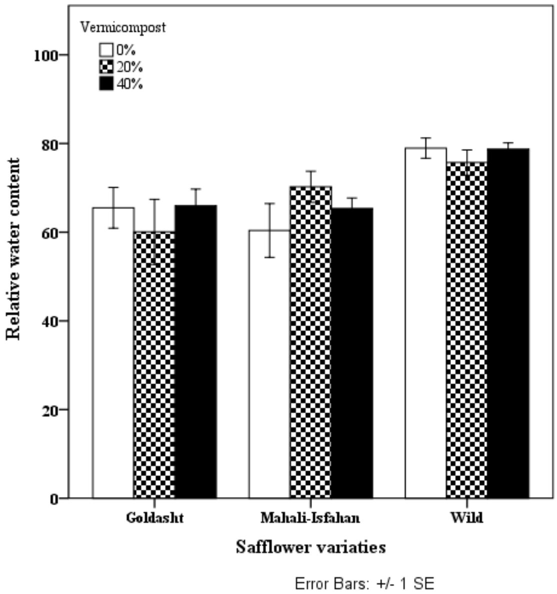

Figure 6) Effect of different rates of vermicompost treatment (0%, 20% & 40%) on mean plant relative water content of safflower (Goldasht, Mahali-Isfahan, and Wild species).

Discussion

In the present study, vermicompost influenced the growth parameters of the safflower plant. The number of leaves, leaf area, and fresh and dry biomass were significantly increased with increasing vermicompost application. These findings agree with a report that showed the growth of a plant in vermicompost can be attributed to humus content which contains humic acid that is excreted by earthworms. Humic acid stimulates plant growth even in small amounts [63]. It has been known that the organic matter in vermicompost can usually provide plants with a balanced source of both macro and micronutrients that can influence the composition and physiology of plants and provide optimum conditions for plant growth [64] through the gradual decomposition of the organic material by microorganisms, and slower release of nutrients and mineralization [65,66]. Plant nutrients become available for plant growth because they are adsorbed by humic acid molecules and released gradually into the soil [32, 67]. The results of the present

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study also agreed with those reports since safflower plant growth progressively continued from the beginning up to harvest. This might be due to the slow release of nutrients from vermicompost, which plants uptake required nutrients continuously for their growth without nutrient deficiency, as reported in other studies [32, 67].

Growth promoting activity of vermicompost in the present study is in line with a study that reported that due to humus content, vermicompost has very high porosity, aeration, drainage, and water-holding capacity than conventional compost, so consequently improving the physical and chemical properties of soil [5]. In addition, vermicompost continually breaks down organic material in the soil by enzymes such as amylase, lipase, cellulase, and chitinase. It releases the nutrients in available forms, such as nitrates, phosphates, and exchangeable calcium and soluble potassium, to the plant roots so plants can readily uptake them [1, 68, 69].

Vermicompost also add beneficial microorganism to the soil, including bacteria, fungi, yeasts actinomycetes which can supply plant growth regulators like auxins, gibberellins, cytokinins, ethylene, and abscisic acid in tangible amounts, which can improve plant growth [70, 71]. In addition, vermicompost has higher nitrates than conventional composts [72, 73], which can increase the growth of the leaf area index of the plant, which results in increased absorption of light leading to more dry matter and yield [74].

In this experiment, urea fertilizer only increased the plant height. This result is in line with some other studies that reported that as the nitrogen doses increased, plant height increased, too [48, 75,77]. They explained that nitrogen fertilizer improves the shoot growth of the plant and causes them to be absorbed some elements, which enhances the growth [48]. In this study, other growth parameters were not influenced by urea fertilizer. One reason could be that the amounts of urea fertilizer were insufficient to influence other safflower growth parameters. However, these amounts were selected based on other studies that showed that these amounts of urea fertilizer affected plant growth.

Interaction between vermicompost and plant cultivars had a significant effect on the phenol content of the plant. This shows that safflower cultivars responded differently to vermicompost treatments regarding plant phenol content. The interaction between urea fertilizer and plant cultivars affected the plant leaf area index. Because urea fertilizer had no significant effect on plant leaf area, we can conclude that the response of plant cultivars to urea fertilizer could be different in the case of leaf area index.

Phenol content was not influenced by any of the main factors in this study. However, the interaction between plant cultivars and vermicompost significantly affected this parameter, which means vermicompost had a synergistic effect on plant phenol content. Phenolic compounds in plants help them to control some pathogens and pests and cause some kinds of resistance against them [37, 78, 79].

According to research evidence, the amounts of phenolic compounds in plants grown under organic conditions are higher than those grown under inorganic conditions [29, 30]. For example, it found that total amounts of phenolic compounds were higher in strawberries (*Fragaria ananasa*, var. Chandler) and corn (Zea mays) grown in organic fertilizer than those grown with inorganic fertilizers [29]. However, more information is required on the application of the appropriate rate of vermicompost with or without organic fertilizers for the highest and most effective phytochemical production for plant defense, increasing the nutritional quality of the plants, and the antioxidant activity of phenol in their products [80].

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Plants with different genotypes, such as cultivars, have different morphological characteristics, nutritional content, and concentration of secondary plant materials. That is why different plant parameters such as plant height, leaf number, fresh biomass weight, leaf area, and leaf relative water content in different cultivars and species exist [81]. Wild safflower species responded more than the other two safflower cultivars to treatments. For example, wild species had the maximum value of leaf area, number of leaves, fresh biomass weight, and relative water content. These features can be used in plant breeding programs. In wild species, the number and area of leaves were more than the other cultivars, resulting in more photosynthesis, more fresh biomass weight production, and more relative water content. However, since plant cultivars did not influence dry biomass weight, it can be concluded that wild species had more water in their leaves, and because of that, it is resistant to drought. According to the climate conditions of Iran, a move towards drought-resistant crop plants such as safflower could be a significant step forward in the country's oil supply, and wild species can be used in the breeding and production of resistant cultivars.

The results confirm a variation in vermicompost effects depending on the plant cultivars. Therefore, specific genotypes or varieties may be more suitable for organic or combined inorganic-organic cropping systems within a given crop than others [71, 82].

Conclusion

In this study, vermicompost treatment had a more influential role in promoting plant growth parameters than urea fertilizer, so vermicompost increased all plant growth parameters of safflower except relative water content but urea fertilizer only increased plant height of safflower. So, applying vermicompost at a 40% rate resulted in the best growth parameters of safflower. So, it can be used instead of urea fertilizer, which is safe for the environment, too. Further studies are needed to evaluate the effect of incorporating vermicompost on the soil mid- and long-term.

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