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Assessment of the Effects of Organic Fertilizer Applications on the Biochemical Quality of Basil

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ABSTRACT

It is important to characterize the biochemical potential of medicinal and aromatic plants, which have significant therapeutic and commercial value. However, fertilizers obtained from natural resources are used in sustainable agricultural practices. In this study, basil (*Ocimum basilicum* L.) was grown from seed with different doses of barnyard manure, vermicompost and chicken manure were added. This work aimed was to investigate and compared the effect of these different fertilizer doses applications on the biochemical potential of basil plants.

Total phenolic content, antioxidant capacity and antimicrobial activity values were statistically significant according to the type and

Keywords: Basil, Manure, Antioxidant capacity, Antimicrobial activity

1. Introduction

doses of fertilizer studied. Linalool was found to be the highest amount of volatile compound in leaf samples of manure treated plants. The highest antioxidant capacity values were determined in the samples where farm manure and chicken manure were applied at low (2.5%) and medium (20%) doses and worm manure was applied at high doses. The highest antibacterial effect was detected in the essential oil extract of leaf samples with 10% vermicompost against *Bacillus cereus*. Among the Gram (-) bacteria, the highest antibacterial effect against *E. coli* was determined in the essential oil extract of leaf samples with 20% farm manure. The essential oil extract of leaf samples with 20% farm manure also showed significant and high degree of antifungal effect against *S. cerevisae*.

The use of excessive amounts of chemical inputs in agriculture to meet the increasing need for food due to the rapid increase in population has become an important ecological problem. Especially in developing countries, the need to obtain more products from a unit area due to malnutrition, famine and economic reasons has led to the use of more chemical inputs. This situation negatively affects the deterioration of the ecological balance and the health of nature and the living creatures living there. For this reason, in recent years, there has been an increase in sustainable ecologically based practices that are in harmony with nature, where high- and high-quality products are obtained from unit area. Especially the interest in medicinal and aromatic plants has increased the need for quality development of these plants. The quality and quantity of active substances in plants may vary according to the genetic characteristics of the plant, climatic conditions and agronomic processes applied. Among these agronomic processes, organic farming, which is at the forefront today, is practiced in almost all countries.

O. basilicum is a plenty popular and thoroughly grown herb global. Organic and inorganic fertilization investigations have been carried out regarding cultivation, breeding, large, herbage efficiency and large essential oil content. *O. basilicum* L., also known as sweet basil, is one of the most economically important aromatic plants belonging to the Lamiaceae family (Baczek at. al. 2019; Sharma et al. 2021) that is native to India and Pakistan but is cultivated as a short-lived annual crop worldwide (Tenore et. al. 2017; Nadeem et al. 2022). The fresh and dried herbs are used as a spice in food and its essential oil in perfume industries for commercial products (Murillo-Amador et al.2013; Santos et al. 2016). There are about 900 medicinal and aromatic plants cultivated for commercial purposes worldwide (Acubuca & Budak 2018). The sweet basil is in high demand in the international market (Egata 2021). Basil is mainly produced in accordance with conventional agriculture. However, its cultivation in organic systems seems to be better adjusted to consumer demands connected with the lack of pesticide residues in foods and their safety. The amount of basil produced organically in Turkey in 2015 was 6 tons (Arslan et al. 2015). The use of human and environmentally friendly production systems in fertilization studies is very important in correcting the natural balance that has been disturbed by wrong methods (Tsvetkov et al. 2018; Feledyn-Szewczyk et al. 2020). It was stated that cultivation of medicinal and aromatic plants with natural, environmentally friendly product quality (Rao et al. 2022). It

has been reported that organic poultry fertilization at an appropriate rate to the soil increases antibacterial content, antioxidant activity, total phenolics, flavonoids and essential oil components in basil (Yaldız et al. 2019 a).

Organic fertilizers from barn, goat, sheep, poultry manures are usually preferred in environmentally friendly fertilizer studies (Yolcu 2011; Loss et al. 2020; Abou-Sreea et al. 2021). Biological and organic fertilizer applications are accepted as alternative methods to chemical fertilizers in sustainable agriculture (Dehghani-Samani et al. 2021; Özbucak & Alan 2024). Organic fertilizers are not only beneficial for plant growth, but also a nutrient-rich environment for later plants (Bernal et al. 2009). The poultry manure application has a positive effect on fresh weight, dry weight and nutrient content development of sweet basil (Yaldız et al. 2019 b). It was reported that different types and doses of vermicompost applications positively affected the product and quality characteristics of lettuce plants (Özbucak & Alan 2024).

Since medicinal and aromatic plants, like other plants, are very sensitive to environmental conditions, great differences are observed between the essential oil ratios and the contents of the main components of the same species of plants growing in different ecologies (Mohamed & Alotaibi 2023). This causes changes in the biological and chemical components of the plant such as quality and quantity of essential oil, antimicrobial and antioxidant properties (Bistgani et al. 2018; Yaldız et al. 2019). It is known that *O. basilicum* L. species contains different chemotypes according to the ratio of essential oil component (Raina & Gupta 2018; Shahrajabian et al. 2020).

This is the first report comparing the effects of vermicompost, farm manure, and chicken manure applications on bioactive components of basil plants grown in greenhouse. In addition, it was tried to determine the appropriate form and dose of organic fertilizer, which has been widely used in recent years, for the growth of basil plants. Therefore, it will undoubtedly be important to determine the potential for sustainable agricultural production by comparing the effects of different organic fertilizer applications on the same plant.

2. Material and Methods

2.1. Material and experimental design

The study was carried out as a pot trial with 3 replications in a plastic greenhouse in 2021. Basil seeds were sown in viols containing peat: perlite at a ratio of (3:1). The first germination was started in 3-4 days. After about 30 days, the seedlings were planted in 3 kg pots filled with soil in a plastic greenhouse. 36 pots were used in the study and two seedlings were planted in each pot. Growing media to which barnyard manure, vermicompost and chicken manure were added at different doses were used in the study. 10%, 20% and 30% doses of vermicompost and barnyard manure, 2.5%, 5% and 10% doses of chicken manure were applied. Soil without fertilizer application was used as control group. At the beginning of the experiment, 300-600-900 g of barnyard manure and vermicompost and 75-150-225 gr chicken manure were added to pots. The pots were placed in the greenhouse to receive the same amount of light. All pots were watered once/twice a week as needed and common tap water was used for irrigation. The plant samples were collected during flowering period

Barnyard, chicken and vermicompost were purchased from a commercial company. Soil analysis was performed at the Tekirdağ Commodity Exchange Analysis Laboratory. According to the analysis the soil used in this study had acidic pH (5.90), humidity 48%, 0.87% organic matter, 0.52 dS/m electrical conductivity, 43 ppm potassium, 3.60 kg/da phosphorus, 3974.25 ppm calcium, 866 ppm magnesium.

Plant samples were dried at 25 °C and grinding using a blender for preparation of methanol and water extracts. Essential oil was obtained from the dry samples obtained in sufficient quantities by method water distillation method in Clevenger apparatus (Karaca et al. 2017). Antimicrobial activity of these oil samples was determined. Volatile organic compounds in dry leaf samples were determined by Shimadzu model - QP2010 Ultrain brand GC-MS (gas chromatography/mass spectrometry).

2.2. Antioxidant capacity

Total phenolic contents (TPC) of water and methanol extracts of *O. basilicum* leaves were determined by Folin-Ciocalteu method (Singleton & Rossi 1965). DPPH free radical scavenging activity test was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in methanol (Blois, 1958). The reducing antioxidant power was calculated following the method based on the reduction of the Fe(III)-TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) complex to form the blue colored Fe(II)-TPTZ complex depending on the antioxidant content of the tested samples (Benzie & Strain 1999).

2.3. Antimicrobial activity

The antimicrobial activities of the studied samples were tested in vitro by paper disk diffusion method. For bactericidal and fungicidal studies, MHA, and PDA media, respectively, were used. MHA medium was used for bactericidal studies and PDA medium was used for fungicidal studies. Known antibiotic such as Gentamicin and Nystatin were used as a standard drug for Microorganism. Antimicrobial activity was measured according to method followed by Ronald (1990).

2.4. Statistical analysis

The Kolmogorov-Smirnov test was used to test whether the data obtained in the study were normally distributed. The homogeneity of the variances of the groups was determined using Levene's test statistic. Considering the existing assumptions, the differences between the group averages were revealed using two-way and three-way ANOVA models. All the results obtained in the study were evaluated under 5% significance level and analyses were carried out through SPSS v24 and R package program.

3. Results and Discussion

The data of volatile component analyses of the leaf parts of basil (*O. basilicum*) grown in different organic fertilizer environments are shown in Table 1. Three volatile organic compounds were identified with a composition percentage above 10 to the control group. These are respectively; linalool (27.28%), Bergamotene <alpha-trans-> (22.19%) and eugenol (14.75%). The highest amounts of Linalool (48.45%-41.08%) were found in the samples where chicken manure was applied at low and medium doses to the control and other groups. However, the amounts of Bergamotene <alpha-trans-> (11.29%-10.44%) in the same doses of chicken manure applications were lower than the control and other groups. Analysis could not be performed due to insufficient sample at 10% dose. Linalool, Bergamotene <alpha-trans-> and Eugenol were the highest volatile compounds among others at all doses of barnyard and vermicompost treatments.

Application	Composition %	Volatile organic compounds
	27.28	Linalool
Control	22.19	Bergamotene <alpha-trans-></alpha-trans->
	14.75	Eugenol
Low	48,45	Linalool
	11,29	Bergamotene <alpha-trans-></alpha-trans->
(Chicken) Medium	41.08	Linalool
	10.44	Bergamotene <alpha-trans-></alpha-trans->
Low	30.19	Linalool
	18.86	Bergamotene <alpha-trans-></alpha-trans->
	14.12	Eugenol
(Barn) Medium	34.75	Linalool
	14.47	Bergamotene <alpha-trans-></alpha-trans->
	12.01	Eugenol
High	38.13	Linalool
	15.54	Bergamotene <alpha-trans-></alpha-trans->
	10.87	Eugenol
Low	28.91	Linalool
	16.47	Bergamotene <alpha-trans-></alpha-trans->
	15.25	Eugenol
(Worm) Medium	33.81	Linalool
	17.85	Bergamotene <alpha-trans-></alpha-trans->
	10.98	Eugenol
High	39.21	Linalool
	17.2	Bergamotene <alpha-trans-></alpha-trans->

Table 1- Distribution of volatile organic compounds of plant leaf samples

In addition to the quality and yield of medicinal and aromatic plants, the type, and the number of active substances they contain are also important (Özer 2022). Therefore, in the production of medicinal and aromatic plants, cultivation and fertilization techniques should be determined and correct applications should be made. Although basil species have different chemotypes in terms of essential oil constituents, there are 4 different classifications in terms of main constituents as they are generally rich in linalool, methyl cinnamate, methyl chavicol and eugenol (Vernin et al. 1984; Simon et al. 1999). In studies carried out in our country, it was determined that 7 different chemotypes were found in basil species collected from Turkey (Telci 2005). The quality of natural products is determined by their composition and functional properties. It is therefore important to identify how the characteristics of plants change depending on the use of different fertilizers (Özbucak et al. 2014). In present study, Linalool, Bergamotene and Eugenol were the most abundant volatile compounds in basil leaves grown in the environments where different organic fertilizers were applied. However, it is observed that the amounts of these components vary depending on the fertilizer application and dose. Linalool was the component with the highest amount in all experimental groups compared to the control. The high proportion of linalool explains that the variety we studied is in the Linalool chemotype. Studies have supported that chemotypes rich in linalool are known as European chemotypes (Vernin et al. 1984; Simon et al. 1999; Raghavan 2006). In the study, it was determined that the Linalool ratio of basil varied between 27.28% and 48.45%. These results are like to some other studies (Milenković et al. 2019; Yaldız et al. 2019). The highest amount of Linalool was observed in 2.5% and 5% chicken manure applications in present study. Linalool results of present study are like Yaldız et al. (2019).

When the volatile components of 10% vermicompost leaf samples are examined, it is seen that the amounts of Linalool and Eugenol are higher than the control. It is noteworthy that the only experiment in which the amount of Eugenol was higher than the control was the 10% vermicompost application. The antimicrobial activity of essential oils generally depends on the chemical composition and quantity of each component. Scientific studies have shown that Eugenol shows excellent antimicrobial activity against a wide variety of Gram (+) and (-) bacteria and fungi (Marchese et al. 2017). Most of the researchers think that the hydroxyl groups of Eugenol bind to proteins and prevent enzyme activation (Burt 2004). However, there are also different mechanisms explaining the activity of Eugenol in the bacterial cell (Devi et al. 2010).

3.1. Antioxidant capacity results

3.1.1. Total phenolic content (TPC) (mg GAE/g sample)

TPC of the methanol and water extracts of leaf parts of the basil samples grown under different doses of chicken, barn, and worm applications was determined. According to the three-way-ANOVA results, interaction of fertilizer type*fertilizer dose*extraction solvent was found statistically significant (P<0.01).

The highest TPC was the samples from the pot where worm manure was applied at 30% ($83.29 \pm 9.66 \text{ mgGAE/g}$ extract) and the pot where chicken manure was applied at 2.5% ($83.11 \pm 8.36 \text{ mgGAE/g}$ extract). In the pots where barnyard manure was used, the relatively highest phenolic substance content was determined in the samples at the 20% dose level ($43.28 \pm 4.67 \text{ mgGAE/g}$ extract). On the other hand, the lowest phenolic substance content was obtained for the water extract samples of the control group (0.57 ± 0.1) and the pot where 30% farmyard manure was applied (0.97 ± 0.4) (Table 2).

Table 2- Descriptive statistics and multiple comparison results for TPC values

zer			
E-star a st	Barn	Worm	Chicken
Extract	$M \pm SD$	$M\pm SD$	$M \pm SD$
Methanol	67.36 ± 9.67	A a x 67.36 ± 9.67	A a x 67.36 ± 9.67 A a x
Water	0.57 ± 0.1	A a y 0.57 ± 0.1	A a y 0.57 ± 0.1 A a y
Methanol	4.47 ± 0.44	A b x 2.22 ± 0.78	B b x 4.15 ± 0.79 A b x
Water	4.21 ± 0.53	A a x 1.78 ± 0.48	A a x 83.11 ± 8.36 B b y
Methanol	13.65 ± 0.71	A b x 2.08 ± 0.82	B b x 23.21 ± 3.69 C c x
Water	43.28 ± 4.67	A b y 2.25 ± 0.49	B a x 4.47 ± 3.3 B a y
Methanol	4.26 ± 0.66	A b x 1.8 ± 0.1	Bbx-
Water	0.97 ± 0.4	A a y 83.29 ± 9.66	Bby-
	<i>Extract</i> Methanol Water Methanol Water Methanol Water Methanol	Extract Barn $M \pm SD$ Methanol 67.36 ± 9.67 Water 0.57 ± 0.1 Methanol 4.47 ± 0.44 Water 4.21 ± 0.53 Methanol 13.65 ± 0.71 Water 43.28 ± 4.67 Methanol 4.26 ± 0.66	ExtractBarn $M \pm SD$ Worm $M \pm SD$ Methanol 67.36 ± 9.67 A a x 67.36 ± 9.67 Water 0.57 ± 0.1 A a y 0.57 ± 0.1 Methanol 4.47 ± 0.44 A b x 2.22 ± 0.78 Water 4.21 ± 0.53 A a x 1.78 ± 0.48 Methanol 13.65 ± 0.71 A b x 2.08 ± 0.82 Water 4.28 ± 4.67 A b y 2.25 ± 0.49 Methanol 4.26 ± 0.66 A b x 1.8 ± 0.1

There is a difference between fertilizer averages without a common capital letter in the same dose and extract (P<0.05)

There is a difference between dose averages that do not have a common lower case letter (a,b,c) at same extract and fertilizer (P<0.05)

There is a difference between extract averages that do not have a common lower case letter (x,y,z) at same dose and fertilizer (P<0.05)

TPC was found to be high and significant in the case of methanol extracts of control group pots (67.36 ± 9.67) (P<0.05). Therefore, any type or dose of fertilizer intervention in the pots led to a decrease in all methanol-extracted phenolic contents. in pots where barn manure was applied as 20%, worm manure as 30% and chicken manure as 2.5%, the phenolic substance content was higher in the case of water extract than in methanol extract.

TPC values calculated for methanol extracts of leaf samples from pots containing low levels of vermicompost were found to be statistically different (P<0.05) from the TPC values of the samples obtained with the other two types of fertilizer (P<0.05). Similar statistical difference was observed in the case of water extract of chicken manure treated samples. In the case of water extract, it was determined that only the phenolic content of barnyard manure differed.

The main objective of the production of medicinal and aromatic plants is to increase the yield of secondary metabolites. Organic fertilizers are known to improve secondary metabolism in essential oil-containing plants by increasing the amount of available nutrients (Emami Bistgani et al. 2018; Rostaei et al. 2018; Rao et al. 2022).

At the same time, organic fertilizer applications can play an important role in increasing plant resilience to stress conditions. In fact, based on this information, it may lead to a decrease in the production of secondary metabolites, as observed by Jakovljević et al. (2017) because it can be interpreted that the plant may not choose the secondary metabolite production route to cope with stress. Numerous studies have noted the effectiveness of organic fertilizers, compost, and compost teas in increasing vegetative growth, biomass, and essential oil yield of sweet marjoram, cumin, fennel, and basil. Improvements in yield and quality following the application of these organic-based materials may be due to increased beneficial microbial communities in the soil, improved nutrient absorption conditions for plants, and induction of plant defence compounds, growth regulators, or phytohormones (Javanmardi & Gjhorbani 2012).

3.1.2. Ferring reducing antioxidant power (FRAP)

It was concluded that the FRAP test results performed on extracts prepared from samples obtained by exposing them to different conditions to evaluate antioxidant activity were statistically significant (P<0.01). According to the three-way ANOVA test in terms of fertilizer type*fertilizer dose*extraction solvent, just like the TPC results (Table 3).

Fertili	zer												
D	F	Barn Worm											
Dose	Extract	$M \pm SD$				$M \pm SD$				$M \pm SD$			
Control	Methanol	851.8 ± 10.16	Α	а	Х	851.8 ± 10.16	Α	b	х	851.8 ± 110.16	Α	а	х
Control	Water	4.32 ± 1.96	Α	а	у	4.32 ± 1.96	Α	а	у	4.32 ± 1.96	А	а	у
Low	Methanol	78.94 ± 3.5	Α	b	Х	31.95 ± 3.67	В	а	х	27.97 ± 5.38	В	b	х
	Water	29.17 ± 7.13	Α	а	у	15.29 ± 5.43	Α	а	У	625.07 ± 109.99	В	b	у
Medium	Methanol	181.49 ± 9.58	Α	b	х	28.29 ± 2.64	В	а	х	213.75 ± 29.83	А	с	х
Medium	Water	297.69 ± 6.87	Α	b	у	15.99 ± 6.57	В	а	У	54.08 ± 20.32	В	а	у
High	Methanol	67.66 ± 2.96	Α	b	X	19 ± 1.69	В	а	x	-			
	Water	8.44 ± 0.48	Α	а	у	633.41 ± 4.12	В	b	У	-			
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Table 3- Descrip	ptive statistics and	l multiple com	parison results f	or FRAP values

There is a difference between fertilizer averages without a common capital letter in the same dose and extract (P<0.05)

There is a difference between dose averages that do not have a common lower case letter (a,b,c) at same extract and fertilizer (P<0.05)

There is a difference between extract averages that do not have a common lower case letter (x,y,z) at same dose and fertilizer (P<0.05)

The highest and lowest FRAP values were observed in the methanol and water extracts of the control pots, respectively. Therefore, any dose of fertilizer intervention in the pots causes a decrease for methanol extracts and an increase for water extracts in terms of FRAP values FRAP values obtained for water extract were higher than those obtained for methanol extract in the samples treated with 20% barnyard manure, 30% vermicompost and 2.5% chicken manure, unlike the control samples. FRAP values of water extract samples were the highest in all pots except the control pot. However, the highest FRAP values were also determined in the methanol extracts of the samples treated with 20% barnyard manure.

3.1.3. DPPH free radical scavenging activity

DPPH radical scavenging activity results as ascorbic acid equivalent were evaluated according to three-way ANOVA model (Table 4). Fertilizer type*fertilizer dose*extraction solvent interaction effect was found statistically significant (P<0.01). Statistically significant difference was observed in the DPPH radical scavenging activity values in the case of methanol and water extracts in all samples except the samples in which 20% stable manure was applied (P<0.05). The highest DPPH scavenging activity was obtained in the case of water extract of the samples obtained from the control pot. It can be said that applying any dose of fertilizer to the pots causes a decrease in the DPPH radical scavenging activity in the water extract samples.

Fertilizer													
Dese	E. () (Barn				Worm				Chicken			
Dose	Extract	$M \pm SD$				$M \pm SD$				$M \pm SD$			
Control	Methanol	0.17 ± 0.09	Α	а	Х	0.17 ± 0.09	Α	а	Х	0.17 ± 0.09	Α	а	х
Control	Water	16.09 ± 4.85	Α	а	У	16.09 ± 4.85	Α	а	у	16.09 ± 4.85	Α	а	у
Low	Methanol	0.62 ± 0.01	Α	b	х	1.19 ± 0.08	В	b	х	1.22 ± 0.05	В	b	х
LOW	Water	2.39 ± 0.48	Α	bc	У	4.32 ± 0.29	В	b	у	0.1 ± 0.03	С	b	у
Medium	Methanol	0.23 ± 0.05	Α	а	х	1.47 ± 0.06	В	b	х	0.19 ± 0.07	Α	а	х
Medium	Water	0.25 ± 0.11	Α	b	х	3.93 ± 0.19	В	b	у	1.54 ± 0.45	С	b	у
Illah	Methanol	0.61 ± 0.03	Α	b	х	2.09 ± 0.19	В	с	х	-			
High	Water	8.11 ± 3.06	А	с	y	0.1 ± 0.02	В	b	y	-			

Table 4- Descriptive statistics and multiple comparison results for DPPH values

There is a difference between fertilizer averages without a common capital letter in the same dose and extract (P < 0.05)

There is a difference between dose averages that do not have a common lower case letter (a,b,c) at same extract and fertilizer (P<0.05)

There is a difference between extract averages that do not have a common lower case letter (x,y,z) at same dose and fertilizer (P<0.05)

In barnyard manure applications, regardless of the solvent, the lowest DPPH activity was reached at 20% dose level. It was observed that DPPH activity reached the highest value in the water extract samples of the pot samples in which the same fertilizer was applied at 30%, and this value was statistically significantly different from all other units except the control pot. It was

observed that the DPPH radical scavenging activity in the water extract of the samples applied with 10% and 20% vermicompost was statistically significant and higher compared to other fertilizer types corresponding to the same dose levels (Table 4).

In accordance with the FRAP test results, a statistically significant difference was observed in terms of DPPH radical scavenging activity values in the case of methanol and water extracts in all samples except the samples in which 20% barnyard manure was applied (P<0.05).

Total phenolic content and antioxidant activity values decreased in the case of methanol extraction after all fertilizer applications, while an increase was observed in all 3 parameters in the case of water extraction. It has been previously reported that fertilizers can have positive and negative effects on phenolic compounds in plants (Gavrić et al. 2021). According to the results, the production of secondary metabolites in the leaves of basil plants was greatly affected by different types of fertilizers. In cases where low and moderate fertilizer applications were made, worm manure was less effective in terms of phenolic content than the other two types of fertilizers, while in cases of high dose fertilizer application, it was concluded that worm manure was more beneficial. The determined FRAP values of the samples prepared by extracting the basil samples grown with water and methanol after the application of control and all fertilizer types at different doses showed statistical differences. In this way, the importance of the effect of the extraction solvent on antioxidant activity was clearly observed. A similar study was carried out on *Ocimum* × *citriodorum* Vis. using worm and chicken manure teas in the ratios of 1:5 and 1:10, and the highest phenolic content was determined when worm manure tea was used in the ratio of 1:5. In this study, where the samples were extracted with acetone, the total antioxidant activity was found to be higher for the same group of extracts compared to others (Javanmardi & Ghorbani 2012).

The correlation coefficient between the total phenolic content and FRAP values of the extracts of the samples obtained after barn and worm manure applications was calculated as 0.91, while in the case of chicken manure application, this value was 0.89. The highest value of the correlation coefficient calculated between phenolic content and DPPH activity values is in the case of the sample exposed to worm manure, while it is 0.76 and 0.88 in the case of samples grown in the presence of barnyard and chicken manure, respectively. In another study where the effect of farm, worm, and chicken manure on the antioxidant activity of basil was compared with chemical fertilizer, the application of poultry manure achieved the highest result among organic fertilizers. Although chemical fertilizer did not cause any change in antioxidant activity compared to the control, the highest DPPH activity data was obtained when chemical fertilizer was used in combination with organic fertilizers (Pandey et al. 2016). Moreover, in the study, the statistical difference arising from the extraction solvent is generally noticeable in all cases. Studies have shown that extraction efficiency is strongly affected by the extraction solvent (Filip et al. 2017; Złotek et al. 2016) It was observed that the total phenolic content values calculated after the extraction of 1 g of basil plant material with 30 mL acetone, water, methanol, and ethanol for 40 minutes were highest in methanol (Do et al. 2020). In the current study, we detected higher phenolic content for methanol extracts, especially in the control group, which is compatible with the literature.

It was concluded that TPC and antioxidant capacity values of basil plants grown with organic, mineral and organomineral fertilizer applications were not significantly affected. This may be due to the weather conditions at the time of the trial. Because weather conditions can have a greater impact on the content of bioactive compounds than the effect of fertilizers (Gavrić et al. 2021). In another study examining the effects of four types of fertilizers, such as biosolids, microorganisms, organic fertilizers, and mineral fertilizers, on the bioactive compounds and antioxidant capacity of basil, it was found that, on the contrary, the bioactive compound content and antioxidant capacity value in basil increased in all applications. In the said study, the highest concentration of total polyphenols was recorded for biosolids fertilization, while total flavonoids and total anthocyanins were better increased by organic and microorganism treatments. However, the total amount of bioactive compounds recorded in dry basil leaves was detected at the highest level in organic and chemical fertilization and microorganism application (Teliban et al. 2020).

Jakovljević et al. (2017) reported that excessive application of chemical fertilizers stimulated the growth of basil plants, but also reduced the content of secondary compounds. To overcome these limitations, future researchers should more carefully consider the potential impact of different fertilizer types and doses, for example, on the composition of essential oils and phenols (Gavrić et al. 2021). In the essential oil analysis of the current study, the highest percentage of linalool was obtained when chicken manure was applied at a rate of 2.5%, and the fact that the highest phenolic content and highest frap values were calculated in the case of the same dose and type of fertilizer application is good evidence of the contribution of the essential oil composition to antioxidant activity.

3.1.4. Antimicrobial activity analysis results

Antimicrobial activities of the leaf samples for the paper disc diffusion method assay propose that the EOs essential oils and methanolic fractions had secondary antibacterial activities. Increasing doses of chicken, worm and barnyard manure applied in the study showed a progressively greater prohibitive influence on the antimicrobial fortune of *O. basilicum* leaf extracts (Table 5). The antimicrobial efficiencies of essential oils and methanolic extracts from *O. basilicum* treated with chicken, worm and barn manure were more powerful against bacteria than fungi and may be potential resources of new antimicrobial agents. The essential oils obtained from the *O. basilicum* treated with barn manures exhibited the highest antibacterial activity against Gram-

negative bacterial strains, such as E. coli, C. freundii and Gram-positive bacterial strains, such as B. cereus. Especially O. basilicum treated with barn manures showed the most effective antifungal effect against S. cerevisiae yeast. The EOs treated with worm demonstrated a more powerful zone demonstration against only Gram-positive bacterial strains, such as B. cereus and S. aureus. In this work, the excessive antibacterial activity was acquired with a small amount of vermicompost applied. At the same time, this application, which investigated the antimicrobial activity of O. basilicum against pathogenic bacteria, obtained the most effective antifungal activity. Right now, investigation we utilization Gram-negative bacteria that are contemplated more indestructible to essential oils than Gram-positive homologues (Nazzaro et al. 2013). This action is imputed to the complicated cell-wall structures of Gram-negative organisms, which do not allow a simple influence of antibiotics and drugs, with the inclusion of phenolic compounds, such as eugenol, Linalool, and other essential oils (Tiwari et al. 2009). The accepted mechanism of activity of essential oils depends on their capability to disturb the bacterial cell wall and the cytoplasmic membrane, so producing a cell lysis and loss of intracellular compounds (Lopez-Romero et al. 2015). The essential oils and the main components of these oils, such as carvacrol, citronellol, geraniol and neroli, exhibited antibacterial activities (Janssen et al. 1986). The essential oils acquired from the O. basilicum L. cured with chicken manure demonstrated the greatest antibacterial activity against S. aureus, L. monocytogenes and E. coli while that acquired by handling of chicken manures did not show the power antifungal efficiency against C. albican and S. cerevisae. Essentially, EOs can diffuse, the phospholipid bilayer of the bacterial cell wall, bind to proteins, and arrest their normal duties owing to their interplays with phenolic molecules (Sakkas et al. 2016). In contrast with beforehand conclusions (Morelli et al. 2017), who did not find any discoverable influence of soil HS practice neither on plant development, nor on O. basilicum antibacterial activity, our conclusions remark that. With chicken, worm, and barn fertilizer compost do impact the subsidiary metabolism of an aromatic plant, such as basil, and promote larger vields of phenolic EO components, such as Linalool, Bergamotene, Eugenol, Cadinene, Muurolol and Terpineol which are included in their antimicrobial activity. O. basilicum L. treated with middle chicken manure application of vermicompost, oil acquired from basil leaves was found to be most influential on microorganisms EO obtained from control leaves showed average effect on micro-organisms, while this value O. basilicum L. treated with little worm and middle barn manure application of showed power effect on microorganisms.

Among Gram (-) bacteria, the highest antibacterial effect on *E. coli* was determined in the essential oil extract of 20% barnyard manure leaf samples. The essential oil extract of 20% barnyard manure leaf samples also showed significant and high antifungal effect on *S. cerevisae*. When the volatile components of 20% samples of barn manure are examined, it is seen that the amount of Linalool is higher than the control. It has been determined by in vivo studies that linalool and linalool-rich essential oils have various biological activities such as antimicrobial, anti-inflammatory, anticancer and antioxidant. The repellent effect of linalool on insects damaging crops has also been investigated, emphasizing the importance of the application of this molecule in environmentally friendly insect pest management (Kamatou &Viljoen 2008). However, scientific studies have determined that linalool has many therapeutic effects (Sezen et al. 2021).

Fertilizer	r Dose Extract Microorganism									
			S. aureus	B. cereus	L. monocytogenes	E. coli	C. freundii	P. aerusinasa	C. albicans	S. cerevisae
Barn	Low	М	17.05 ± 0.01 Abx	$6 \pm 0 Aa$	15.75 ± 0.01 Abx	15.25 ± 0.01 Abx	$12 \pm 0.01 \text{Aax}$	12 ± 0.01 abx	13 ± 0.01 Abx	12.5 ± 0.01 Abx
		EO	$6 \pm 0 Aby$	$6 \pm 0 Aa$	$6 \pm 0 Aby$	$6 \pm 0 Aby$	$6 \pm 0 Aay$	$6 \pm 0 Aby$	$6 \pm 0 Aby$	6 ± 0 Aby
	Medium	М	$12.34 \pm 0.01 Acx$	-6 ± 0Aa	$14 \pm 0.01 Acx$	$13.5 \pm 0.01 Acx$	$10\pm0.01 Abx$	$10.5 \pm 0.01 Ac$	12.75 ± 0.01Acx	9.75 ± 0.01Acx
		EO	$6 \pm 0 Aby$	17.5 ± 0.01 Ab	6 ± 0Aby	$20.5 \pm 0.01 \mathrm{Acy}$	$9 \pm 0.01 Aby$	$6 \pm 0 Ab$	14 ± 0.01Acy	21.25 ± 0.01 Acy
	High	М	13.25 ± 0.01 Adx	$6 \pm 0 Aa$	$11 \pm 0.01 \mathrm{Adx}$	$11.25\pm0.01 \text{Adx}$	11.25 ± 0.01Adx 9.75 ± 0.01Acx		: 0.01d 13 ± 0.01Abx	
		EO	$6 \pm 0 Aby$	$13.75 \pm 0.01 Ac$	$6 \pm 0 Aby$	$12 \pm 0.01 \text{Ady}$	19.4 ± 0.01Acy	$6\pm0\mathrm{Ab}$	9.25 ± 0.01Ady	12.75 ± 0.01 Ady
Worm	Low	М	12.64 ± 0.01 Bbx	$6 \pm 0 Aa$	$10\pm0.01 Bbx$	$13 \pm 0.01 Bax$	$11.25 \pm 0.01 Bax$	10.75 ± 0.01 Bbx	11.5 ± 0.01 Bbx	13.25 ± 0.01 Bbx
		EO	$6 \pm 0 Aby$	$26 \pm 0.01 Ba$	6 ± 0 Aby	13.5 ± 0.01 Bby	$13.75 \pm 0.01 Bay$	$6 \pm 0 Aby$	10.13 ± 0Bby	20 ± 0.01Bby
	Medium	М	$6.94 \pm 0.01 Bcx$	-6 ± 0Aa	$6 \pm 0 Aa$	$8.95 \pm 0.78 Bbx$	$11\pm0.01Bbx$	$6\pm0\mathrm{Aa}$	12.75 ± 0.01Acx	10 ± 0.01 Bcx
		EO	$6 \pm 0 Aby$	$20\pm0.01Bb$	$6\pm0\mathrm{Ab}$	$11.75\pm0.01Bcy$	$10\pm0.01Bby$	$6\pm0\mathrm{Ab}$	12.5 ± 0.01Bcy	$14 \pm 0.01 Bcy$
	High	М	15.09 ± 0.01Bdx	$6 \pm 0 Aa$	$14 \pm 0.01 Bcx$	$9 \pm 0.01 Bbx$	10.75 ± 0.01 Bcx	$6 \pm 0 Aa$	14 ± 0.01 Bdx	$8 \pm 0.01 Bdx$
		EO	$8.15 \pm 0.01 Bcy$	$8.75 \pm 0.01 Bc$	11.5 ± 0.01Bcy	$6 \pm 0 B dy$	$21.75\pm0.01Bcy$	$6\pm0\mathrm{Ab}$	7.25 ± 0.01Bdy	$10.5 \pm 0.01 \text{Bdy}$
Chicken	Low	М	$9.24 \pm 0.01 Cbx$	$6 \pm 0 Aa$	-	$14.5 \pm 0.01 \text{Abx}$	$11.25 \pm 0.01 Bax$	$6\pm0\mathrm{Aa}$	13.75 ± 0.01Cbx	12 ± 0.01 Cbx
		EO	11.59 ± 0.01Bby	$6 \pm 0 Aa$	$6 \pm 0 Ab$	$6 \pm 0 Aby$	$6 \pm 0 Aay$	$6 \pm 0 Ab$	7 ± 0.01Cby	10 ± 0.01 Cby
	Medium	М	16.57 ± 0.01Ccx	$6\pm0Aa$	$15 \pm 0.01 Bb$	15 ± 0.01 Ccx	11.75 ± 0.01 Cbx	11.75 ± 0.01 Bb	$12 \pm 0.01 Bax$	$9 \pm 0.01 Ccx$
		EO	$6 \pm 0 Acy$	7 ± 0.01 Cb	$6\pm0\mathrm{Ab}$	8.75 ± 0.01 Ccy	$9.75\pm0.01Cby$	$6\pm0\mathrm{Ab}$	9 ± 0.01 Ccy	14 ± 0.01Ccy
	Gentamici	n	41,9± 0.01BAc	40,99± 0.0BAc	49,99± 0.01 BAc	41,9± 0.01 BAc	37,99± 0.01 BAc	34,76± 0.01 BAc	NT	NT
	Nystatin		NT	NT	NT	NT	NT	NT	17.67± 0.01	17.45± 0.01
ANOVA	(3-way)	F. Value	F(5,44)=507691.16	F(3,16)=2208055.56	F(3,40)=75775.79	F(5,44)=1141.01	F(3,32)=131138.1	F(2,38)=13494.32	F(5,44)=84648.37	F(5,44)=367485.12
results, only for interaction term		P. Value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5- Antimicrobial Activity Analysis Results

M: Methanol, EO: Essential oil, NT: Not tested, *Listeria monocytogenes* ATCC®7677, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC®10876) *Pseudomonas aeruginosa* ATCC®27853, *Citrobacter freundii* ATCC® 43864 (-), *Escherichia coli*, yeast *Saccharomyces cerevisiae* ATCC 976, and fungi, *Candida albicans* ATCC®10231). The significant values are indicated in red.

4. Conclusions

Few investigations have been reported on behavior regarding the organic chicken, worm, and barn fertilization effect on essential oil content and the quality of basil. Particularly, medium chicken, low worm, and medium barn manure doses had the largest antibacterial efficiency, as well as antioxidant activity, total phenolics, flavonoid and essential oil components. These fertilizers could thence be contemplated as appropriate substitutes for chemical fertilizers when expanding medicinal plants that are gaining both an incremented significance and requisition. We recommend that the effective chemical compounds existing in *O. basilicum* L could fluctuation a role in the treatment of various bacterial contaminations. Hence, wider, and detailed research may be requisitioned to discover the potential use of *O. basilicum* L for treatment of contagious diseases.

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