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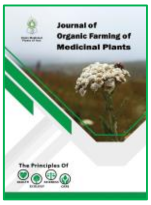

Original Article

Evaluation of intercropping basil and forage turnip on yield, essential oil content, phenols and flavonoids of sweet basil (*Ocimum basilicum* L.)

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ABSTRACT

Intercropping systems and soil amendments usage are the environmental friendly strategies to improve the quality of plants. A study was conducted to investigate the yield and some quality traits of sweet basil (*Ocimum basilicum* L.) in intercropping system with forage turnip (*Brassica rapa* L.) with the use of vermicompost and biochar as soil amendments. The research was conducted during 2018 and 2019 using split-plot randomized complete block design with three replications. The treatments in the main plots were: A_1: 90% forage turnip + 10% basil; A_2: 80% forage turnip + 20% basil; A_3: 70% forage turnip + 30% basil, also two vermicompost levels (15 ton ha^{-1}, 18.5 ton ha^{-1}) and two biochar levels (control, 5 ton ha^{-1}) were placed in subplots. The results showed that intercropping of basil and forage turnip at level of 70% forage turnip + 30% basil with biochar and vermicompost at 18.5 ton ha^{-1} was the best performance compared to other treatments. That treatment raised dry matter yield (62.54%), essential oil (63.15%), essential oil yield (191.76%), and total flavonoids (36.18%), compared to 90% forage turnip + 10% basil and vermicompost at 15 ton ha^{-1}, without biochar. In intercropping treatments, 70% forage turnip + 30% basil increased soluble protein (88.88%), and improved total phenols (103.98%), compared to 90% forage turnip + 10% basil. Application of vermicompost reduced soluble protein (50%), and improved total phenols (27.36%), and biochar usage raising soluble protein (75%) and increased total phenols (60.56%), compared to control. Generally, intercropping in level of 70% forage turnip + 30% basil and the application of vermicompost 18.5 ton ha^{-1} and biochar increased the yield, yield compounds and quality of basil and it can be suggested to farmers as environmental friendly methods to improve basil yield and quality.

1. Introduction

Sustainable Intercropping forage-medicinal plants plays an important role in farming systems that can improve the light absorption and soil conservation compared to monoculture (Huss et al. 2022). In the context of sustainable agricultural systems, intercropping is a more profitable and cost-effective agricultural system always has been the keen attraction center for farmers and researchers worldwide (Lv et al., 2021). In addition, intercropping promotes crop stability and environmental security though agro-ecosystem benefits (soil quality conservation, biodiversity and pest control) and provides alternative products to increase farmer profitability (Gudisa Megersa & Tadese Banjaw, 2024; Maitra et al., 2021).

Medicinal plants have been proposed as the best drugs options for livestock feeds with low side effects and prices. Medicinal plants remain the most important and sometimes the only source of therapeutics and a suitable option for livestock due to reduced production costs, prevent the antibiotics' usage, alleviate disadvantages of resistant bacteria and residual effects in food, and as a result increased public health (Salmerón-Manzano et al. 2020; Oluwafemi et al. 2020). Basil (*Ocimum basilicum* L.) is one of the medicinal plants that is anti-cancer, antimicrobial, and antifungal. Moreover, it has anti-inflammatory, anti-oxidative, and anti-microbial activities due to its abundant secondary metabolites (Choi et al. 2020; Shahrajabian et al. 2020). Some studies reported that intercropping improved yield and yield compounds of basil with tomato (*Solanum lycopersicum*) (Chala Mamo, 2021), also, basil and lemongrass (*Cymbopogon citratus* (DC.) Stapf) (Ashish et al. 2022).

Intercropping of medicinal plants with forage crops is a compatible manner to reduce the chemical drugs disadvantages for livestock (Dogan Das & Denek 2021). Forage turnip (*Brassica rapa* L.) is an acceptable crop for livestock that has the strength to adapt to different climatic conditions and grow in different seasons of the year with high energy and protein (Dogan Das & Denek 2021; Tan & Yolcu 2020).

Biochar as a soil amendment is a carbon-rich material that prevents nutrient leaching and improves the crop yield and quality (Arshad et al. 2021; Ochiai et al. 2021). Other studies supported that using biochar improved yield and quality of basil (Nocentini et al., 2023), Thyme (*Thymus vulgaris* L.) (Mahmoud et al. 2022). Nevertheless, the application of biochar only to soil is not certain to enhance soil quality and crop yields.

For this reason, the co-application of biochar with other organic amendments, such as vermicompost and compost, has acquired attention recently due to its favorable effects on plant growth and production under open field and greenhouse conditions (EL-Mogy et al., 2024). Vermicompost reduces soil nutrients by its water holding capacity and nutrient contents (Voko et al. 2022; Paczka et al. 2021).

Research has shown that vermicompost usage improved the qualitative parameters of sage (*Salvia officinalis*) (Greco et al. 2021), and rosemary (*Rosmarinus officinalis* L.) (Loera-Muro et al. 2021). Some studies on basil have been based on compare monocultures and intercropping, the researchers focused on intercropped of basil and forage turnip to produce a forage crop for livestock. The aim of this study was to evaluate the yield and some quality traits of basil with different levels of forage turnip under soil amendments applications (biochar and vermicompost).

2. Materials and Methods

2.1. *Experimental design and agronomic practices*

This experiment was performed at the research farm of the Faculty of Agriculture and Natural Resources, Islamic Azad University of Karaj (Karaj, Iran), over two-years (2018-2019).

The geographic coordinates of the experimental farm are 35˚49’ N and 51˚6’ E, with an altitude of 1321 m above sea level. Meteorological information of this area is illustrated in Table 1. This farm has for the last 10 years been managed as an agro ecological farming system, without the use of any agrochemical inputs.

The temperature and humidity conditions for the two years of the experiment are shown in Fig. 1.

The soil was sampled before planting from a depth of 0-30 cm, and the soil characteristics are shown in Table 2.

Table 1. Meteorological information of Karaj (Karaj, Iran)

Meteorological information	
climate zone	warm and dry Mediterranean
Summers	warm and dry
Winters	dry moisture regime
Dry days in year	150-180
Average yearly precipitation	243 mm
Average maximum annual temperature	28˚C in July
Average minimum annual temperature	1˚C
Average 30-years soil temperature	14.5˚C

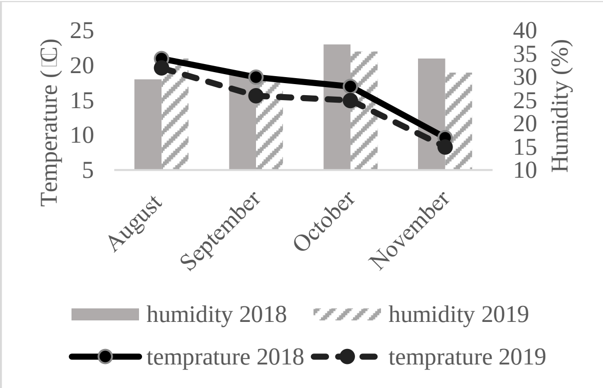


Fig. 1 Climatology curve for Karaj city from August to November (2018-2019).

Table 2. Physicochemical properties of the soil in the field before planting (0–30 cm depth)

Year	O.M (%)	EC (ds.m^{-1})	K (mg.kg^{-1})	P (mg.kg^{-1})	N (mg.kg^{-1})	pH	Soil texture
2019	0.38	1.7	324	8.23	6.64	7.6	S.C
2018	0.42	1.6	332	8.48	5.12	7.8	S.C

O.M: Organic matter, EC: Electrical Conductivity, S.C: Sandy Clay

The experiment was conducted as a split-plot factorial with a completely randomized design with three replications. The experiment was carried out in two years (2018-2019), using the same location in both years and with the exact same treatment combinations. The treatments applied in the main plots were: intercropping at three levels (A_1: 90% forage turnip + 10% basil; A_2: 80% forage turnip + 20% basil; and A_3: 70% forage turnip + 30% basil). Treatments with vermicompost (V_1: 15 ton ha^{-1}; V_2: 18.5 ton ha^{-1}) and biochar (B_1: control, B_2: 5 ton ha^{-1}) were placed in subplots. The sweet basil cultivar was Mobarake and the forage turnip cultivar was PacFB05. The forage turnip density was 120,000 plants ha^{-1} in the monocultures and in the intercropping treatments the forage turnip density was reduced by sowing 10, 20 or 30 % of basil, with the percentages relating to the number of seeds sown. Basil density was 30 plants in m^{-2}. In the intercropping treatments, the forage turnip density was reduced by sowing 10, 20 or 30% of basil, with the percentages relating to the number of seeds sown. The basil was sown between turnip rows.

In both years, sowing was done by hand on July 24, at the same time for both species. Each plot included four rows, with a length of 2m and a distance of 30cm 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 without using any chemical inputs. No pesticides were used during the growth of the plants.

The applied biochar has been made by the pyrolysis method, from forest trees wood. The results of the chemical analysis of biochar are shown in Table 3. The amounts of vermicompost applied were based on the nitrogen required of the plant by the crop (V_1: 15 ton ha^{-1}), and at a rate 25% higher than this (V_2: 18.5 ton ha^{-1}). Before application, the physical/chemical characteristics of the vermicompost were measured, and the results are illustrated in Table 4. One day before sowing (July 23 in both years), the biochar and vermicompost were distributed on top of each row and mixed into the soil to a depth of 30 cm.

Table 3. Chemical analysis of biochar

Year	N (%)	P (%)	K (%)	Iron (%)	Calcium (%)
2019	0.31	0.18	0.38	0.42	4.31
2018	0.34	0.16	0.35	0.45	4.25
Year	Oxygen (%)	Carbon (%)	Silicon (%)	Aluminum (%)	
2019	43.71	48.83	1.43	0.18	
2018	42.26	47.24	1.32	0.17	

Table 4. Physicochemical properties of the vermicompost

Year	C/N	O.C (%)	O.M (%)	EC ($ds.m^{-1}$)	K (%)
2019	13.22	22.89	40.18	7.5	0.55
2018	14.45	25.17	41.25	7.8	0.50
Year	P (%)	N (%)	pH	Humidity	
2019	0.43	1.64	7.06	4.06	
2018	0.48	1.78	7.8	4.58	

O.C: Organic Carbon, O.M: Organic matter, EC: Electrical Conductivity

2.2. *Yield and yield components of basil*

After 90 days, the basil and forage turnip plants were harvested. However, this study focused on measuring the basil, and all traits reported here related to the basil only. At the time of harvest, fresh and total dry matter yields (DMY) (leaf + stem) were measured. Plant samples were harvested from a $1m^2$ area of each plot. Fresh weight were measured with digital scale. Dry matter yields samples were placed at room temperature (at 25°C) for 14 days. The number of secondary stems in plants, leaf number in plants, and the number of flowers in plants were counted manually. Leaf area was also measured using a LAI meter (LI-COR, Model LI-3100 AREA METER).

2.3. *Essential oil amount*

The essential oil content was measured by extracting it from the dry plant (leaves and basil flowers) using the distillation method with water by the Clevenger device (for 3 hours), and finally, the essential oil content was calculated in milliliters per 100 g of dry matter. Essential oil content was obtained from the product of the dry matter yield in the essential oil content.

2.4. *Soluble protein*

To measure the amount of total protein in the plant, 5 ml extract was added to the test tubes, followed by 100 microliters of protein extract, and the solution was stirred rapidly. After 5 minutes, its absorbance was read at a wavelength of 595 nm with a spectrophotometer (Bio Quest C250 model) (Bradford, 1976). The protein was calculated using an albumin standard curve.

2.5. *Total flavonoids and phenols*

The aluminum chloride colorimetric method was used to determine the amount of flavonoids. The solutions were left at room temperature for 30 minutes. The absorbance of each reaction compound was measured at 415 nm with a spectrophotometer. A standard curve was prepared with methanolic quercetin solutions and obtained according to a linear relationship in mg dry weight (Chang et al., 2002). The amount of total phenolic compounds was determined using the Folin-Ciocaltio reagent. To measure the amount of total phenolics, about 1 g of powdered dry leaves of the plant was ground in 14 ml of methanol for 2 minutes and the resulting solution was centrifuged after passing through filter paper. Their absorbance was read at 765 nm with a spectrophotometer (Verza et al., 2007).

2.6. *Statistics*

The data from the experiment was analyzed based on split-plot on randomized complete block design by using SAS statistical software package (ver. 9.4). The comparison of treatment means was made using Duncan test at a 5% probability level.

3. Results

3.1. Fresh weight, dry matter yield (DMY), and secondary stem in plant

The analysis of variances showed that the fresh weight, and DMY were influenced significantly by the interactions between intercropping ×biochar, intercropping ×vermicompost, biochar× vermicompost, as well as the triple interactions between intercropping × biochar × vermicompost were significant ($p < 0.01$). The lateral stem in plant was influenced significantly by the main effects of intercropping, biochar and vermicompost ($p < 0.01$) (Table 6). In both years, with increased proportions of basil, the fresh weight, DMY, and secondary stem in plant of the basil increased and the use of biochar and vermicompost improved the fresh weight, DMY, and secondary stem in plant in both of the experimental years (Table 7 and 9). The lowest fresh weight (4517.56 kg ha^{-1}), was observed in the interaction of 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} and the highest fresh weight (2778.92 kg ha^{-1}) was seen in the interaction of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} (Table 7). The lowest DMY (1208.23 kg ha^{-1}), was observed in the interaction of 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} and the highest DMY 1963.91 kg ha^{-1} was seen in the interaction of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} (Fig. 2). In intercropping treatments, 70% forage turnip + 30% basil (41.68%) improved lateral stem in plant compared to 90% forage turnip + 10% basil. Application of vermicompost 18.5 ton ha^{-1} increased lateral stem in plant (18.57%), and biochar usage raising lateral stem in plant (20.95%) compared to control (Table 9).

3.2. *Leaf number in plants, leaf area and flower number in plant*

The analysis of variances indicated that the leaf number in plants and the flower number in plant were influenced significantly by the interactions between intercropping ×biochar, intercropping ×vermicompost, biochar× vermicompost, as well as the triple interactions between intercropping × biochar × vermicompost were significant ($p < 0.01$) (Table 6 and 8). The leaf area was influenced significantly by the main effects of intercropping, biochar and vermicompost ($p < 0.01$) (Table 6). Treatment of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} increased leaf number in plants (21.90%), and improved flower number in plant (96.49%) compared to 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} (Table 7). In intercropping treatments, 70% forage turnip + 30% basil (5.8%) improved leaf number compared to 90% forage turnip + 10% basil. Application of vermicompost 18.5 ton ha^{-1} increased leaf number (13.50%), and biochar usage raising leaf number (13.77%) compared to control (Table 9).

3.3. *Essential oil content*

The results of the ANOVA for essential oil was significantly influenced by the interactions between intercropping × biochar, intercropping × vermicompost, biochar × vermicompost, as well as the triple interactions between intercropping × biochar × vermicompost were significant ($p < 0.01$) (Table 8). The mean values indicated that with increased proportions of basil, the essential oil of the basil improved, and the use of biochar and vermicompost at 18.5 ton ha^{-1} increased essential oil in both of the experimental years. The lowest essential oil (1.14 %) was observed in the interaction of 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} and the highest essential oil (1.86 %) was related to the interaction of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} (Table 7). The lowest essential oil yield (12.51 kg ha^{-1}), was observed in the interaction of 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} and the highest essential oil yield (36.52 kg ha^{-1}) was seen in the interaction of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} (Fig. 3).

3.4. *Soluble protein, total flavonoids and total phenols*

The analysis of variances indicated that the soluble protein, and total flavonoids were influenced significantly by the main effects of intercropping, biochar and vermicompost. Also, the interactions between intercropping ×biochar, intercropping × vermicompost, biochar× vermicompost, as well as the triple interactions between intercropping × biochar × vermicompost were significant ($p< 0.01$) for total flavonoids (Table 8). The total phenols were influenced significantly by the main effects of intercropping, biochar and vermicompost ($p< 0.01$) (Table 8). Treatment of 70% forage turnip + 30% basil × biochar × vermicompost 18.5 ton ha^{-1} improved total flavonoids (36.18%), compared to 90% forage turnip + 10% basil × vermicompost at 15 ton ha^{-1} (Table 7). In intercropping treatments, 70% forage turnip + 30% basil increased soluble protein (91.20%), and improved total phenols (103.98%), compared to 90% forage turnip + 10% basil. Application of vermicompost 18.5 ton ha^{-1} promoted soluble protein (37.12%), and improved total phenols (27.36%), and biochar usage raised soluble protein (62.65%) and increased total phenols (60.56%), compared to control (Table 9).

4. Discussion

In this study, Fresh yield increased with biochar and vermicompost usage. Biochar improved fresh and dry weight by increasing the plant's access to nitrogen and other nutrient. On the other hand, vermicompost can increase plant weight due to the availability and increase of soil nitrogen. It seems that vermicompost fertilizer increased the dry weight of the plant's aerial parts by increasing plant growth by providing the required nutrients. Also, combination of biochar and vermicompost increased the plant weight by improving the qualitative characteristics of the soil, which can be said to be due to the availability of nitrogen in vermicompost and increasing plant access to this element through biochar (Shushupti et al. 2022; Mumivand et al. 2023; Ouertatani 2021; Greco et al. 2021). Other researchers confirmed that intercropping with maize

Table 5. Bartlett test results

	Fresh weight	DMY	Lateral stem in plant	Leaf number in plants	Leaf area	Flower number in plant	Essential oil	Essential oil yield	Soluble protein	Total flavonoids	Total phenols
P	0.486	0.651	0.528	0.268	0.675	0.345	0.252	0.439	0.534	0.741	0.562
chi-square	0.237	0.389	0.843	0.723	0.351	0.481	0.421	0.145	0.373	0.562	0.449

DMY: dry matter weight

Table 6. ANOVA for basil (*Ocimum basilicum* L.) fresh weight, dry matter weight (DMY), lateral stem in plant, number in plants and leaf area

Source	DF	Fresh weight	DMY	Lateral stem in plant	Leaf number in plants	Leaf area
Year	1	3.49 ^{ns}	1.27^{ns}	0.97 ^{ns}	11.31 ^{ns}	5.62 ^{ns}
Replication (year)	4	8.61	4.26	3.81	21.36	4.38
Intercropping (a) leaf	2	172.83**	97.24**	94.52**	94.53**	175.29**
Year × a	2	12.55 ^{ns}	1.70^{ns}	8.31^{ns}	2.84^{ns}	3.64^{ns}
Replication (year × intercropping)	8	10.46	4.41	10.25	5.68	8.41
Biochar (b)	1	236.81**	103.94**	91.73**	88.43**	159.46**
Year × b	1	6.57^{ns}	1.80 ^{ns}	6.82^{ns}	2.97^{ns}	12.28^{ns}
Vermicompost (c)	1	189.53**	68.79 **	64.79**	79.58**	149.81**
Year × c	1	2.65^{ns}	3.21 ^{ns}	3.46 ^{ns}	5.35 ^{ns}	3.33 ^{ns}
a × b	2	311.82**	51.82 **	8.57^{ns}	112.56**	4.53^{ns}
Year × a × b	2	3.56^{ns}	2.36 ^{ns}	3.48 ^{ns}	9.85 ^{ns}	5.39 ^{ns}
a × c	2	459.26**	74.21 **	11.26^{ns}	142.79 **	1.24^{ns}
Year × a × c	2	10.89^{ns}	4.15 ^{ns}	10.32 ^{ns}	8.56 ^{ns}	6.71 ^{ns}
b × c	1	456.73**	92.09 **	5.07^{ns}	129.73**	10.59^{ns}
Year × b × c	1	8.94^{ns}	2.28 ^{ns}	1.34 ^{ns}	5.68 ^{ns}	4.35 ^{ns}
a × b × c	2	561.43**	101.73 **	67.85 ^{ns}	143.86**	8.56 ^{ns}
Year × a × b × c	2	10.35^{ns}	8.11^{ns}	1.13^{ns}	11.72 ^{ns}	10.11^{ns}
Error	36	13.68	10.11	13.86	18.21	19.34
C.V (%)		14.32	12.83	8.21	10.46	13.27

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

Table 7. Interaction of intercropping, vermicompost and biochar on fresh weight, leaf and flower number in plants, essential oil, total flavonoids and flower number in plant

Treatments			Fresh weight (kg.ha^{-1})	Leaf number in plants	Flower number in plant	Essential oil (%)	Total flavonoids (mg g^{-1})
Intercropping	Vermicompost (ton.ha^{-1})	Biochar (ton.ha^{-1})					
70% Forage turnip + 30% Basil	18.5	5	4517.56 ^{a}	67.33^{a}	38.67^{a}	1.86^{a}	98.41^{a}
		0	4139.28 ^{b}	63.67 ^{b}	34.26^{b}	1.68 ^{b}	94.75 ^{b}
	15	5	3599.45 ^{c}	60.43 ^{c}	31.37^{c}	1.52 ^{c}	94.56^{b}
		0	3364.95 ^{d}	60.67^{c}	31.28^{c}	1.38 ^{d}	82.93^{d}
80% Forage turnip + 20% Basil	18.5	5	4093.46 ^{b}	63.28 ^{b}	34.53^{b}	1.72 ^{b}	94.42^{b}
		0	3688.55 ^{c}	60.41 ^{c}	31.43^{c}	1.55 ^{c}	88.96 ^{c}
	15	5	3372.38 ^{d}	57.55 ^{d}	27.67^{d}	1.38 ^{d}	83.24^{d}
		0	3128.47 ^{e}	57.35^{d}	23.45 ^{e}	1.28 ^{e}	77.56 ^{e}
90% Forage turnip + 10% Basil	18.5	5	3624.51 ^{c}	60.52 ^{c}	31.76 ^{c}	1.56 ^{c}	89.18^{c}
		0	3481.52 ^{cd}	57.71^{d}	27.12^{d}	1.44 ^{cd}	89.32^{c}
	15	5	3412.28 ^{cd}	57.67^{d}	27.42^{d}	1.42 ^{cd}	83.07 ^{d}
		0	2778.92^{f}	55.23 ^{f}	19.68^{f}	1.14 ^{f}	72.26^{f}

Dissimilar letters indicate significant differences according to Duncan's test at the 5% level.

Table 8. ANOVA for basil (*Ocimum basilicum* L.) fresh weight, dry matter weight (DMY), secondary stem in plant, leaf number in plants and leaf area

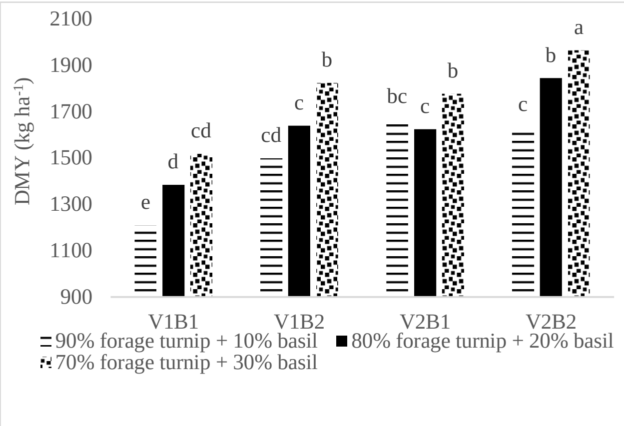
Source	DF	Flower number in plant	Essential oil	Essential oil yield	Soluble protein	Total flavonoids	Total phenols
Year	1	1.68 ns	0.43 ns	1.13 ns	1.74 ns	4.32 ns	3.37 ns
Replication (year)	4	12.35	3.42	5.49	8.26	12.51	6.59
Intercropping (a)	2	159.43**	21.15 **	30.66**	74.51**	174.83 **	65.21**
Year × a	2	3.75 ns	1.86 ns	1.48ns	3.27ns	3.56 ns	2.45ns
Replication (year × intercropping)	8	8.62	5.23	6.79	15.83	12.41	12.75
Biochar (b)	1	128.59 **	56.97 **	40.22**	94.16**	389.48 **	49.58**
Year × b	1	2.38 ns	1.30 ns	3.16 ns	5.42ns	23.54 ns	2.31ns
Vermicompost (c)	1	106.84 **	76.21 **	39.53 **	81.59**	536.86**	72.53**
Year × c	1	4.21 ns	1.91 ns	4.32 ns	12.34 ns	23.52 ns	4.65 ns
a × b	2	178.26 **	39.85 **	48.79 **	10.28ns	621.90*	5.59ns
Year × a × b	2	10.35 ns	1.73 ns	2.36 ns	13.51 ns	17.45 ns	6.75 ns
a × c	2	256.07 **	30.99 **	55.89 **	9.18ns	798.55 **	7.21ns
Year × a × c	2	8.35 ns	2.69 ns	3.35 ns	13.56 ns	14.73 ns	11.08 ns
b × c	1	141.79 **	29.31 **	63.72 **	15.03ns	854.43**	12.34ns
Year × b × c	1	4.38 ns	1.25 ns	1.73 ns	8.76ns	14.79ns	9.46 ns
a × b × c	2	249.28 **	11.76 **	21.06 **	11.19 ns	896.01 **	10.53 ns
Year × a × b × c	2	11.49ns	3.24 ns	4.73 ns	27.91ns	59.15 ns	11.08 ns
Error	36	19.73	5.42	8.21	35.83	49.81	19.42
C.V (%)		11.56	7.22	8.76	10.43	9.65	12.04

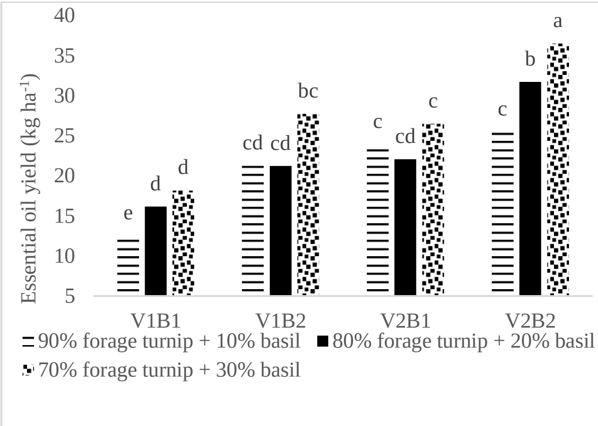
**Significant at $p < 0.01$. ns, no significance (at $p < 0.05$)

Table 9. Effect of intercropping, vermicompost and biochar on lateral stem in plant, leaf area, soluble protein, and total phenols

Treatments	Lateral stem in plant	Leaf area	Soluble protein (mg g^{-1} FW)	Total phenols (mg g^{-1})
Intercropping				
70% Forage turnip + 30% Basil	17.47[a]	36.12[a]	1.74[a]	2.61[a]
80% Forage turnip + 20% Basil	14.35[b]	32.74[b]	1.23[b]	1.93[b]
90% Forage turnip + 10% Basil	12.33[c]	30.32[c]	0.91[c]	1.28[c]
Vermicompost (ton ha^{-1})				
18.5	16.28[a]	35.73[a]	1.81[a]	2.56[a]
15	13.73[b]	31.48[b]	1.32[b]	2.01[b]
Biochar				
5 ton ha^{-1}	15.41[a]	34.45[a]	1.35[a]	2.28[a]
Control	12.74[b]	30.28[b]	0.83[b]	1.42[b]

Dissimilar letters indicate significant differences according to Duncan's test at the 5% level.





V1: Vermicompost 15 ton ha^{-1}, V2: Vermicompost 18.5 ton ha^{-1}, B1: No biochar, B2: Biochar 5 ton ha^{-1}

Fig. 2 The interaction of intercropping, vermicompost and biochar on Dry matter yield (DMY). Means with the same letter are not significantly different.

Fig. 3 The interaction of intercropping, vermicompost and biochar on essential oil yield. Means with the same letter are not significantly different.

promoted biomass production of basil (Lamlom & Hafez, 2018).

The essential oil yield increased by improving the use of vermicompost and also application of biochar. Because vermicompost and biochar prepare nitrogen for basil and increased leaf area (Mumivand et al. 2023). Also, light is one of the most important factors for photosynthesis, which has a great effect on increasing the percentage of yield compounds and essential oil. In this study the height of intercropping plants (basil and forage turnip) were the same and forage turnip was not exposed to shading stress and also increased absorption of photosynthetically active radiation. Thus, basil can use light as well as forage turnip and improved essential oil (Husain et al. 2020). Vermicompost contains growth hormones and nitrate, phosphorus, potassium, calcium and magnesium, which are essential minerals for plants. Vermicompost makes these nutrients easily available to plants and provides suitable conditions for plant growth. On the other hand, biochar prevents nutrient leaching and increases the availability of nutrients for plants. Therefore, it improves dry matter yield and yield compounds (Voko et al. 2022; Greco et al. 2021). Our outcomes are also similar to the work of Mumivand et al. (2023), they reported that application of biochar improved peppermint (*Mentha × piperita* L.) yield and yield compounds. Other researchers suggested that the intercropping pattern of 3basil:2common beans after use of vermicompost can improve the Essential oil productivity and quality of basil (Rezaei-Chiyaneh et al., 2021).

The use of vermicompost and biochar increased soluble protein in basil plants due to the increase in nitrogen available to the plant, because the amount of nitrogen in the plant has a direct relationship with the amount of protein in the plant (Greco et al., 2021). Vermicompost can improve plant growth and essential oil of plants due to its porous structure, high water retention capacity, hormone-like substances and plant growth regulators, and high amounts of macro and micro elements. Phenolic and flavonoid compounds are the main ingredients of medicinal plants. These compounds are considered as effective anti -oxidizing sources. Adding compost and vermicompost to the soil improves the physical conditions and vital processes of the soil while creating a suitable substrate for root growth, increasing growth, increasing dry matter production, flower yield, accelerating metabolic reactions, increasing the production and accumulation of metabolites, and ultimately improving the percentage and yield of essential oils. Previous research results indicate that essential oils contain terpenoid compounds and their constituent units require essential elements such as nitrogen and phosphorus. Therefore, vermicompost consumption increase the percentage and yield of essential oils by affecting the absorption of nitrogen and phosphorus (Nocentini et al., 2022; Libutti et al., 2020; Chookalaii et al., 2020). Therefore, intercropping is a modern farming system that ensures multiple benefits like increasing plant yield, environmental security, production sustainability. Intercropping systems have enough potential to enable sustainability in agriculture by crop diversification, efficient resource management and soil fertility restoration. But intercropping has certain limitations. Further study should be focused on finding solutions for these limitations (Maitra et al., 2021; Huss et al. 2022).

5. Conclusion

The results of this study indicated that application of biochar and vermicompost promoted basil yield, yield compounds and quality, because of their potential like water and food holding capacity and prevent nutrient leaching. In intercropping, with increased proportions of basil, yield, yield compounds and quality of basil improved, especially in 70% forage turnip + 30% basil. It can be said that it happens due to the increase in the percentage of basil and the competition of plants for better use of growth resources such as light, water and food. Finally, intercropping in level of 70% forage turnip + 30% basil and the application of vermicompost 18.5 ton ha^{-1} and biochar promoted the yield, yield compounds and quality of basil. Generally, due to environmental friendly farming, more usage of time and place, use more production resources efficiently, it is better to use intercropping with biochar and vermicompost.

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References

Arshad, U., Azeem, F., Mustafa, G., Bakhsh, A., Toktay, H., McGiffen, M., Amjad, Nawaz, M., Naveed, M., Amjad, & Ali, M. (2021). Combined application of biochar and biocontrol agents enhances plant growth and activates resistance against meloidogyne incognita in tomato. *Gesunde Pflanzen*.73, 591–601. https://doi.org/10.1007/s10343-021-00580-4.

Ashish, K., Jnanesha, A.C., Lal, R.K., & Dubey, B.K. (2022). Intercropping and mixed herb distillation for high-quality oil yield using lemon-scented basil (*Ocimum africanum Lour*.) cv. CIM-Jyoti and lemongrass (*Cymbopogon flexuous (Nees ex Steud*.)

cv. Krishna. *Acta Ecologica Sinica*. 49(4), 269-273. https://doi.org/10.1016/j.chnaes.2021.10.007.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*. 72(2), 248-254.

Chala Mamo, M. (2021). Response of Basil (*Ocimum basilicum* L.) Growth and Yield to Planting Densities and Row Arrangements in Tomato-Basil Intercropping System. *Agricultural Research*. 5(2), 117-124. DOI: https://doi.org/10.20961/agrotechresj.v5i2.54333

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal Food and Drug Analysis*. 10(3), 178-82.

Choi, J., Heo, Y., Bae, S., Kim, S., & Moon, J. K. D. (2020). Discriminating the Origin of Basil Seeds (*Ocimum Basilicum* L.) Using Hyperspectral Imaging Analysis. *LWT - Food Science and Technology*. 118, 108715. DOI: 10.1016/j.lwt.2019.108715.

Chookalaii, H., Riahi, H., Shariatmadari, Z., Mazarei, Z., & Seyed, Hashtroudi, M. (2020). Enhancement of total flavonoid and phenolic contents in *Plantago major* L. with plant growth promoting Cyanobacteria. *Journal of Agricultural Science and Technology*. 22(2), 505-518. http://dorl.net/dor/20.1001.1.16807073.2020.22.2.8.6.

Dogan Das, B., & Denek, B. (2021). Effect of Wheat Straw and Molasses Supplementation on Quality, Lamb Performance, and Digestibility of Forage Turnip (*Brassica rapa*) Silage. *Iranian Journal of Applied Animal Science*. 11(3), 547-555. Doi: 20.1001.1.2251628.2021.11.3.13.9

EL-Mogy, M.M., Adly, M.A., Shahein, M.M., Hassan, H.A., Mahmoud, S.O., & Abdeldaym, E.A. (2024). Integration of Biochar with Vermicompost and Compost Improves Agro-Physiological Properties and Nutritional Quality of Greenhouse Sweet Pepper. *Agronomy*. 14(11), 2603. https://doi.org/10.3390/agronomy14112603

Greco, C., Comparetti, A., Fascella, G., Febo, P., La Placa, G., Saiano, F., Mammano, M.M., Orlando, S., & Laudicina, V.A. (2021). Effects of Vermicompost, Compost and Digestate as Commercial Alternative Peat-Based Substrates on Qualitative Parameters of *Salvia officinalis*. *Agronomy*. 11, 98. https://doi.org/10.3390/agronomy11010098

Gudisa Megersa, H., & Tadese Banjaw, D. (2024). Intercropping System: Enhancing Productivity and Sustainability in Hot Pepper (*Capsicum annum* L.) and Basil (*Ocimum basilicum* L.) Cultivation: A Review. *Global Academic Journal of Agriculture and Bio sciences*. 6, 40-47.

Huss, C.P., Holmes, K.D., Blubaugh, C.K. (2022). Benefits and risks of intercropping for crop resilience and pest management. *Journal of Economic Entomology*. 115, 1350–1362.

Hussain, S., Liu, T., Iqbal, N., Brestic, M., Pang, T., Mumtaz, M., Shafiq, I., Li, S.H., Wang, L., Gao, Y., Khan, A., Ahmad, I., Allakhverdiev, S.I., Liu, W., & Yang, W. (2020). Effects of lignin, cellulose, hemicellulose, sucrose and monosaccharide carbohydrates on soybean physical stem strength and yield in intercropping. *Photochemical and Photobiological Sciences.* 19, 462–472. https://doi.org/10.1039/c9pp00369j.

Lalay, G., Ullah, S., & Ahmed, I. (2021). Physiological and biochemical responses of *Brassica napus* L. to drought-induced stress by the application of biochar and Plant Growth Promoting Rhizobacteria. *Microscopy Research and Technique*. 85(4), 1267-1281. https://doi.org/10.1002/jemt.23993.

Lamlom, M.M., & Hafez, Y.A. (2018). Evaluation of some maize cultivars under different intercropping patterns with basil. *Journal of Productivity and Development*. 23(2), 235- 259.

Libutti, A., Trotta, V., & Rita Rivelli, A. (2020). Biochar, Vermicompost, and Compost as Soil Organic Amendments: Influence on Growth Parameters, Nitrate and Chlorophyll Content of Swiss Chard (*Beta vulgaris* L. var. cycla). *Agronomy*. 346(10), 1-18. https://doi.org/10.3390/agronomy10030346

Loera-Muro, A., Troyo-Diéguez, E., Murillo-Amador, B., Barraza, A., Caamal-Chan, G., Lucero-Vega, G., & Nieto-Garibay, A. (2021). Effects of Vermicompost Leachate versus Inorganic Fertilizer on Morphology and Microbial Traits in the Early Development Growth Stage in Mint (*Mentha spicata* L.) And Rosemary (*Rosmarinus officinalis* L.) Plants under Closed Hydroponic System. *Horticulturae*. 7, 100. https://doi.org/10.3390/horticulturae7050100

Lv, W., Zhao, X., Wu, P., Lv, J., & He, H. A. (2021). Scientometric analysis of worldwide intercropping research based on web of science database between 1992 and 2020. *Sustainability*. 13, 2430-2446.

Mahmoud, A.W.M., Esmail, S.E.A., El-Attar, A.B., Othman, E.Z., & El-Bahbohy, R.M. (2022). Prospective Practice for Compound Stress Tolerance in Thyme Plants Using Nanoparticles and Biochar for Photosynthesis and Biochemical Ingredient Stability. *Agronomy*. 12(5), 1069. https://doi.org/10.3390/agronomy12051069

Maitra, S., Hossain, A., Brestic, M., Skalicky, M., Ondrisik, P., Gitari, H., Brahmachari, K., Shankar, T., Bhadra, P., Palai, J.B., Jena, J., Bhattacharya, U., Duvvada, S.K., Lalichetti, S., & Sairam, M. (2021). Intercropping—A Low Input Agricultural Strategy for

Food and Environmental Security. *Agronomy*. 11, 311-343. https://doi.org/10.3390/agronomy11020343

Mumivand, H., Izadi, Z., Amirizadeh, F., Maggi, F., & Morshedloo, M.R. (2023). Biochar amendment improves growth and the essential oil quality and quantity of peppermint (*Mentha* × *piperita* L.) grown under waste water and reduces environmental contamination from waste water disposal. *Journal of Hazardous Materials*. 446, 130674. https://doi.org/10.1016/j.jhazmat.2022.130674

Nocentini, M., Mastrolonardo, G., Michelozzi, M., Cencetti, G., Lenzi, A., Panettieri, M., & Knicker Giacomo Certini, H. (2023). Effects of biochar and compost addition in potting substrates on growth and volatile compounds profile of basil (*Ocimum basilicum* L.). *Science of food and agriculture*. 104(3), 1609.1620.

Ochiai, S., Iwabuchi, K., Itoh, T., Watanabe, T., Osaki, M., & Taniguro, K. (2021). Effects of Different Feedstock Type and Carbonization Temperature of Biochar on Oat Growth and Nitrogen Uptake in Co application with Compost. *Soil Science and Plant Nutrition*. 21, 276-285. https://doi.org/10.1007/s42729-020-00359-y.

Ouertatani, S. (2021). Study of the Effect of the Biochar Amendment on the Physic-Chemical Properties of a Soil Cultivated in Green Mint (*Mentha Viridis* L.) Agriculture Productivity in Tunisia under Stressed Environment. *Springer Water*. *Springer*. 173-192. DOI: 10.1007/978-3-030-74660-5_8.

Oluwafemi, R.A., Isiaka Olawale, A., & Alagbe, J.O. (2020). Recent trends in the utilization of medicinal plants AS Growth promoters in poultry nutrition - a review. Scholars. *Journal of Agriculture and Veterinary Science*. 4, 5-11.

Paczka, G., Mazur-Paczka, A., Garczynska, M., Hajduk, E., Kostecka, J., Bartkowska, I., & Butt, K.R. (2021). Use of Vermicompost from Sugar Beet Pulp in Cultivation of Peas (*Pisum sativum* L.). *Agricultural Science*. 11, 1-11 https://doi.org/10.3390/agriculture11100919.

Rezaei-Chiyaneh, E., Amani Machiani, M., Javanmard, A. Mahdavikia, H., Maggi, F., & Morshedloo, M.R. (2021). Vermicompost Application in Different Intercropping Patterns Improves the Mineral Nutrient Uptake and Essential Oil Compositions of Sweet Basil (*Ocimum basilicum* L.). *Journal of Soil Science and Plant Nutrition*. 21, 450–466. https://doi.org/10.1007/s42729-020-00373-0

Salmerón-Manzano, E., Garrido-Cardenas, J.A., & Manzano-Agugliaro, F. (2020). Worldwide Research Trends on Medicinal Plants. *International Journal of Environmental Research*. 17, 3376. https://doi.org/10.3390/ijerph17103376

Shahrajabian, M.H., Sun, W., & Cheng, Q. (2020). Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review. *International journal of food properties*. 23(1), 1961-1970. https://doi.org/10.1080/10942912.2020.1828456

Shushupti, O., Orpa, R.S., Tarannum, T., Naima, N.C., Jamin Haque Suchi, S., & Khalilur Rahman, M.D. (2022). Influence of various commercially available organic manures on growth, yield and nutrient accumulation in mint plants (*Mentha sp*.). *Journal of Biodiversity Conservation and Bioresource Management*. 7(1), 73-84. DOI: 10.3329/jbcbm. v7i1.57125

Tan, M., & Yolco, H. (2020). Current Status of Forage Crops Cultivation and Strategies for the Future in Turkey: A Review. *Journal of Agricultural Science*. 27(2), 114-121. https://doi.org/10.15832/ankutbd.903732

Verza, S.G., Kreinecker, M.T., Reis, V., Henriques, A.T., & Ortega, G.G. (2007). Valuation of the analytical variables of the Folin-Ciocalteu method for determining the content of total tannins using as a model the aqueous extract of leaves of *Psidium guajava* L. *Quím*. 30(4), 815-820.

Voko, M.P., Kulkarni, M.G., Ngoroyemoto, N., Gupta, S.H., & Finnie, J.F. (2022). Vermicompost Leachate, Seaweed Extract and Smoke-Water Alleviate Drought Stress in Cowpea by Influencing Phytochemicals, Compatible Solutes and Photosynthetic Pigments. *Journal of plant growth regulation*. 2, 1-30. https://doi.org/10.21203/rs.3.rs-975337/v1

Zhao, F., Zhang, Y., Li, Z., Shi, J., Zhang, G., Zhang, H., & Lijuan, Y. (2020). Vermicompost improves microbial functions of soil with continuous tomato cropping in a greenhouse. *Journal of Soils and Sediments*. 20, 380–391. https://doi.org/10.1007/s11368-019-02362-y