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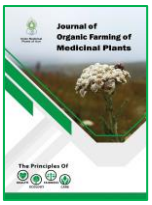

Original Article

Evaluation of organic fertilizers and soil amendments on yield and yield compounds of roselle (*Hibiscus sabdariffa* L.)

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ABSTRACT

The application of organic inputs can be a suitable alternative to chemical fertilizers due to their many properties and reduction of environmental effects. Therefore, in order to evaluate the effects of organic fertilizers, research was conducted as a factorial base on a completely randomized block design in three replications on roselle (*Hibiscus sabdariffa* L.) in 2019 in Nazarabad, Karaj, Iran. The treatments included cow manure levels (0, 5 and 10 ton ha^{-1}), vermicompost levels (0 and 15 ton ha^{-1}) and biochar levels (0 and 10 ton ha^{-1}). The results of this study showed that the application of cow manure, biochar and vermicompost promoted yield and yield compounds of roselle compared to control. In addition, the integrated application of cow manure, biochar and vermicompost effects on traits better than the main effects. The highest dry matter weight (1116.82 g m^{-2}), fruit dry weight (456.82 g m^{-2}), and soil respiration (0.37 µmol m^{-2} s^{-1}), were related to the integrated application of cow manure, biochar and vermicompost. Thus, to promote roselle yield and yield compounds, the usage of organic fertilizers and soil amendments (cow manure, biochar and vermicompost) is a suitable alternative to chemical fertilizers.

1. Introduction

Organic amendments improve soil and plant properties. Fertilization is an essential component of plant system management, chiefly under sustainable farming. Increasing soil organic matter causes soil fertility. Application of biochar as a soil amendment has several advantages for plants, because biochar can prevent nutrient leaching, especially nitrogen, also increase soil water and soil fertility, finally promote the quality and quantity of crops (Arshad et al., 2021; Allam et al., 2020; Fang et al., 2021). Usage of biochar increased the activity of microorganism of the soil and also improved soil respiration, physical and chemical properties of the soil (Zheng et al., 2018; Agegnehu et al., 2015). Many studies have shown that use of biochar improved the quality and quantity of basil (*Ocimum basilicum* L.) (Jabborova et al., 2021), Peppermint (*Mentha piperita* L.) (Sadowska et al., 2020) and roselle (*Hibiscus sabdariffa*) (Liu et al., 2021).

Cow manure increases the organic matter of the soils. Cow manure is full of elements particularly nitrogen. Nitrogen plays an important role in yield of plants and it is the most required nutrient element for plants. Cow manure improve absorption of elements in the soil and finally promote plant growth (Durán-Lara et al., 2020). Cow manure application increased coriander (*Coriandrum sativum* L.) (Kadhim, 2021) and Thyme (*Thymus vulgaris* L.) yield and yield compounds (Sharaf EL-Din et al., 2019).

Vermicompost is an organic fertilizer that include nutrients and minerals for plants. It can increase the quality and quantity yield of plants. Vermicompost can increase structure and biological activity of soils, therefore to improve sustainability of soil resources and production, and decrease environmental issues (Paczka et al., 2021; Voko et al., 2022). Other researchers demonstrated that vermicompost increased yield and yield compounds of Japanese mint (*Mentha arvensis* L.) (Bajeli et al., 2016) and lemon balm (*Melissa officinalis* L.) (Koozehgar kaleji et al., 2020).

Roselle (*Hibiscus sabdariffa*) is a medicinal plant belongs to the Malvaceae family. Its leaves are used as an edible vegetable, its seeds are a rich source of protein, and its aerial sepals are used to prepare various drinks, ice cream, chocolate, and cake (Dhar et al., 2015) and also roselle could be consumed as juice, tea, jam and foods. Its sepals can be used as medicine (Rozan et al., 2017). Sepals contain organic oxalic,

citric and tartaric acids as well as vitamin C, protein, minerals and anthocyanin (Wu et al., 2018). Organic fertilizers are an important source to sustain the soil organic matter, productivity and growth of plants. Results a study confirmed that usage of organic fertilizers had positive effects on the growth, yield and antioxidant content of roselle (Norhayati et al., 2019). The use of chemical fertilizers and their negative effects on the biological cycles of agricultural systems made environmental issues. On the other hand, the issue of providing high quality products (especially medicinal plants) for the ever-increasing population of the world is necessary to increase the crops production. For this purpose and environmental protection, this research was conducted using organic inputs on rosella.

2. Material and Method

2.1 Field experiment

This experiment was performed at the farm of Nazarabad (Karaj, Iran), in 2019. The geographic coordinates of the experimental farm are 35˚49' N and 51˚6' E, with an altitude of 1321 m above sea level. The amberothermic graph for four months of the experiment was indicated in Figure 1.

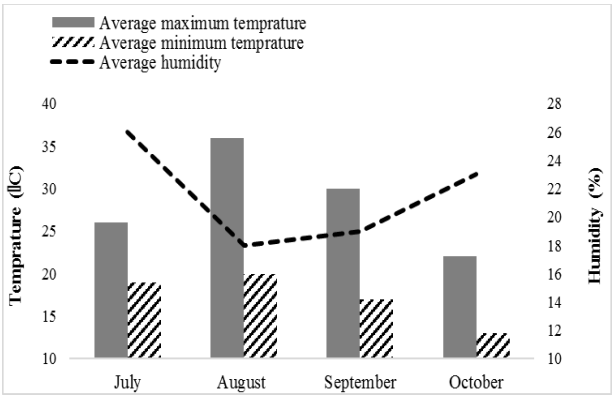

Fig.1. Climatology curve for Nazarabad city from July to October 2019.

The soil was sampled before planting from a depth of 0-30 cm, and the soil characteristics is showed in Table 1. Biochar, vermicompost and cow manure characteristics are illustrated in Table 2, Table 3 and Table 4, respectively.

Table 1. Physicochemical properties of farm soil

Texture	Clay (%)	Silt (%).1.	Sand (%)	phosphorus (ppm)
Sandy Clay	30	29	41	9.12
pH	Organic Matter %	Electrical conductivity	Nitrogen (mg kg^{-1})	potassium (ppm)
7.3	0.69	1.50	8.25	316

Table 2. Results of chemical and physical properties of biochar.

Phosphorus (%)	Potassium (%)	Nitrogen (%)	Fe (%)	Nitrogen (%)
0.16	0.35	0.34	0.45	0.34
Aluminum (%)	Oxygen (%)	Carbon (%)	Silicon (%)	Calcium (%)
0.17	42.26	47.24	1.32	4.25

Table 3. Physicochemical properties of the vermicompost

K (%)	P (%)	N (%)	pH	Moisture (%)
0.50	0.48	1.78	7.8	4.58
C/N	O.C (%)	O.M (%)	EC (ds m^{-1})	
14.45	25.17	41.25	7.8	

Table 4. Physicochemical properties of the cow manure

EC (ds.m^{-1})	K (ppm)	P (ppm)	N (%)	pH
5.84	765	93	1.21	7.62

The experiment was conducted as a factorial base on completely randomized block design with three replications. The treatments included cow manure levels [0 (control), 5 and 10 ton ha^{-1}], vermicompost levels [0 (control) and 15 ton ha^{-1}) and biochar levels [0 (control) and 10 ton ha^{-1}]. Sowing was done by hand on 20 July 2019. The used biochar has been made by pyrolysis method, from forest trees wood. The amounts of vermicompost applied were based on the nitrogen required of plants (15 ton ha^{-1}). One day before sowing (19 July 2019), the biochar, cow manure and vermicompost were distributed on top of each row and was mixed into the soil to a depth of 30 cm. The plants were planted as seedlings and length of each seedling was 20 cm. Each plot included four rows, with a length of 4m and distance of 50 cm between the lines. The outer rows were considered the border, and the two inner rows were used to evaluate various plant traits. The plots were regularly irrigated at intervals of 7 days. Weeds were controlled manually by hand and without using any chemical inputs. No pesticides were used during the growth of the plants. After three months (20 October 2019), the plants were harvested.

2.2 Sample collection and measurement

At the time of harvest, total anthocyanin, total flavonols, soil respiration, chlorophyll index, relative water content (RWC), plant height, number of branches, total dry weight, number of fruits and fruit dry weight were measured.

Total anthocyanin and total flavonols were extracted from 1 g of dried sepals by adding 10 mL of ethanol (95%), HCl 1.5 M. Then the sample was transferred to 50 mL beaker, covered and kept overnight in the refrigerator at a temperature of 4°C. Absorption of solution was measured by spectrophotometer at 535 nm

for total anthocyanin and 374 nm for total flavonols (Lee & Francis 1971).

Total anthocyanin = [A535×V×100]/ [98.2×W] (Eq.1)

Total flavonols = [A374×V×100]/ [76.5×W] (Eq2)

Where, V = total volume extract (ml), W = weight sample (g).

To measure the dry matter yield of the plants (total dry weight and fruit dry weight), they were dried in the oven at 70°C for 3 days. Chlorophyll index measured by SPAD (SPAD-502, Konica Minolta, Japan). The relative water content was calculated through Equation 3 (Ferrat & Loval, 1999).

RWC= [Fw-DW]/[Sw-DW] ×100 (Eq.3)

Where, FW = leaf wet weight, DW = leaf dry weight and SW = saturated leaf weight.

For measuring soil respiration, 20 grams of each soil sample was poured into a container. In another container, 10 ml of NaOH 2 M was poured. Both containers were placed in a larger container and the lid of the large container was closed and the samples were stored in a dark environment at 25°C for 20 days. After that, the samples were taken out of the container and titrated with HCL acid, and soil respiration was calculated using Equation 4 (Isermeyer, 1952).

Soil respiration = 2.2×100×(c-s)/ Sw×100 (Eq. 4)

Where, C = the amount of acid used for the control, S = the amount of acid used for the sample, SW = the initial weight of wet soil, 2.2 = the conversion factor (1 ml of 0.1 M HCl is equivalent to 2.2 mg of CO_2).

2.4 Statistical analysis

Data analysis was completed using SAS software (Ver. 9.4). Excel software was used to plot the graphs. The mean values were compared using Duncan's multiple range test at a 5% probability levels.

3. Results

3.1 Total anthocyanin and total flavonols

According to analysis of variances, the main effects of the cow manure, biochar, vermicompost, as well as, the interactions between them were significant ($p < 0.01$).

The triple interactions of treatments were significant at $p < 0.05$ (Table 5). In mean values, increasing concentration of cow manure improved total anthocyanin and total flavonols. Usage of biochar and vermicompost improved total anthocyanin and total flavonols over to control.

The highest total anthocyanin (211.04 µg g^{-1} DW) and total flavonols (17.25 µg g^{-1} DW), was observed in interaction of cow manure, biochar and vermicompost and the lowest total anthocyanin (148.52 µg g^{-1} DW) and total flavonols (9.11 µg g^{-1} DW) was recorded in control.

Interaction of organic fertilizers promoted total anthocyanin (42.09 %) and total flavonols (89.35 %) over control (Figure 2 and Figure 3).

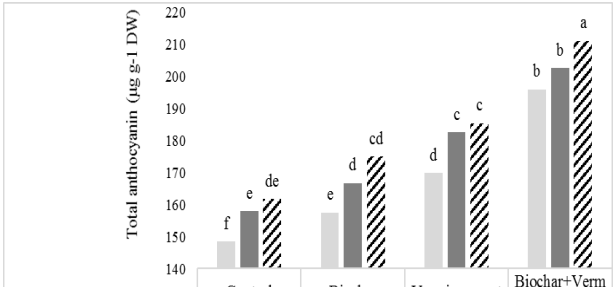

Fig.2. The interaction of cow manure, biochar and vermicompost on total anthocyanin. Means with the same letter are not significantly different.

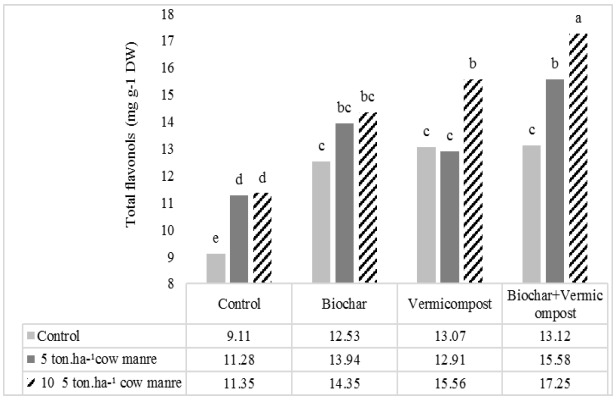

Fig.3. The interaction of cow manure, biochar and vermicompost on total flavonols. Means with the same letter are not significantly different.

Table 5. Analysis of variance (means of squares) for yield and yield compounds of roselle and soil respiration.

Source	DF	Total anthocyanin	Total flavonols	Soil respiration	Chlorophyll index	Relative water content	Plant height	Number of branches	Dry matter weight	Fruit dry weight	Number of fruits
Replication	2	21.68 ns	10.36 ns	0.27 ns	1.57 ns	32.90 ns	6.36 ns	4.21 ns	5.71 ns	6.92 ns	5.74 ns
Cow manure (c)	2	111.36 **	79.92 **	0.92 **	3.02 **	165.24 **	23.14**	18.56 **	19.27 **	19.24 **	21.83**
Biochar (b)	1	102.75 **	45.53 **	1.05 **	3.25 **	353.36 **	32.55**	36.12 **	19.43 **	20.68 **	18.93 **
Vermicompost	1	143.24 **	21.44 **	0.81 **	3.61 **	598.70 **	17.34**	21.85 **	18.52 **	23.68 **	17.46 **
b×c	2	111.63 **	35.79 **	0.99 **	2.98 **	652.84 **	42.59**	28.33 **	17.52 **	19.13 **	21.67 **
b×v	2	95.07 **	89.68 **	0.83 **	3.16 **	470.13 **	14.53**	19.82 **	55.12 **	69.87 **	35.90 **
c×v	1	154.83 **	67.14 **	1.06 **	3.56 **	364.71 **	38.27**	21.94 **	24.16 **	24.13 **	44.72 **
b×c×v	2	48.32 *	70.21 *	0.87 **	0.97 **	980.94 *	94.03*	97.89 *	76.71 **	5.64 *	4.95 **
Error	22	14.79	19.48	0.73	21.12	32.12	12.32	10.36	13.27	12.45	18.22
C.V (%)		8.35	7.26	4.98	11.53	13.24	10.29	9.44	14.98	8.97	12.83

*Significant at $p < 0.05$, **$p < 0.01$. ns, no significance.

3.2 Soil respiration

Analysis variances results showed that, the significance of the main effects of the cow manure, biochar and vermicompost. Also, the interactions of them were significant ($p < 0.01$) (Table 5). In mean values, when cow manure content improved, soil respiration promoted too. Usage of biochar and vermicompost increased soil respiration over to control. The highest soil respiration (0.35 µmol m^{-2} s^{-1}), was recorded in interaction of cow manure, biochar and vermicompost and the lowest of this trait (0.14 µmol m^{-2} s^{-1}), was observed in control. Usage of organic fertilizers increased soil respiration (150 %) over to control (Figure 4).

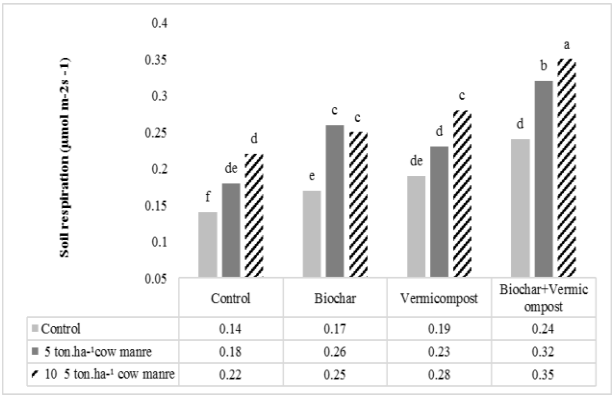

Fig.4. The interaction of cow manure, biochar and vermicompost on soil respiration. Means with the same letter are not significantly different.

3.3 Chlorophyll index

Analysis variances for chlorophyll index demonstrated that the significance of the main effects of the cow manure, biochar and vermicompost. As well as, the interactions of these treatments were significant ($p < 0.01$) (Table 5). In mean values, chlorophyll index increased with increasing cow manure concentration. Application of biochar and vermicompost increased chlorophyll index over to control.

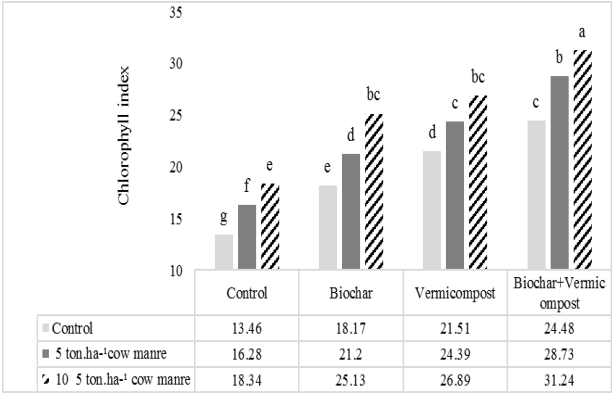

Fig.5. The interaction of cow manure, biochar and vermicompost on chlorophyll index. Means with the same letter are not significantly different.

The highest chlorophyll index (31.24), was observed in interaction of cow manure, biochar and vermicompost and the lowest of this trait (13.46) was achieved control. Chlorophyll index improved (132.09 %) by organic fertilizers usage (Figure 5).

3.4 Relative water content

According to analysis variances, the significance of the main effects of the cow manure, biochar, vermicompost and also, the interactions between them were significant ($p < 0.01$). The triple interactions of the cow manure, biochar, vermicompost were significant at $p < 0.05$ (Table 5). In mean values, increasing concentration of cow manure improved relative water content. Usage of biochar and vermicompost improved relative water content over to control. The highest relative water content (74.57 %), was observed in interaction of cow manure, biochar and vermicompost and the lowest relative water content (48.11 %), was recorded in control. Usage of organic fertilizers increased relative water content (54.87 %) over to control (Figure 6).

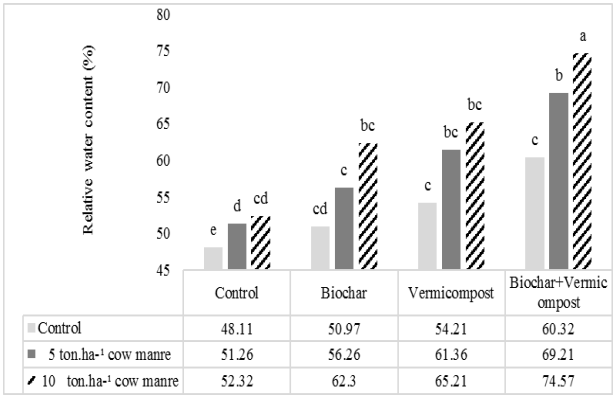

Figure 6. The interaction of cow manure, biochar and vermicompost on relative water content. Means with the same letter are not significantly different.

3.5 Plant height and number of branches

The results of the ANOVA for plant height and number of branches showed that the significance of the main effects of the cow manure, biochar, vermicompost and also, the interactions between these treatments were significant ($p < 0.01$). The triple interactions of the cow manure, biochar, vermicompost were significant at $p < 0.05$ (Table 5).

The mean values indicated that increasing amount of cow manure, promoted plant height and number of branches too. Application of biochar and vermicompost increased plant height and number of branches over to control.

The highest plant height (125.92 cm) and number of branches (13 branches), was observed in interaction of cow manure, biochar and vermicompost and the lowest plant height (87.56 cm) and number of branches (7 branches) was observed in control. Usage of organic fertilizers increased plant height (43.80 %) and number of branches (85.71 %) over to control (Figure 7 and Figure 8).

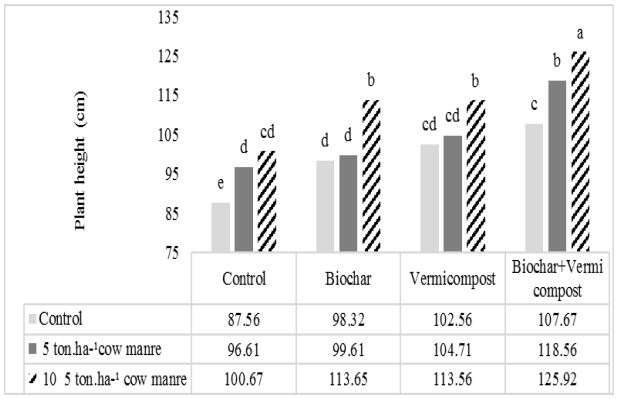

Fig.7. The interaction of cow manure, biochar and vermicompost on plant height. Means with the same letter are not significantly different.

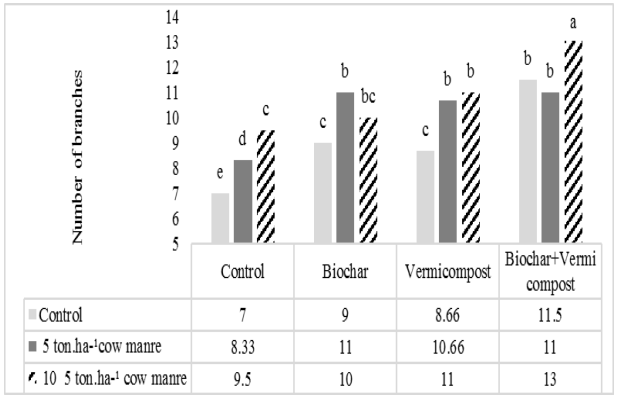

Fig.8. The interaction of cow manure, biochar and vermicompost on number of branches. Means with the same letter are not significantly different.

3.6 Dry matter weight and fruit dry weight

Analysis of variances in this study indicated that the significance of the main effects of the cow manure, biochar, vermicompost. As well as, the interactions between treatments were significant on dry matter weight and fruit dry weight ($p < 0.01$) (Table 5). The mean values showed that dry matter weight and fruit dry weight promoted by increased cow manure concentration. Usage of biochar and vermicompost increased dry matter weight and fruit dry weight compared to control. The highest dry matter weight (1116.82 g m^{-2}) and fruit dry weight (456.82 g m^{-2}) was observed in interaction of cow manure, biochar and vermicompost and the lowest dry matter weight (650.34 g m^{-2}) and fruit dry weight (324.34 g m^{-2}) was achieved in control. Dry matter weight (71.72 %) and fruit dry weight (40.84 %) improved by usage of organic fertilizers compared to control (Figure 9 and Figure 10).

3.7 Number of fruits

According to variances analysis of this study, the significance of the main effects of the cow manure, biochar and vermicompost. Also, the interactions of treatments were significant ($p < 0.01$) (Table 5). The mean values demonstrated that increasing amount of cow manure promoted number of fruits. Usage of biochar and vermicompost increased number of fruits compared to control. The highest number of fruits (40.5 fruits), was recorded in interaction of cow manure, biochar and vermicompost and the lowest one (15 fruits) was observed in control. Application of organic fertilizers promoted number of fruits (170 %) compared to control (Figure 11).

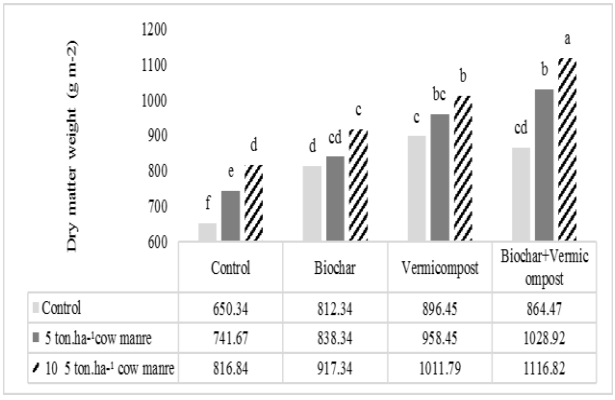

Fig.9. The interaction of cow manure, biochar and vermicompost on dry matter weight. Means with the same letter are not significantly different.

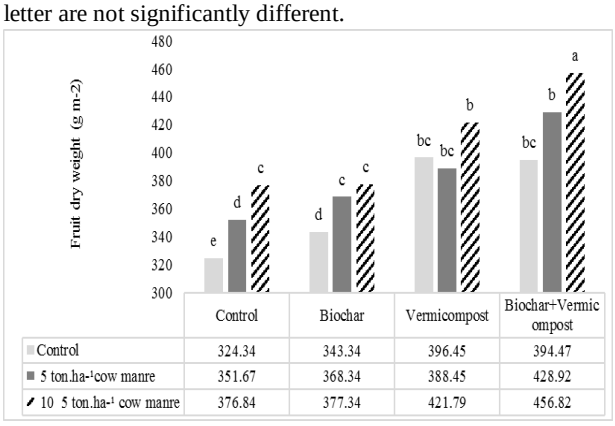

Fig.10. The interaction of cow manure, biochar and vermicompost on fruit dry weight. Means with the same letter are not significantly different.

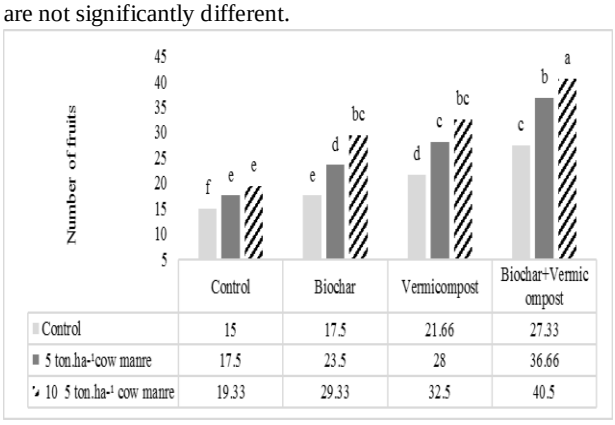

Fig.11. The interaction of cow manure, biochar and vermicompost on number of fruits. Means with the same letter are not significantly different.

4. Discussion

In this study, organic fertilizer usage improved total anthocyanin and total flavonols of roselle. Vermicompost and cow manure are full of elements and increased the availability of nutrients for plants, which

enhanced the higher photosynthetic ctivity and in turn, corresponded to the higher amounts of total anthocyanin and total flavonols. On the other hand, light intensity and temperature are two important environmental factors for red pigments development such as anthocyanin (Naser et al., 2016). Similarly, Norhayati et al. (2019), found that roselle treated with organic fertilizers promoted the total anthocyanin content. In addition, other researchers confirmed that biochar usage as a soil amendment improved roselle total flavonols with increasing the activity of soil microbes that increased the nutrients release and enhanced soil physiochemical characteristics (Liu et al., 2021).

In this study, besides plant yield and yield compounds, and also some quality properties, soil respiration were under study, as this property is crucial to soil fertility and also is critical variables to consider in plant production. Results of this study demonstrated that use of biochar, cow manure and vermicompost improved soil respiration. The microbial activity of the soil largely determines the biogeochemical cycles, the processes of organic matter, fertility and quality of soils. Nevertheless, agricultural management, especially usage of fertilizers, may have important influence on the structure of the soil microbial. Vermicompost and biochar are soil amendments. The results of the present study were consistent with other study and they reported that biochar increased soil respiration and increased soil microbial biomass (Sheng & Zhu, 2018). More respiration in soils treated with biochar may be due to the organic property of biochar, which provides the environment for the spread of more bacteria (Zheng et al., 2018; Cui et al., 2021). Other researchers reported that the use of organic fertilizer due to the improvement of soil structure and retention of water creates a suitable environment for plant growth and provides more available water to the plant and as a result increases the relative water content of leaves. On the other hand, the use of biochar, cow manure and also vermicompost leads to an increase in the plant's water availably, and with the increase in the soil's water holding capacity, the plant is less likely to face dehydration, which increases the relative water contents of the leaves (Zheng et al., 2018; Voko et al., 2022).

The results of present study demonstrated that chlorophyll index increased by usage of organic fertilizers. Nitrogen is the main elements for chlorophyll synthesis and vermicompost and cow manure are full of nitrogen. In addition, biochar prevents nitrogen leaching. Thus, usage of these treatments improved chlorophyll synthesis (Jabborova et al., 2021; Kadhim, 2021). Stomata plays an essential role in water relations and plant photosynthesis, and their reaction to different conditions is one of the main factors affecting the growth, development and production of plants. The chlorophyll index indicates the photosynthetic status of the plant, which can have higher values despite the better nutritional conditions of the plants. Similarly, the increased chlorophyll content to the vermicompost application was recorded in the coriander (*Coriandrum sativum* L.) (Sakthivel et al., 2020), and biochar usage increased roselle chlorophyll by improving the efficiency of photosynthesis besides increased absorption of essential nutrients (Liu et al., 2021).

Increasing chlorophyll in plants increases photosynthesis. Nitrogen is an important element for plant height and plant yield too. Vermicompost and cow manure provide nitrogen and also other nutrients such as phosphorus, potassium for plants. Which are all the important minerals for plant growth. Biochar, cow manure and vermicompost are rich with organic matter which will increase the growth of some advantages of microorganisms. Microorganisms have a good ability to produce different organic acids compounds that helping in nutrients availability and improving plant growth (Durán-Lara et al., 2020; Voko et al., 2022; Cui et al., 2021). Similarly, other researchers recommended that vermicompost promoted coriander height and dry matter yield (Sakthivel et al., 2020). Use of biochar prevent nitrogen leaching and provide better nitrogen for plants, so it helps to increased plant height and plant yield (Zheng et al., 2018). Studies suggested that biochar can promoted peppermint height (Sadowska et al., 2020).

Usage of vermicompost and also cow manure improved the plant biomass by enhancing the soil biological activities and supplying more nutrients uptake for plants (Paczka et al., 2021). Biochar is very effective in improving the soil physiochemical properties; thus, it can help to increased dry matter yield and yield compounds (Fang et al., 2021). Similarly, biochar improved number of fruits of rosella by preventing minerals leaching (Bajeli et al., 2016). Other studies suggested that biochar had positive effects on rosella and increased fruit dry weight of rosella (Liu et al., 2021). Due to the presence of micronutrient elements, vermicompost and cow manure increase the organic matter of the soil and improve the ability to absorb zinc, copper, iron, phosphorus, potassium and nitrogen of the soil.

\The presence of these elements in the soil and its absorption by the roots increases the vegetative growth and the production of leaves in the plant, which causes the level of light absorption and photosynthesis, and also the production of hydrocarbon substances in the leaves; thereby, increased fruit yield, fruit number and number of branches (Liu et al., 2021; Sharaf EL-Din et al., 2019).

5. Conclusion

According to the findings of this study, application of organic inputs (biochar, cow manure, and vermicompost), and also combined usage of them improved quality and quantity of roselle by providing nutrient and minerals for plants. Biochar prevents nitrogen leaching and keeps water and nutrients around the plant roots, which increases the yield and yield compounds of roselle. Organic inputs promoted soil respiration too, that was indicating suitable soil growth conditions for ensuring plant yield and quality. Accordingly, due to environmental protection and social health, it is better to use organic inputs instead of chemical inputs and using organic fertilizers is a good choice for famers.

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