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Research Article

The Roles of Chicken Manure and Biochar Applications in Enhancing the Morphological, Yield, Crude Protein Content and Antioxidant Activities of Basil

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ABSTRACT

This study was conducted to investigate the effects of chicken manure (10 and 20 t ha^{-1}) and biochar (40 and 80 t ha^{-1}) applications on the morphological, yield, protein content, antioxidant activity, total phenolic and total flavonoid contents of basil (*Ocimum basilicum* L.). Pot experiments under climate chamber conditions at 24 °C (a day length of 16 h) and 16 °C (8 h in a night) showed that A8 and A4 applications significantly increased plant height (PH) values compared to A9 application. Branch number (BN) values had no statistically significant differences in the first and second harvest, but these values showed statistically differences in the harvests mean of the BN. For fresh weight (FW) values, while the A3 application had the 80.00% increase than A9 application, A8 application showed higher FW value compared to A9 application with the increasing of 0.51% and 0.24% in the first and total harvest, respectively. Dry weight (DW) values significantly increased by 2%, 7% and 9% with application of A3 (40 t ha^{-1} biochar×10 t ha^{-1} chicken manure) compared to the control (A9) application. The highest crude protein, DPPH, FRAP and total phenolic and flavonoid contents were found from applications of A7, A2, A9, A6, and A4, respectively. The principal coordinate analysis (PCA) revealed over 52% total variations depending on the first two PCs. Cluster heat map analysis showed that most of the applications took place in the main group B. The results suggest that chicken manure and biochar applications influenced the morphology, yield, protein content, and antioxidant activities of basil. Therefore, these applications can play a crucial role in sustainable basil cultivation, serving as a safe source of mineral matter, particularly for organic farming.

Keywords: *Organic agriculture, Sustainability, Ocimum basilicum*

Tavuk Gübresi ve Biyokömür Uygulamalarının Fesleğenin Morfolojik, Verim, Protein İçeriği ve Antioksidan Aktivitelerini Artırmadaki Rolü

ÖZ

Bu çalışma, tavuk gübresi (10 ve 20 t ha-1) ve biyokömür (40 ve 80 t ha-1) uygulamalarının fesleğenin (Ocimum basilicum L.) morfolojik, verim, antioksidan aktivitesi, toplam fenolik ve toplam flavonoid içerikleri üzerine etkilerini araştırmak amacıyla yürütülmüştür. 24 ◦C (16 saatlik bir gün uzunluğu) ve 16 ◦C'de (8 saat gece) iklim odası koşullarında saksı deneyleri, A8 ve A4 uygulamalarının bitki boyu (PH) değerlerini A9 uygulamasına kıyasla önemli ölçüde artırdığını göstermiştir. Dal sayısı (DS) değerleri birinci ve ikinci hasatta istatistiksel olarak önemli bir fark göstermemiş, ancak bu değerler DS'nin hasat ortalamasında istatistiksel olarak farklılıklar göstermiştir. Yaş ağırlık (YA) değerlerinde, A3 uygulaması A9 uygulamasına göre %80.00 artış gösterirken, A8 uygulaması A9 uygulamasına göre daha yüksek YA değeri göstermiş, ilk ve toplam hasatta sırasıyla %0.51 ve %0.24 artış

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olmuştur. Kuru ağırlık (KA) değerleri, A3 uygulamasıyla (40 t ha-1 biyokömür×10 t ha-1 tavuk gübresi) kontrol (A9) uygulamasına göre sırasıyla %2, %7 ve %9 oranında artmıştır. En yüksek ham protein, DPPH, FRAP ve toplam fenolik ve flavonoid içerikleri sırasıyla A7, A2, A9, A6 ve A4 uygulamalarında bulundu. Temel Bileşen Analizler (TBA), ilk iki TB'ye bağlı olarak %52'den fazla toplam varyasyon olduğunu ortaya koymuştur. Kümeleme ısı haritası analizi, uygulamaların çoğunun B ana grubunda gerçekleştiğini göstermiştir. Sonuçlar, tavuk gübresi ve biyokömür uygulamalarının fesleğenin morfolojisini, verimini, protein içeriğini ve antioksidan aktivitelerini etkilediğini göstermiştir. Bu nedenle, bu uygulamalar özellikle organik tarım için güvenli bir mineral madde kaynağı olarak sürdürülebilir fesleğen yetiştiriciliğinde önemli bir rol oynayabilirler.

Anahtar Kelimeler: *Organik tarım, Sürdürülebilirlik, Ocimum basilicum*

I. INTRODUCTION

The use of organic fertilizers is gaining importance for environmental safety, including the intensive farming of livestock and poultry. When properly managed, these manures can serve as an effective source of nitrogen, contributing to sustainable crop production [1]. One of the most important environmental manure in poultry manures is chicken manure. Chicken manure is an organic fertilizer that is particularly rich in nutrients, especially nitrogen and phosphorus. Its high organic matter content also enhances the physical structure of the soil, making it more conducive to plant growth [2]. In addition, chicken manure is a valuable source of both macro and micro-nutrients, particularly nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) [3]. Its application enhances soil physical properties and supports long-term soil fertility. As an affordable and accessible fertilizer, chicken manure helps reduce nutrient loss, making it an effective tool for sustainable farming [4,5]. Previous studies reported that applications of 10-12.5 t ha^{-1} chicken manure had positive impacts on fresh and dry weights, antioxidant activity, as well as total phenolics, flavonoids and essential oil components of basil [1,6]. Similarly, 10 t ha^{-1} and 17.5 t ha^{-1} chicken manures increased the capric acids and Fe, Cu and Mn concentrations in bitter melon, respectively [7]. Another study by Cheng and Lehmann [8] indicated that applying 10-15 tons of chicken manure per hectare is optimal for basil growth under controlled conditions. However, higher application rates may sometimes result in negative effects, such as nutrient leaching or increased soil salinity. Similar with chicken manure, biochar is another type of environmentally friendly fertilizer. Biochars derived from various sources have been shown to positively impact plant growth, development, yield, and nutrient contents of different plants. Many studies have demonstrated that biochar applications can improve soil quality and productivity, leading to enhanced plant growth [9]. The positive effects of biochar on plant growth are linked to its ability to increase soil water retention, cation-exchange capacity, and specific surface area [10]. As a negatively charged substance, biochar retains water and essential nutrients, promoting better soil fertility [11]. Additionally, biochar has been shown to increase total soil carbon content, as well as the concentrations of magnesium (Mg), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and soil enzyme activity [12].

Sweet basil (*Ocimum basilicum* L.) is a highly valued spice from the Lamiaceae (Labiatae) family. Widely cultivated for its essential oil production, sweet basil is economically significant and is popular in various regions, including India, Türkiye, Iran, Japan, and China [13]. Traditionally, basil has been used in folk medicine for a wide range of treatments. Research has highlighted its diverse protective effects, such as radiation protection, preventive potential against certain chemicals, anti-inflammatory properties, central nervous system stimulant activity, bactericidal effects, modulation of glutathione levels, and enhancement of cognitive function. Furthermore, it has shown promise in ulcer protection and various other therapeutic applications [14-16]. In addition, the last previous study reported that the extract from the basil (dino cultivar) showed a positive effect on Alzheimer's disease (AD) based on the properties examined. It can be suggested that basil extract may have potential as a treatment for AD [17].

Based on the above information, this study was conducted to examine the morphology and yield properties of basil, as well as its protein content, antioxidant activity, total phenolic content, and total flavonoid content in response to applications of different doses of chicken manure and biochar.

II. MATERIALS AND METHODS

A. MATERIALS AND METHODS

A. 1. Plant Material and Growing Conditions

The experiment was conducted in a climate room during the vegetation of between November 2023-June 2024 at the Agriculture Faculty of Bolu Abant İzzet Baysal University, Bolu, Türkiye. The dino basil cultivar seeds used in the study were obtained from medicinal and aromatic plant department. 10 seeds were sown in plastic pots filled with 3.5 kg of soil at the 24 °C-65% humidity in climate chamber in November 2023. Plants in each pots were tinned to 4 plants after germination. The experiment was carried out according to completely randomized complete block design with two factors; biochar and chicken manure were placed in the main plot, and sub-plot was two levels of biochar applications (40 and 80 t ha^{-1}) and chicken manure (10 and 20 t ha^{-1}) with a control (without any fertilizer applications). All applications were repeated three times. Detailed information on the experiment design parameters were given in Table 1.

Table 1. *Detailed information of the used applications.*

Application description	Used code	Quantity of application
Biochar-1	A1	40 t ha^{-1}
Biochar-2	A2	80 t ha^{-1}
Biochar-1×Chicken manure-1	A3	40 t ha^{-1}×10 t ha^{-1}
Biochar-1×Chicken manure-2	A4	40 t ha^{-1}×20 t ha^{-1}
Biochar-2×Chicken manure-1	A5	80 t ha^{-1}×10 t ha^{-1}
Biochar-2×Chicken manure-2	A6	80 t ha^{-1}×20 t ha^{-1}
Chicken manure-1	A7	10 t ha^{-1}
Chicken manure-2	A8	20 t ha^{-1}
Control	A9	No fertilization

Application of biochar and chicken manure levels for main and sub-main plots were applied with sowing. During the vegetation period, no fertilizers or chemicals were applied to the plant, except for application of water and the fertilizers applied in the experiment. Plants were grown under climate chamber conditions at 24 °C during the day and 16 °C at night for 9 weeks. The seeds were planted in plastic pots (17.7 cm diameter, 21.5 cm depth) containing 3.5 kg of soil. Each pot was watered every two days. Prior to each harvest, plant height, branch number, and fresh weight were measured. The fresh plants were then dried in a drying oven at 35°C until their moisture content reached 12-14% to calculated the dry weight. The field soil was used in the experiment, and the parameters of used soil in the experiment showed low organic matter (1.06%), middle phosphorus (75.8 kg ha^{-1}), rich potassium (947.4 kg ha^{-1}) content. Also other properties for soil were found as 7.37% $CaCO_3$, 0.04% total soluble salts, clay loam and neutral pH value (7.46). The used chicken manure and biochar parameters were given in Table 2. All parameters of the chicken manure were found higher than biochar except moisture and EC values.

Table 2. *Chemical parameters of the used chicken manure and biochar.*

Analysis parameters	Unit	Chicken manure	Biochar
pH		7.08	7.00
EC	µmho cm^{-1}	6.68	20.00
Moisture	%	6.00	7.80
Organic matter	%	89.00	-

Table 2 (cont). *Chemical parameters of the used chicken manure and biochar.*

N	%	0.70	0.45
P	%	0.13	0.01
K	%	0.44	0.02
Ca	%	4.32	0.005
Mg	ppm	0.77	0.002
Fe	ppm	0.09	-
Zn	ppm	0.05	0.022

A. 2. Crude Protein Analysis (%)

Crude protein content was determined using the Kjeldahl method with slight modifications. A 0.5 g leaf sample was grinded and hydrolyzed with 20 mL of sulfuric acid (H_2SO_4) and 3.5 g of a selenium catalyst tablet in a hot block. The sample was first heated at 240 °C for 25 minutes, followed by heating at 380 °C for 3 hours. After the digestion process, the samples were cooled, and sufficient distilled water and sodium hydroxide (NaOH) were added to the hydrolysate. The resulting solution was then titrated and neutralized. The total nitrogen content was determined, and the nitrogen ratio obtained from the Kjeldahl method was multiplied by a factor of 6.25 to calculate the crude protein content [18].

A. 3. Extract Preparation of Samples

Approximately 5 g of basil samples were grinded and extracted with 50 ml of 80% aqueous methanol (v/v) by shaking on a shaker at room temperature for 60 minutes. Then the extracts were centrifuged at 10,000 rpm for 10 minutes and prepared for analysis.

A. 4. Determination of Antioxidant Activity (%)

The radical scavenging activity of the basil methanol extract, rich in phenolic content, was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [19]. One mL sample of the extract was mixed with 2 mL of DPPH radical solution (1 mg DPPH dissolved in 100 mL methanol). After thorough mixing and incubation at room temperature for 5 minutes, absorbance values (Δ517 nm) were measured using a spectrophotometer. As a control, 2 mL of DPPH solution was mixed with 1 mL of distilled water. The free radical scavenging activity was calculated using the following equation:

RSA (%) = [(Δ517 nm control − Δ517 nm sample) / Δ517 nm

A. 5. Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP method was performed according to the procedure reported by Camlica and Yaldiz [19]. First, the FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6; 3.1 g sodium acetate trihydrate + 16 ml glacial acetic acid + distilled water), 10 mM 2,4,6-tris (2-pyridyl)-striazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio to create the working reagent. Next, 1 ml of the FRAP reagent was added to 100 µL of basil extract. After incubating for 30 minutes, absorbance values were measured using a spectrophotometer at a wavelength of 595 nm. To estimate the activity capacity of the sample, a Trolox calibration curve (TE) was generated, and the results were recorded as mg TE per g of sample.

A. 6. Determination of Total Phenolic Content

Total phenolic content was determined according to the method reported by Camlica and Yaldiz [19]. To 100 µL of basil extract, 0.4 ml of distilled water and 0.5 ml of diluted Folin-Ciocalteu reagent were added. The mixture was left for 5 minutes, after which 1 ml of 7.5% sodium carbonate (w v^{-1}) was added. Absorbance was measured at a wavelength of 765 nm using a spectrophotometer after a 2-hour incubation. A gallic acid (GA) calibration curve was used to estimate the phenolic content. The results were expressed as mg GA equivalent per 100 g of sample (mg GA g^{-1}).

A. 7. Determination of Total Flavonoid Content

Total flavonoid content was determined following the protocol reported by Camlica and Yaldiz [19]. A mixture of 1 ml extract, 4 ml distilled water, and 300 µL $NaNO_2$ (0.3%) was shaken for 5 minutes. Then, 300 µL $AlCl_3$ (10%) and 200 µL 1 M NaOH were added, and the mixture was well mixed. Finally, 2.4 ml distilled water was added, and the mixture was shaken again. The absorbance of the total flavonoid content was measured at 510 nm. Quercetin (QE) was used as the standard to determine the total flavonoid amount, and the results were expressed as mg QE per g of dry sample (mg QE g^{-1}).

B. STATISTICAL ANALYSIS

The collected data were analyzed using Analysis of Variance (ANOVA) with the JMP statistical software. The means for each property were calculated, and significant differences were identified using the Least Significant Difference (LSD) test at a 5% probability level. The principal coordinate analysis (PCA) were performed by using JMP and XLSTAT programs, and the heat map analysis were conducted by using the Clustvis program.

III. RESULTS AND DISCUSSION

A. MORPHOLOGICAL, YIELD, CRUDE PROTEIN CONTENT AND ANTIOXIDANT VALUES

The plant height (PH) values exhibited significant variations within the harvests among the applications, ranging from 15.60 to 32.89 cm (Table 3). The PH values showed notable fluctuations in response to biochar and chicken manure applications during the vegetation period, and increased in the first harvest and subsequently decreased in the second harvest. Significant differences were found among the application for the PH values in the first harvest. The PH values for the first harvest ranged from 20.56 to 32.89 cm, and A8 and A5 applications had the highest PH values. The lowest PH values were found in A5 and A6 applications in the first harvest. Compared to control (A9) application, the applications of A8, A4 and A3 showed higher values as 23.83%, 12.95% and 2.48%. In the second harvest, statistically significant differences were found among the applications in PH values. The PH values changed between 16.00-28.83 cm, and the maximum and minimum values were noted in A8 and A6 applications, respectively. While A8 application increased the PH value, A7 application decreased it. However, higher PH values were obtained in the combination of A1 with A8 compared to the combination of A1 and A6. It is clearly noted that alone biochar or combination with chicken manure applications revealed positive impact on the PH of basil in the first and second harvest results except A6 application. Compared to means of the first and second harvest, the PH values of showed variation from 18.19 cm to 30.86 cm with mean of 22.19 cm. The highest mean plant height (PH) values were observed in the A8 and A4 applications, while the lowest values were recorded in the A6 and A7 applications. Compared to the A9 application, the A8 application showed a significant increase of 41.69%.

Although the application of A2 (biochar at 80 t ha^{-1}) resulted in a 4.13% increase, A1 (biochar at 40 t ha^{-1}) showed a 15.73% decrease compared to the A9 application. These results were somewhat similar to those of Jabborova et al. [20], who reported that biochar derived from black cherry wood enhanced basil growth, increasing plant height by 48%. It was reported that biochar applications as 2 and 6% showed limited growing in the first fifteen days of vegetation period, and the plant height value was observed with the 6% added biochar application in the sixty days of experiment nearly 14 cm [21]. Another study reported that plant height of basil changed between 16.2-19.2 cm grown under different biochar applications [22]. Teliban et al. [23] reported that plant height values of different basil genotypes ranged from 15.40 cm to 23.10 cm under different growing conditions. Moreover, when comparing the PH obtained in this study with previous studies, the results were found to be similar to the PH values reported by Tas et al. [24] (32.5-44 cm) and Karaca et al. [25] (17.46-45.33 cm). The obtained results were found partly similar with the previous studies except reported by Tas et al. [24] and Danish et al. [21]. The differences can be explained by soil properties, growing conditions, application doses and genetic material.

Table 3 showed that no significant differences were noted among the applications in the first and second harvest for the branch number (BN). The BN values ranged from 5.92 to 12.00 no plant^{-1}. Data regarding BN showed that A6 application (80 t ha^{-1} biochar×20 t ha^{-1} chicken manure) significantly decreased BN by 26.83% over the A9 application in the first harvest. Maximum BN was recorded with A9 application which resulted in 10.19% and 12.46% increase over the A8 and A1 applications, respectively. Furthermore, A1 application significantly increased the BN of basil compared to A2 application. In the second harvest, the BN values changed between 5.92-10.83, and the highest and lowest values were found from A9 and A4 applications, respectively. Compared to A9 application, A2 application showed 14.62% decrease with the second highest BN value. Contrary to the 1st harvest, BN values were found to be lower in the A1 application, compared to the biochar A2 application in the second harvest. Similar findings were found between the 10 t ha^{-1} and 20 t ha^{-1} chicken manure applications for the BN values in the first and second harvests.

Table 3. *Plant height and branch number values of the basil grown under different biochar and chicken manure.*

Applications	**Plant height (cm)**			**Branch number (no plant^{-1})**		
	1. harvest	**2. harvest**	**Mean**	**1. harvest**	**2. harvest**	**Mean**
A1	20.89c	16.75b	18.82c	10.67ns	6.00ns	8.33b
A2	24.11bc	21.25ab	22.68bc	10.22	9.25	9.74ab
A3	27.22abc	20.00ab	23.61abc	10.44	6.00	8.22b
A4	30.00ab	22.75ab	26.38ab	10.33	6.25	8.29b
A5	20.56c	20.58ab	20.57bc	9.44	9.67	9.56ab
A6	20.78c	15.60b	18.19c	8.78	5.92	7.35b
A7	21.22c	16.00b	18.61c	10.44	8.33	9.39ab
A8	32.89a	28.83a	30.86a	10.89	7.00	8.94ab
A9	26.56abc	17.00b	21.78bc	12.00	10.83	11.42a
Mean	24.91	19.86	22.39	10.36	7.69	9.03
LSD (5%)	6.88	10.67	7.32	3.94	5.22	2.81

[1]There is no statistical difference between the means shown with similar letters in the same column. LSD: Least Significant Difference at the 0.05 level. ns: Not significant

The BN values obtained in this study were similar to those reported by Yaldız et al. [6], who found that the BN of basil ranged from 5.93 to 9.67 number/plant under poultry manure applications, as well as to those reported by Yaldız and Çamlıca [26], who observed BN values ranging from 5.93 to 9.08 no plant^{-1} in different basil genotypes of varying origins. Also, the obtained BN values were found lower than findings of Qazizadah et al. [27], who reported that BN values changed between 13.83-16.70 in per plant grown under chitosan application levels at different maturity stages. It is clear that basil plants exhibited similar or different BN values based on growing conditions, genotypes, or other factors compared to previous studies. Therefore, the differences in BN values observed in the study by Qazizadah et al. [27] may be explained by variations in growing conditions.

All biochar applications significantly decreased the fresh and dry weight values of basil compared to the control (A9) application, with the A3 having a more pronounced effect (Table 4). The fresh weight (FW) values changed between 0.96-5.54, 0.60-2.30 and 2.24-6.14 g plant^{-1} in the first, second and total harvest of basil, respectively. Application of A8 and A9 showed the highest FW values, while A6 and A4 applications had the minimum FW values in the first harvest. In the second harvest, FW values ranged from 0.60 to 2.30 g plant^{-1}, and A3 application had the maximum value, followed by A6 and A9 applications with 1.28 g plant^{-1}. A8 and A7 applications showed the lowest FW values among the chicken manure and biochar applications. Significant differences were found among the applications according to total FW values of basil ($p<0.05$). The total FW values changed between 2.24-6.14 g plant^{-1} depending on the different chicken manure and biochar fertilizer doses. A8 and A6 applications had the highest and lowest total FW values, respectively. When the total FW values obtained from chicken manure and biochar applications were compared to the A9 application, the total FW value for the A8 application was 24.30% higher, whereas the total FW value for the A6 application was 55.00% lower. Specifically, A8 and A3 applications increased the FW values in the first and the second harvests, while the application of A8 increased the FW values in total FW compared to A9 application.

Chicken manure and biochar applications had statistically positive impacts on the dry herb weight (DW) values of basil ($p<0.05$) in all harvests (Table 4). The DW values ranged from 0.15 to 0.57 g plant^{-1} in the first harvest. The maximum DW weight of 0.57 g was recorded in A8 application which was 0.15 g more than the second highest DW in application of A3 ($p<0.05$) and 0.17 g higher than the control (A9)- a weight increase of more than 42%. In the second harvest, different observations were found for the DW of basil grown under chicken manure and biochar applications. The maximum value for DW was found from A6 application, and A7 application had the next highest increase, but other applications except A8 were found statistically similar to A7 application. The lowest DW values were noted in A8 application with 0.06 g plant^{-1}, although it had maximum value in the first harvest. In the total DW values, statistically significant differences were found among the applications. The values ranged from 0.30 to 0.72 g plant^{-1}, and the highest values showed difference in the applications according to the first and second harvests. A3 application had the highest DW value, and compared to A9 application, it was higher with the increasing 14.29%.

Table 4. *Impact of applications on the yield of basil.*

Applications	Fresh weight (g plant^{-1})			Dry weight (g plant^{-1})		
	1. harvest	2. harvest	Total	1. harvest	2. harvest	Total
A1	1.80cde	0.81bc	2.61c	0.19cde	0.11ab	0.30c
A2	1.62cde	1.13bc	2.75c	0.20cde	0.22ab	0.42abc
A3	2.49bc	2.30a	4.79ab	0.42b	0.30ab	0.72a
A4	1.32de	0.96bc	2.28c	0.33bc	0.21ab	0.39bc
A5	2.56bc	0.88bc	3.44bc	0.15e	0.21ab	0.54abc
A6	0.96e	1.28b	2.24c	0.18de	0.37a	0.51abc
A7	2.42cd	0.78bc	3.20c	0.31bcd	0.24ab	0.55abc
A8	5.54a	0.60c	6.14a	0.57a	0.06b	0.63ab
A9	3.66b	1.28b	4.94a	0.40b	0.23ab	0.63ab
Mean	2.49	1.11	3.60	0.31	0.22	0.52
LSD (5%)	1.17	0.60	1.44	0.15	0.26	0.33

[1]There is no statistical difference between the means shown with similar letters in the same column. LSD: Least Significant Difference at the 0.05 level.

The positive impact of biochar on plant growth is generally attributed to its improvement of soil physical, chemical, and biological properties, including nutrient availability and water retention [28]. When the obtained results were compared with previous studies, FW values were found to be lower than the values reported by Chang et al. [29] (29.2-35.3 g), while FW values showed partly similarities with the values reported by Jadczak et al. [30] (4.19-28.35 g). In another study, Abdipour et al. [31] reported that cow manure biochar (CBM) was applied to basil at levels of 0%, 1%, 2%, and 3%. The results showed that a 3% CBM application significantly increased both the fresh (about 0.5-4 g plant^{-1}) and dry herb (about 0.2-0.9 g plant^{-1}) values of basil. These differences can be explained by genotype differences, cultivation conditions, and the effects of genotype characteristics.

A study by Amin et al. [32] found that basil plants grown in soil amended with chicken manure exhibited higher dry weight compared to control plants. This was attributed to the higher nutrient availability in the soil, especially nitrogen, which promotes leaf and stem growth, and phosphorus, which is critical for root development. Gomez et al. [33] reported that plants grown with chicken manure had well-developed root systems, which in turn contributed to increased dry weight due to improved water and nutrient absorption.

In a climate chamber experiment by Zhang et al. [34], basil plants grown in soil amended with biochar at a rate of 5% showed a significant increase in dry weight compared to those grown without biochar, suggesting that biochar enhances growth under controlled conditions. Similarly, a study by Cheng and Lehmann [8] indicated that moderate biochar application improved basil dry weight, with better results observed when the biochar was combined with organic fertilizers, further enhancing soil fertility and promoting plant growth. In a study by Atkinson et al. [35], it was found that biochar application led to improved soil pH and nutrient availability, contributing to the increased dry weight of basil plants. Moreover, the slow-release nature of nutrients from biochar could sustain plant growth over time, enhancing biomass production.

There have also been studies reporting that biochar applications promoted plant growth [36] depending on the raw material used in production, production temperature, and the characteristics of the soil-applied, as well as studies reporting that it was ineffective [37] and even had a negative effect [38]. The obtained DW values results were lower than the values reported by Chang et al. [29] (3.4-5.4 g). These differences can be explained by genotype differences and cultivation conditions.

In the present study, 9 different applications on basil were analyzed for the crude protein content (PC) values grown under chicken manure and biochar applications, and showed significant differences at $p<0.05$ level (Table 5). In the first harvest, the crude PC values ranged from 6.91 to 20.11%. The highest PC was found from the A7 application and followed by A9 and A5 applications. The lowest crude PC was noted in A6 and A2 applications. The control application (A9) had the higher values with the biochar and biochar×chicken manure applications with the 20 t ha^{-1} chicken manure application (A8) in the first harvest. Significant differences were found among the applications based on the crude PCs of basil in the second harvest. The PCs values ranged from 10.80% to 20.28%, and the highest and lowest values were obtained from applications of A1 and A9, respectively (Table 5). Alone biochar and biochar×chicken manure applications increased the PCs of basil, except A5 application. The crude PC values according to mean of the harvests changed between 10.83-18.69% among the applications. A7 application had the highest crude PC content and followed by A1 (16.73%) and A5 (16.67%) applications. A4 application had the minimum crude PC values and followed by A2 (11.38%) and A6 (12.33%) among the applications (Table 4). It is clearly noted that increasing chicken manure and biochar applications decreased the PCs of basil in the first and second harvest. Compared to A9 application, A7, A1 and A5 applications increased the crude PC of basil as 26.54, 13.27 and 12.86%, respectively.

Table 5. *Crude protein content values of basil grown under chicken manure and biochar applications in different harvests.*

Applications	Protein content (%)		
	1. harvest	**2. harvest**	**Mean**
A1	13.18d	20.28a	16.73b
A2	8.07g	14.69d	11.38ef
A3	11.08e	17.65b	14.37c
A4	9.12f	12.54e	10.83f

Table 5 (cont). *Crude protein content values of basil grown under chicken manure and biochar applications in different harvests.*

A5	17.44c	15.90cd	16.67b
A6	6.91h	17.75b	12.33de
A7	20.11a	17.27bc	18.69a
A8	13.1d	12.64e	12.87d
A9	18.74b	10.80f	14.77c
Mean	13.08	15.5	14.29
LSD (5%)	0.43	1.65	0.97

[1]There is no statistical difference between the means shown with similar letters in the same column. LSD: Least Significant Difference at the 0.05 level.

The previous studies revealed that genetic variability, growing conditions and chemical or organic applications caused the chemical properties of basil. Siti Mahirah et al. [39] and Yilmaz and Alibaş [40] reported that protein contents of basil in different drying methods ranged from 3.22 to 18.72% and from 19.21 to 31.50%. In another study, Nurzyńska-Wierdak et al. [41] noted that protein contents of herbs in different basil cultivars changed between 8.20-20.00%. The obtained protein contents (6.91-20.28%) from this study were found partly similar with the previous studies.

Antioxidant activities of different chicken manure and biochar applications on basil were evaluated as DPPH and FRAP in the first and second harvests (Table 6). In the first harvest, the first antioxidant activity was DPPH and its values showed statistically differences in the first harvest and the values changed between 20.98-98.44%. The highest DPPH value was observed from application of A3 and followed by A2 (92.66%) and A1 (73.68%). The lowest DPPH values were found from A4 and A8 applications. Compared to A9 condition, while A3 and A2 applications increased the DPPH values with 80.59% and 69.99%, A4 and A8 applications decreased the DPPH values by 61.51% and 49.77% in the first harvest. In the second harvest, DPH values showed high variability and changed between 26.73-69.37%. A2 application had the maximum DPPH values and A4 application had the minimum DPPH value. As in the 1st harvest, it was determined that A2 had the highest value, while A4 application had the lowest value. The results showed that alone biochar and biochar×10 t ha^{-1} chicken manure applications had positive impact on DPPH values of basil in the first harvest (Table 6). The highest biochar and chicken manure applications had the positive effect on the DPPH values of the basil in the second harvest. However, interaction of the highest biochar and chicken manure application revealed the lowest DPPH value in both harvests. The mean DPPH values of the basil grown under different chicken manure and biochar applications ranged from 23.85 to 81.02% with an average of 54.88%. The highest and lowest DPPH values according to mean values of the first and second harvest were found from A2 and A4 applications.

The second antioxidant activity was FRAP and it showed wide variations in the first and second harvests. In the first harvest, the FRAP values changed between 46.88-106.12 mg TE g^{-1} (Table 6). The highest FRAP value was found from A5 application with 106.12 mg TE g^{-1}, and followed by A6 application with 104.99 mg TE g^{-1} and A2 application with 74.32 mg TE g^{-1}. The lowest FRAP values were seen in A3 and A7 applications. In the second harvest, the FRAP values of the basil grown under different chicken manure and biochar applications ranged from 24.84 to 115.95 mg TE g^{-1}, and the highest and lowest FRAP values were obtained from A9 and A1 applications, respectively. Although the highest dose of biochar application proved effective, the highest FRAP value for basil in the second harvest was observed in the control (A9) treatment. This value was 27.00% higher than that of the next highest application. The FRAP values, based on the means of both harvests, showed statistically significant differences, ranging from 37.13 mg TE g^{-1} to 92.35 mg TE g^{-1}. Similar with the second harvest, A9 application had the highest FRAP value, while A7 application had the lowest value.

Table 6. *Antioxidant activities of the basil grown under chicken manure and biochar applications.*

Applications	DPPH (%)			FRAP (mg TE g^{-1})		
	1. harvest	2. harvest	Mean	1. harvest	2. harvest	Mean
A1	73.68b	52.97bc	63.32b	62.88e	24.84h	43.86e
A2	92.66a	69.37a	81.02a	74.32c	91.15b	82.74c
A3	98.44a	46.68c	72.56a	42.49h	33.49e	37.99f
A4	20.98d	26.73d	23.85f	94.66b	30.12f	62.39d
A5	31.33d	52.25bc	41.79e	106.12a	67.33d	86.72b
A6	64.65bc	51.16c	57.91bc	104.99a	71.78c	88.39b
A7	64.31bc	45.61c	54.96bcd	46.88g	27.39g	37.13f
A8	27.38d	65.46ab	46.42de	57.22f	30.40f	43.81e
A9	54.51c	49.62c	52.06cd	68.74d	115.95a	92.35a
Mean	58.66	51.09	54.88	73.14	54.77	63.93
LSD (%)	12.09	14.14	9.13	3.85	1.91	2.11

[1]There is no statistical difference between the means shown with similar letters in the same column. LSD: Least Significant Difference at the 0.05 level.

Antioxidants help reduce the risk of chronic diseases, including cancer and heart disease. The primary sources of naturally occurring antioxidants are whole fruits, grains, and vegetables [42]. It has been reported that there is a relationship between the content of total phenolic compounds and their antioxidant capacity [43,44]. Previous studies reported that DPPH values of basil changed depending on the applications, genetic variation, growing conditions and environmental differences. Nadeem et al. [45] reported that DPPH value of basil leaves cultivated in Pakistan was found between 32.4-82.4%. Yaldiz and Camlica [42] was found the DPPH values of different origin basil genotypes between 8.14-54.69%. In a separate study, Ma and Le [46] reported that the antioxidant capacity of Thai basil leaves was measured at 39.06%. Uyoh et al. [47] reported that the DPPH value for *Ocimum basilicum* leaf extract was 92.37%, and that the DPPH values of various concentrations of Ocimum extracts and reference compounds ranged from 83.53% to 87.59%. The obtained DPPH values from this study were in accordance with the previous studies.

Total phenolic contents of basil showed statistically significant differences among the chicken manure and biochar applications for basil in both harvests (Table 7). The TPC values ranged from 14.66 to 104.93 mg GAE g^{-1}, and the highest TPC values were found in A9 and A4 applications. Applications of A2 and A8 had the minimum TPC with 14.66 and 56.88 mg GAE g^{-1}. While the TPC values of alone biochar applications were found closest, alone chicken manure applications showed high differences. In the second harvest, TPC values changed between 6.10-104.75 mg GAE g^{-1}, and A6 and A5 applications had the highest and lowest values, respectively. It was seen that the highest biochar and the lowest chicken manure applications had positive effect on TPC values of basil in the second harvest. However, combination of biochar and chicken manure applications showed opposite impact on the TPC values compared to pure applications. The mean TPC values of the harvests ranged from 16.38 to 86.10 mg GAE g^{-1}, and A6 and A7 applications had the highest values. A8 and A5 applications had the minimum TPC values in basil. The A9 application decreased the TPC values by 33.14%, and 13.15% compared to A6 and A7 applications, respectively.

Chicken manure and biochar applications had significant impacts on the total flavonoid contents (TFC) of basil in both harvest. In the first harvest, the highest TFC values were observed in A6 and A1 applications, while the lowest TFC values were found in A8 and A9 applications (Table 7). Pure applications of biochar showed differences as well as chicken manure applications, and the highest TFC values were found in the lower applications in the first harvest. The greatest reduction in TFC occurred in the A8 application, compared to A9 application. In the second harvest, the TFC values ranged from 1.06 to 22.39 mg QE g^{-1}, and the highest and lowest values were found from the applications of A4 and

A3, respectively. A9 application exhibited the higher rises in TFC values compared to A4 (63.15%) and A5 (31.48%) applications in the second harvest. In contrast, the highest TFC values were found in the higher applications in the second harvest. The mean TFC values according to harvest results showed statistically significant differences among the applications. The highest and lowest TFC values were found in A8 and A4 applications according to mean values of the both harvests, respectively.

Table 7. *Total phenolic and flavonoid contents of basil grown under chicken manure and biochar applications.*

Applications	**Total phenolic (mg GAE g^{-1})**			**Total flavonoid (mg QE g^{-1})**		
	1. harvest	**2. harvest**	**Mean**	**1. harvest**	**2. harvest**	**Mean**
A1	57.66d	19.66d	38.66ef	39.36b	3.36de	21.36d
A2	56.84d	56.69b	56.76c	15.09de	9.46c	12.28e
A3	60.32d	30.36c	45.34de	27.42c	1.06f	14.24e
A4	77.48b	18.03d	47.75d	49.35a	22.39a	35.87a
A5	69.13c	6.10e	37.61f	37.09b	12.04b	24.56c
A6	67.46c	104.75a	86.10a	51.12a	4.74d	27.93b
A7	66.52c	66.06b	66.29b	12.41ef	2.14ef	7.27f
A8	14.66e	18.09d	16.38g	9.61f	2.47ef	6.04f
A9	104.93a	10.22de	57.57c	18.03d	8.25c	13.14e
Mean	63.87	36.66	50.27	28.83	7.32	18.08
LSD (%)	5.25	10.09	6.69	4.15	2.18	2.60

[1]There is no statistical difference between the means shown with similar letters in the same column. LSD: Least Significant Difference at the 0.05 level.

Phenolic compounds with bioactive properties are known to contribute to the nutritional value of plants and play a crucial role in their environmental adaptation and stress resistance. Additionally, the cultivation system has been reported to influence the phenolic content of plants, along with various other factors [48]. It has been suggested that biochar amendment, as an alternative cultivation system, could alter the phenolic compound content of plants by modifying nutrient availability in the growing medium [49]. For example, Jabborova et al. [20] found that biochar produced from cherry tree wood increased basil's total flavonoid content and antioxidant activity. These differing findings highlight that effect of biochar on phenolic content may depend on its source material. Total phenolic and flavonoid contents of basil showed differences based on the genetic variability, growing conditions, extraction methods, environmental factors. A study conducted by Uyoh et al. [47] reported that total phenolic content of *Ocimum basilicum* grown in Nigeria extracts was found as 27.41 mg GAE g^{-1} DW and the total flavonoid content was noted as 22.88 μg RE mg^{-1}. In another study conducted by Elmas et al. [50] reported that total phenolic and flavonoid contents of basil grown under different bio and chemical fertilizer ranged from 14.99 to 32.90 mg GAE g^{-1} and from 4.44 to 26.29 mg QE g^{-1}, respectively. Yaldiz et al. [1] reported that total phenolic and total flavonoid contents were noted between 11.95-47.38 mg GAE g^{-1} and between 4.29-18.08 mg QE g^{-1}, respectively. The total phenolic and flavonoid contents obtained in this study were consistent with those reported in previous studies.

B. PRINCIPAL COORDINATE ANALYSIS (PCA)

The first eight factors in the scree plot, derived from the original data, accounted for 100% of the total variation (Figure 1a, b). Principal component analysis (PCA) revealed that the first six principal components (PC1 to PC6) had eigenvalues greater than 1.0 and collectively explained 94.58% of the total variation. Specifically, PC1 accounted for 32.43%, and PC2 explained 19.80% of the total variation (Figure 1a, b).

The high variability observed across all four PC axes indicates considerable diversity among the twelve traits. Within the first principal component (PC1), traits with larger absolute values, closer to unity, had a stronger influence on clustering compared to traits with values closer to zero. The major contributing traits to PC1 included the presence of FW-1, DW-1, FW-M, PH-M and PH-1 properties. The positive and negative correlations between the components and variables are indicated by the corresponding positive and negative loadings.

For the second principal component (PC2), the primary contributors were TFC-2, DPPH-M and DPPH-1. In PC3, BN-2 emerged as the principal contributor to variation, while the PC-M was the key factor for PC4. Traits with higher coefficients in PC1 and PC2 were of particular significance, as these two components together accounted for 52.23% of the overall variation. Positive and significant correlations were found among the eight traits such as FW-1, FW-M, DW-1, DW-M, BN-1, BN-2, BN-M, PC-1. Plant height traits as PH-1, PH-2 and PH-M were found at the same axis, and the significant correlations were observed among them (Figure 1). FRAP-M was correlated with TFC-1, TFC-2, TFC-M and FRAP-1, and rest of traits were correlated with each other. A8 application had positive effect on the PH-1, PH-2 and PH-M, A4 application showed significant effect on TFC in PCA.

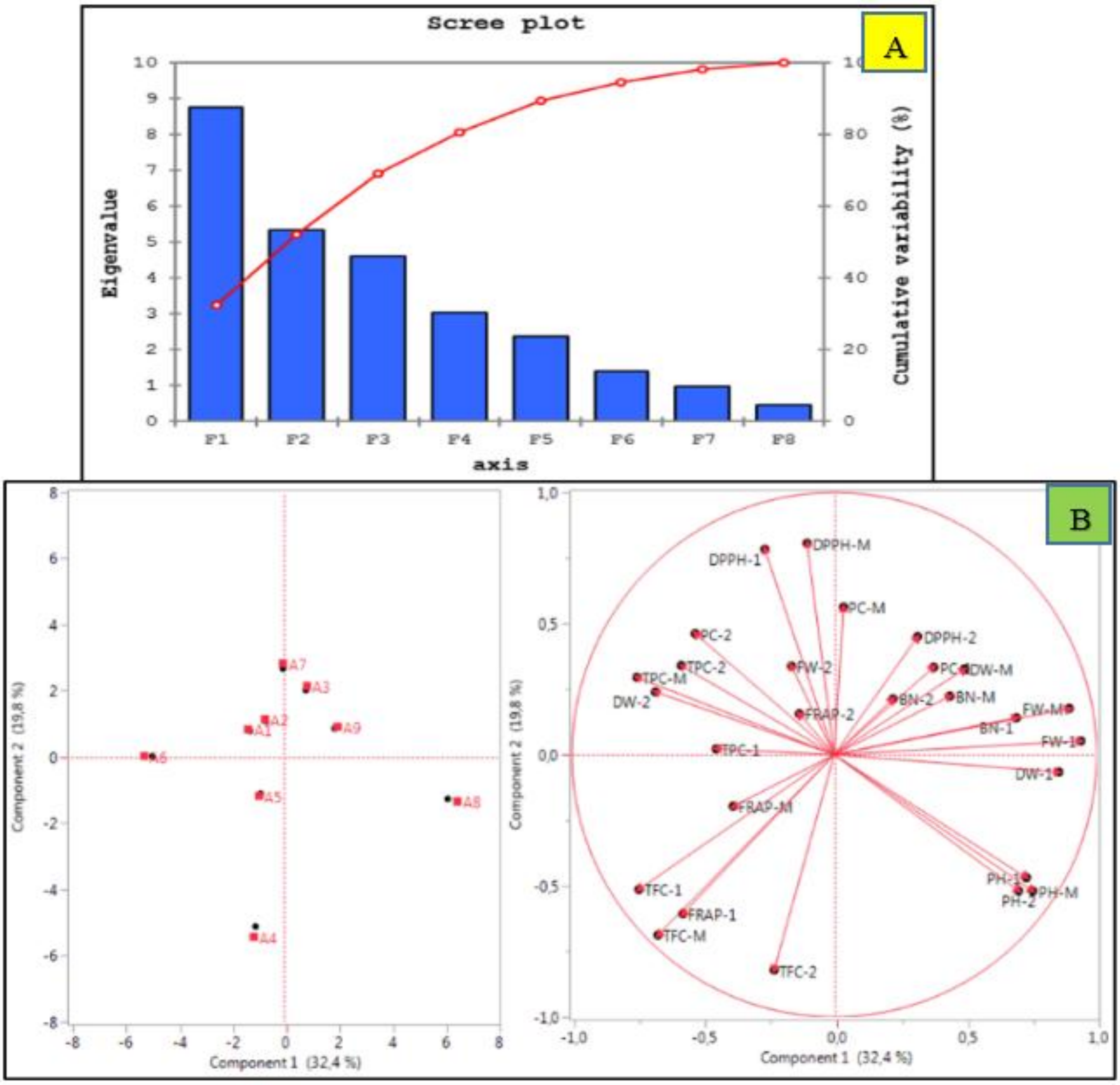

Figure 1. *Scree plot (A) and PCA (B) of the study depending on the examined properties. PH: Plant height, BN: Branch number, FW: Fresh weight, DW: Dry weight, PC: Protein content, DPPH: 1,1-diphenyl-2-picrylhydrazyl, FRAP: Ferric reducing antioxidant power, TPC: Total phenolic content, TFC: Total flavonoid content.*

The PCA results obtained from this study was found similar with Amato et al. [51], who reported that the variables contributed most to PC1 were those related to the morphology and yield of oregano, including stem height, inflorescence height, total fresh yield, and the dry yield of inflorescences and leaves.

C. HEAT MAP ANALYSIS

The heat map analysis (Figure 2) revealed two main clusters: one corresponding to the A3 and A8 applications and the other to all the applications. A3 and A4 applications (main group A) divided into two subgroups as A1 and A2, and separated with the values of plant height with dry and fresh weight except DW-2.

The main group B divided into two subgroups as B1 and B2, and B1 group separated from other group depending on the DW-2, TPC-M and TPC-2. Notably, the plant height values (PH-1, PH-2 and PH-M) and branch number values (BN-1, BN-2 and BN-M) clustered in the same group, while the other properties showed differences. Applications of A6, A4 and A9 exhibited the highest values of TPC-2, TFC-2 and BN-M in the main group B, respectively. In particular, the A9 application clustered separately because of its lower values of BN-1, BN-2, BN-M, FRAP-2, FRAP-M and TPC-1 traits. The applications A3, A4 and A6 showed the highest DPPH-1, TFC-2 and TPC-2 values, respectively. It was clearly noted that the traits about plant heights (PH-1, PH-2 and PH-M) were separated from other traits depending on the A8 application (20 t ha^{-1} chicken manure). As a result, the heat map clearly highlighted which application contributes to the increase in each trait's value.

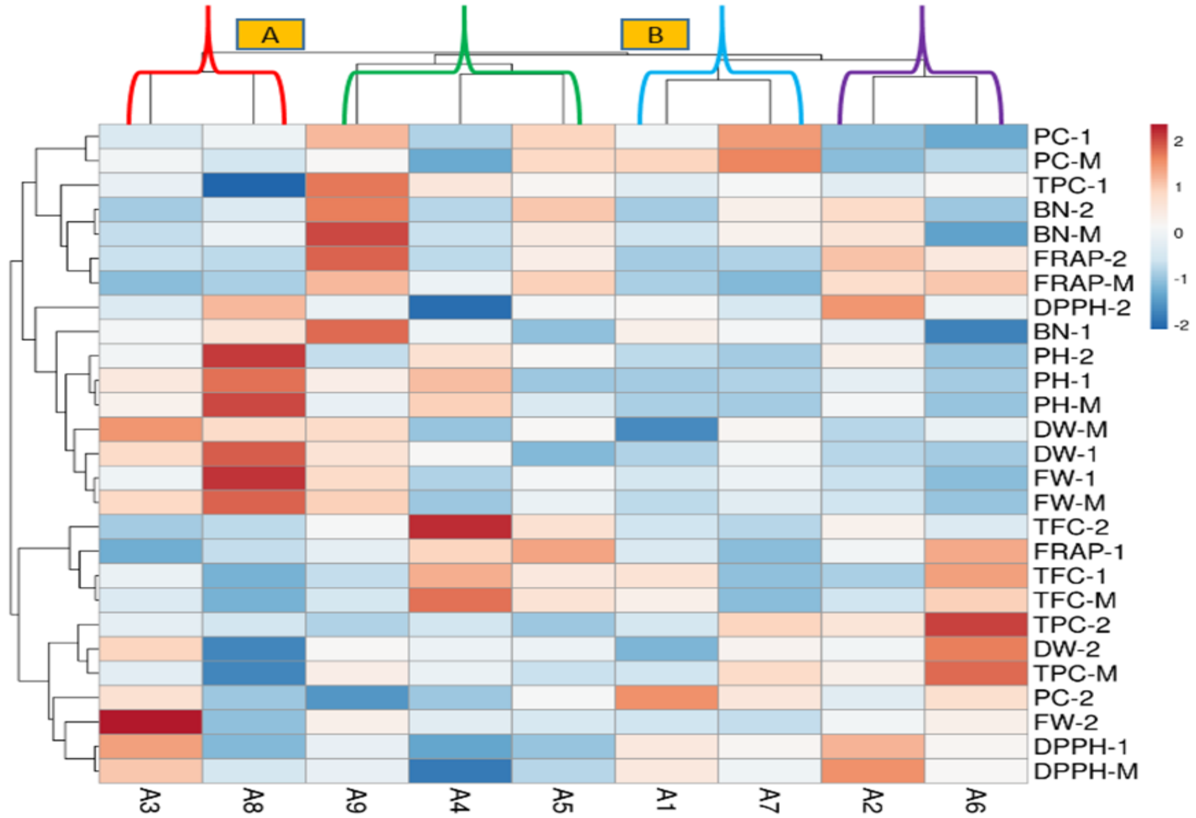

Figure 2. *Heat map analysis summarizing oregano responses to chicken manure and biochar applications (from A1 to A9). PH: Plant height, BN: Branch number, FW: Fresh weight, DW: Dry weight, PC: Protein content, DPPH: 1,1-diphenyl-2-picrylhydrazyl, FRAP: Ferric reducing antioxidant power, TPC: Total phenolic content, TFC: Total flavonoid content*

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The heat map analysis results showed similarity with the previous study reported by Amato et al. [51].

IV. CONCLUSION

Biochar and chicken manure applications had a significantly positive impact on plant height, branch number, fresh and dry weights, as well as on antioxidant activity and protein content of basil grown in a climate room conditions. Chicken manure and biochar application promoted the development of morphological, yield, and antioxidant activity properties and thus production availability for basil. Furthermore, increasing biochar applications caused an increase of DPPH content, while the increasing chicken manure application enhanced the plant height and total fresh weight. The PCA and heat map analysis showed important results according to applications.

Chicken manure and biochar applications increased basil production and some quality properties with a significant increase. These results showed that the impacts of used materials as growth-promotings are related to the increase in nutrient intake besides its other properties, such as developing the soil physical properties and increasing the water holding capacity according to results of the present study, both chicken manure and biochar applications can be used to improve the productivity of basil.

In conclusion, the positive effects are attributed to the ability of chicken manure and biochar to improve soil structure, support microbial activity, and enhance nutrient availability. These benefits make chicken manure and biochar valuable amendments for sustainable agriculture.

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