Effect of three different organic fertilizers on growth, yield, and essential oil content of basil (*Ocimum basilicum* var. *pilosum*)

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ABSTRACT

Basil (*Ocimum basilicum* var. *pilosum*) is an herbaceous plant species exhibiting various economic and medicinal values. This study aimed to determine the effect of different organic fertilizers within creasing application rates on the plant growth, fresh yield, and essential oil content of basil grown in Thu Duc city. The treatments were a factorial combination of three kinds of organic fertilizers (cow manure fertilizer (CMF)–control, worm castings manure fertilizer (WCF) and Komix organic fertilizer (KOF)) and five levels of application (5-control, 10, 15, 20 and 25 tons/ha). The field experiment was arranged in split-plot design with three replications. Generally, when applying WCF and KOF with the amount of 5, 10, 15, 20, and 25 tons/ha, the indexes resulted in higher plant height, stem diameter, number of primary branches, number of leaves and chlorophyll index CMF. Among the three types of fertilizers, WCF gave higher results than KOF and CMF. Specifically, the tree height reached 37.9 cm, the base diameter was 5.5 mm, and the number of leaves was 13.3 branches/plant. There was no significant difference in plant growth when increasing the fertilization rate from 15 to 25 tons/ha. The results showed that the application of 25 tons/ha of WCF gave to best results of fresh weight (93.3 g/plant), theoretical yield (24.9 tons/ha), actual yield (14.7 tons/ha), essential oil content (0.47 mL)/100 g), and yield of essential oil (117 L/ha). The economic efficiency analysis showed that the highest total profit after three harvests (VND 737,570,000 per ha) was obtained at the treatment of 25 tons/ha WCF, while that of 5 tons/ha achieved the highest real benefit-cost ratio (3.26).

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1. Introduction

Basil (*Ocimum basilicum* var. *pilosum*) is an herbaceous species belonging to the group of basil. Basil is a typical spice of the South-Central provinces and the Central Highlands, especially famous in Phu Yen province. Basil contains essential oils and oleoresin that are used as a flavouring agent in food, in cosmetics and ingredients in traditional medicine (Tambun et al., 2017).

Organic fertilizer contains both macro and micronutrients, which helps to reduce dependence on chemical fertilizers. Bufalo et al. (2015) indicated that applying the appropriate amount of organic fertilizer for basil created favorable conditions for photosynthesis in plants and the accumulation of nutrients. Therefore, increasing plant yield and essential oil content. In Vietnam, the organic fertilizer market is diverse with multiple different types, such as cow dung manures, green manures, microbial fertilizers, and mineral organic fertilizer, however, the utilization of them on basil is not well understood in the study area. Therefore, the objectives of this study were to determine the appropriate types and application levels of CMF, WCF, and KOF for maximizing the yield and essential oil content of basil grown on loamy sand soil in Thu Duc City.

2. Materials and Methods

2.1. Experimental design

The experiment was carried out at the Experimental field of the Faculty of Agronomy, Nong Lam University, Ho Chi Minh City from September 2020 to January 2021. The two-factor experiment was laid out in a split-plot design with three replicates. The main plot (H) consisted of three kinds of organic fertilizers (CMF-control, WCF, and KOF). The sub-plot (L) included five levels of application rates (5-control, 10, 15, 20, and 25 tons/ha). The basil was planted at a density of 266,667 plants/ha. The spacing between blocks, plots, rows and plants were 1.0, 0.5, 0.25, and 0.15 m, respectively. The plot area was 6 m^2 (6.0 m $\times$ 1.0 m) and total experimental area was 270 m^2. All treatments were fertilized with the same dose of 500 kg lime, 135 kg N, 75 kg P_2O_5, and 120 kg K_2O per hectare (ha).

The physicochemical properties of soil and organic fertilizers are presented in Table 1. Soil sample in the experiment tested in Southern Forestry Science Institute. The data showed that the soil is clay loam, moderately acidic, low in soil organic matter, total N but high in total P_2O_5 and K_2O. The organic fertilizers used in the experiment had organic matter of 22.0 - 88.68%. Total N, P_2O_5 and K_2O of organic fertilizers ranged from 1.06 - 2.50%, 0.07 - 4.0% and 1.31 - 6.0%, respectively (Table 1).

Table 1. Selected physicochemical properties of soil and organic fertilizer used in the experiment

Properties	Soil[1]	CMF[2]	WCF[3]	KOF[4]
Texture				
Sand (%)	45			
Silt (%)	28			
Clay (%)	28			
pH_{H_2O}	5.58			
MC (%)		22.19	15.0	25.0
OM (%)	1.28	88.68	22.0	22.0
Total N (%)	0.06	1.06	1.57	2.50
Total P_2O_5 (%)	0.15	0.07	1.24	4.0
Total K_2O (%)	2.5	1.31	0.67	6.0

CMF = cow manure, WCF = worm castings fertilizer, KOF = Komix organic fertilizer, OM=organic matter, MC = moisture content.
[1]Forest Science Institute of South Vietnam, 2021; [2]Research Institute for Biotechnology and Environment. [3]SFARM, 2020; [4]TSJSC, 2012.

2.2. Data collection and statistics

Growth parameters, yield components, yield and essential oil content were collected randomly from ten plants in the middle three rows of each base plot. In detail, the plant height, the number of leaves, stem diameter, the number of primary branches before harvest, the number of leaves on the main stem, and the number of shoots was calculated 20 days after planting (DAP). The average fresh weight of stems and leaves (g/plant) was calculated in 90 DAP: Measured the average weight fresh of stems and leaves of 10 target plants, cut with a sickle at 3 cm from the ground (P_1). Theoretical fresh yield (ton/ha) = P_1 (g/plant) x 10^{-6} $\times$ Planting density (plant/ha). Actual harvested yield (ton/ha) = [Weight of fresh stems and leaves of each base plot (kg) x 10^{-3} $\times$ 10,000]/Area of the base plot (m^2).

For the analysis of the essential oil content of the basil, 100 g/pot of branches and leaves of 10 target plants, collected at the time point when the plants on the experimental plot had buds and begun to bloom, were used. The samples after being harvested were kept for 2 - 3 hours at room temperature, then the essential oil was distilled by steam distillation (Khang & Khien, 2001). Essential oil content was calculated by the percentage of essential oil obtained from the mass of the starting material after being extracted by steam distillation in 90 DAP. The calculation was based on the formula: Content of essential oil in leaves, stems (%) = (Volume of essential oil)/(Weight of sample) × 100. Theoretical yield of essential oil (L/ha): Calculated as essential oil content of leaves and stems (mL/100 g) × Theoretical fresh yield (ton/ha) × 10.

For the analysis of the economic efficiency, total cost (VND/ha/three harvests) = seeds + fertilizers + pesticides + labor + electricity, water; Total revenue (VND/ha/three harvests) = Actual yield (kg/ha) x selling price (VND/kg); Total profit (VND/ha/three harvests) = Total revenue - Total cost, and benefit - cost ratio = Total profit/Total cost, were measured.

Data were subjected to analysis of variance (ANOVA) using R software. Treatments means were separated using, Duncan's rank test at the 5% level of significance.

3. Results and Discussion

3.1. Effect of types and amounts of organic fertilizers on growth of basil (*Ocimum basilicum* var. *pilosum*)

As shown in Table 2, among three kinds of fertilizer, the treatment using worm castings fertilizer (WCF) produced the highest (39.7 cm) while plant height was highest when basil was fertilized with fertilizer 25 tons/ha (38.9 cm) in five levels of organic fertilizer. The height of basil plants was affected by the interaction between the type and amount of organic fertilizer, ranging from 28.8 to 41.8 cm. The highest plants were seen in the treatment using 25 tons/ha (41.8 cm) of WCF and there was a very significantly different ($P \leq 0.01$) from the plants in the treatment using 5 tons/ha of CMF (28.8 cm).

The basil with the largest stem diameter was found in the treatment using WCF (5.5 mm) and it was statistically significantly different from the other two treatments at Table 2. The use of fertilizer at a rate of 25 tons/ha created the largest stem diameter (5.5 mm) but it was not statistically different from the treatment using 20 tons/ha (5.4 mm). In addition to, the basil plants had a stem diameter ranging from 4.8 - 6.0 mm depending on different types and amounts of organic fertilizers. The basils in the treatment using WCF at a rate of 25 tons/ha had the largest stem diameter (6.0 mm) and there was very significantly different ($P \leq 0.01$) from the basils fertilized with CMF at a rate of 5 tons/ha (4.8 mm).

The study also revealed that the application of different amounts of organic fertilizers generated differences in the number of primary branches on basil. The highest number of primary branches (13.4 branches) were observed on the plants that applied 25 tons/ha of organic fertilizers and gradually reduced when applied the organic fertilizers with the lower rates (Table 2). The number of primary branches in the experiment were lower than that in the experiment of Topalov et al. (1966), which ranged from 24.6 to 25.6 branches/plant. Dinh Hai An noted that at the time of 60 DAP, the number of primary branches fluctuated from 20 to 30 branches/plant when studying the effects of manure and foliar fertilizers on the growth, development and essential oil content of the plant.

The data in Table 2 showed that the basil plants in the treatments using WCF at a rate of 25 tons/ha and 20 tons/ha showed the highest number of leaves (16.8 leaves) in Table 2 and there was significantly different from those observed in the treatment using 5 tons/ha and 10 tons/ha of CMF and KOF (14.8 and 15.0 leaves).

Statistical analysis in Table 2 showed significant differences in the number of shoots, ranging from 14.5 - 20.2 shoots. Basils in the treatment were applied WCF with the amount of 25 tons/ha had the highest number of post-regenerated shoots (20.2 shoots), whereas the basils fertilized with 5 tons/ha of KOF produced the lowest number of shoots (14.5 shoots).

3.2. Effect of types and amounts of organic fertilizers on fresh yield and essential oil yield of basil (*Ocimum basilicum* var. *pilosum*)

In Table 3, the treatment with the highest weight of fresh stems and leaves using earthworm organic fertilizer (80.2 g/plant) was statistically significantly different from the treatment using Komix fertilizer (69.1 g/plant) and cow manure fertilizer (66.5 g/plant). Using 5 tons/ha for the lowest weight of fresh stems, branches, and leaves (62.0 g/plant). Using WCF at 25 tons/ha continued to show the highest effects on the average fresh weight of stems and leaves of basil (93.3 g/plant). Whereas, the lowest average fresh weight of stems and leaves was observed on basils in the treatment with 5 tons/ha of CMF (55.0 g/plant).

Table 2. Effect of sources and application levels of organic fertilizers on plant growth of basil (*Ocimum basilicum* var. *pilosum*)

Parameters (20 DAP)	Organic fertilizers (H)	Application rates (L) (ton/ha)					
		5 (control)	10	15	20	25	Average H
Plant height (cm)	CMF (control)	28.8^{c}	29.7^{bc}	33.5^{abc}	34.2^{abc}	35.9^{abc}	32.4^{b}
	WCF	34.9^{abc}	35.9^{abc}	39^{a}	39.1^{a}	41.8^{a}	37.9^{a}
	KOF	36.3^{abc}	37.3^{ab}	38.4^{ab}	37.3^{ab}	39.0^{a}	37.7^{a}
	Average L	33.3^{c}	34.3^{bc}	36.6^{ab}	36.9^{a}	38.9^{a}	
	CV = 12.8%; FH = 3.5*; FL = 1.7*; FHL = 1.9**						
Stem diameter (mm)	CMF (control)	4.8^{c}	4.9^{bc}	5.1^{bc}	5.1^{bc}	5.1^{bc}	5.0^{b}
	WCF	5.2^{abc}	5.4^{abc}	5.3^{abc}	5.8^{ab}	6.0^{a}	5.5^{a}
	KOF	4.8^{c}	5.1^{bc}	5.2^{abc}	5.4^{abc}	5.5^{abc}	5.2^{b}
	Average L	4.9^{c}	5.1^{c}	5.2^{bc}	5.4^{ab}	5.5^{a}	
	CV = 8.6%; FH = 1.1*; FL = 1.1*; FHL = 1.1**						
Primary branches (branch/plant)	CMF (control)	12.2^{ab}	11.5^{b}	12.2^{ab}	13.0^{ab}	13.4^{a}	12.5^{b}
	WCF	12.7^{ab}	13.3^{a}	13.4^{a}	13.5^{a}	13.5^{a}	13.3^{a}
	KOF	12.9^{ab}	13.0^{ab}	13.2^{a}	13.1^{ab}	13.4^{a}	13.1^{a}
	Average L	12.6^{b}	12.6^{b}	12.9^{ab}	13.2^{ab}	13.4^{a}	
	CV = 6.6%; FH = 2.6*; FL = 4.5*; FHL = 3.0*						
Number of leaves (leave/plant)	CMF (control)	14.8^{d}	14.8^{d}	15.2^{cd}	15.5cd	15.7^{bcd}	15.2^{b}
	WCF	15.5^{cd}	15.9$^{a\text{-}d}$	16.1^{abc}	16.8^{a}	16.8^{a}	16.2^{a}
	KOF	15.0^{d}	15.1^{cd}	15.5^{cd}	15.5^{cd}	16.6^{ab}	15.5^{ab}
	Average L	15.1^{d}	15.3^{cd}	15.6^{bc}	15.9^{ab}	16.4^{a}	
	CV = 3.8%; FH = 1.3*; FL = 0.6**; FHL = 0.5**						
Number shoots (shoot)	CMF (control)	15.4^{bc}	17.0^{abc}	17.1^{abc}	17.5^{abc}	18.5^{ab}	17.1
	WCF	15.9^{bc}	16.7^{bc}	16.9^{abc}	17.5^{abc}	20.2^{a}	17.4
	KOF	14.5^{c}	15.3^{bc}	16.5^{bc}	16.9^{abc}	17.3^{abc}	16.8
	Average L	15.3^{c}	16.3^{bc}	16.8^{abc}	18.4^{ab}	18.7^{a}	
	CV = 10.7%; FH = 0.5^{ns}; FL = 0.7*; FPL = 0.5*						

Values within the same group followed by the same letters are no significant different at $P \leq 0.05$ according to the Duncan's multiple range test; * and ** are significantly different at the 5% and 1% levels, respectively. CMF = cow manure, WCF = worm castings fertilizer. KOF = Komix organic fertilizer.

Data in Table 3 showed that the treatment using 25 tons/ha of WCF generated the highest theoretical fresh yield with 24.9 tons/ha. Applying CMF at a rate of 5 tons/ha produced the lowest theoretical fresh yield (14.7 tons/ha). This result was in accordance with a report by Anwar et al. (2005), who reported that fertilizing 5 tons/ha of vermicompost with 50 kg N + 25 kg P_2O_5 + 25 kg K_2O per hectare gave basil the highest yield of 16.85 tons/ha. The highest dry yield was 4.05 tons/ha and the highest essential oil yield was 121.30 L/ha.

The highest theoretical yield (21.9 tons/ha) was discovered when applied 25 tons/ha of organic fertilizer and it was statistically significantly different from using 5 tons/ha (15.8 tons/ha). As Table 3 revealed using WCF at a rate of 25 tons/ha created the highest actual fresh yield with 14.7 tons/ha. On the contrary, using CMF at a rate of 5 tons/ha produced the lowest actual fresh yield with 8.4 tons/ha.

With the equal amounts of different fertilizers, WCF showed higher essential oil content than the other two fertilizers, specifically the highest in the amount of fertilizer 15 and 25 tons/ha with 0.47% at Table 3.

In short, different types and levels of fertilizer

Table 3. Effect of sources and application levels of organic fertilizer on yield components and yield of basil (*Ocimum basilicum* var. *pilosum*)

Parameters (90 DAP)	Organic fertilizers (H)	Application rates (L) (ton/ha)					
		5 (control)	10	15	20	25	Average H
Fresh weight (g/plant)	CMF (control)	55.0^{c}	57.3^{c}	65.3^{bc}	75.3^{abc}	79.7^{abc}	66.5^{b}
	WCF	66.3^{abc}	70.3^{abc}	87.3^{ab}	77.7^{abc}	93.3^{a}	80.2^{a}
	KOF	64.7^{bc}	65.0^{bc}	67.0^{abc}	73.0^{abc}	75.3^{abc}	69.1^{b}
	Average L	62.0^{c}	64.2^{bc}	70.0^{b}	80.5^{a}	82.8^{a}	
	CV = 12.6%; $F_H = 1.1^{*}$; $F_L = 2.8^{**}$; $F_{HL} = 1.3^{*}$						
Theoretical yield (ton/ha)	CMF (control)	14.7^{c}	17.4^{bc}	18.8^{abc}	20.1^{abc}	20.1^{abc}	18.2^{b}
	WCF	17.4^{bc}	19.5^{abc}	21.3^{abc}	23.3^{ab}	24.9^{a}	21.3^{a}
	KOF	15.3^{c}	17.3^{bc}	17.7^{abc}	17.9^{abc}	20.7^{abc}	17.8^{b}
	Average L	15.8^{d}	18.1^{c}	19.3^{bc}	20.4^{ab}	21.9^{a}	
	CV = 2.6%; $F_H = 1.1^{*}$; $F_L = 2.8^{**}$; $F_{HL} = 1.3^{*}$						
Actual fresh yield (ton/ha)	CMF (control)	8.4^{b}	10.8^{ab}	11.8^{ab}	11.9^{ab}	12.0^{ab}	11.0^{b}
	WCF	10.9^{ab}	11.3^{ab}	12.8^{ab}	13.8^{ab}	14.7^{a}	12.7^{a}
	KOF	9.0^{ab}	10.2^{ab}	11.1^{ab}	12.0^{ab}	13.4^{ab}	11.1^{b}
	Average L	9.4^{d}	10.8^{c}	11.9^{b}	12.6^{ab}	13.4^{a}	
	CV = 5.8%; $F_H = 1.7^{*}$; $F_L = 0.5^{**}$; $F_{HL} = 1.4^{*}$						
Essential oil content (mL/100g)	CMF (control)	0.30^{c}	0.33^{bc}	0.40^{ab}	0.40^{ab}	0.40^{ab}	0.37^{a}
	WCF	0.36^{bc}	0.40^{ab}	0.40^{ab}	0.47^{a}	0.47^{a}	0.42^{a}
	KOF	0.36^{bc}	0.36^{bc}	0.36^{bc}	0.37^{bc}	0.40^{ab}	0.37^{a}
	Average L	0.34^{bc}	0.36^{c}	0.39^{ab}	0.41^{a}	0.42^{a}	
	CV = 13.6%; $F_H = 4.5^{*}$; $F_L = 1.1^{*}$; $F_{HL} = 0.5^{*}$						
Theoretical yield of essential oil (L/ha)	CMF (control)	55.2	62.8	75.5	77.7	80.7	70.4b
	WCF	67.2	77.6	83.7	89.1	95.6	82.6^{a}
	KOF	66.4	60.3	67.4	72.3	82.4	69.8^{b}
	Average L	62.9^{c}	66.9^{c}	75.5^{b}	79.7^{ab}	86.2^{a}	
	CV = 9.5%; $F_H = 0.1^{*}$; $F_L = 0.8^{*}$; $F_{HL} = 1.1^{ns}$						

Values within the same group followed by the same letters are no significant different at $P \leq 0.05$ according to the Duncan's multiple range test; * and ** are significantly different at the 5% and 1% levels, respectively. CMF = cow manure fertilizer, WCF = worm castings fertilizer. KOF = Komix organic fertilizer.

affected the theoretical yield of essential oils. The treatment of 25 tons/ha of fertilizer generated the highest theoretical yield of essential oils 82.6 L/ha. The theoretical yield of essential oil fluctuated from 55.2 to 95.6 L/ha when considering the interaction between the two factors (Table 3.

For the benefits of using WCF, vermicompost contain more nutrients than the other organic product from which it is processed (Buchanan et al., 1988) and thus it acts as a rich source of nutrients for plant growth and promotion (Ismail, 2000). Vermicompost decreases the amount of heavy metal incorporated to soil compare to compost as described by Lim et al. (2016). It has been also stated that vermicompost may have more compounds serve as a plant hormone which enhances plant growth and development compared to compost (Najar et al., 2015; Coulibaly & Bi, 2010). Our study results were in accordance with Shahriari et al. (2015), who reported that application of vermicomposting at doses of 10 tons/ha, 5 tons/ha, and 0 tons/ha to basil, the corresponding values were higher: plant height 54.3 cm versus 52.46 cm and 44.98 cm; dry weight 13.03 g/plant compared with 9.72 g/plant and 7.64 g/plant; the number of leaves/plant is 182.7 - 162.2 and 118.4, respectively; at the same time, the ratio and yield of essential oil also increased correspondingly when the amount of vermicompost was increased.

3.3. Effect types and amounts of organic fertilizers on economic efficiency of basil (*Ocimum basilicum* var. *pilosum*)

Results at Table 4 showed that when selling with price of VND 25.000 per kg basil, applying worm castings manure at the amount of 25 tons/ha gained the highest total revenue (VND 1,002,500,000 per ha/3 harvests) and total profit (VND 737,570,000 per ha/3 harvests); Total cost was highest (VND 439,930,000 per ha/3 harvests) in the treatment using 25 tons/ha of cow manure. However, the benefit-cost ratio when growing basil in the treatment using worm castings manure at a rate of 5 tons/ha was the highest (3.26).

4. Conclusions

The field experiment examined the effects of three types of organic fertilizers with increasing application rates on the plant growth, yield, and essential oil content of basil grown in Thu Duc City. Generally, the application of WCF and KOF resulted in better plant height, stem diameter, number of primary branches, number of leaves, and chlorophyll index than CMF. The results showed that the application of 25 tons/ha of WCF yielded the best results in fresh weight (93.3 g/plant), theoretical yield (24.9 tons/ha), actual yield (14.7 tons/ha), essential oil content (0.47 mL)/(100 g), and yield of essential oil (117 L/ha). The economic efficiency analysis showed the highest total profit after three harvests per hectare (VND 737,570,000 per ha/3 harvests). However, the highest real benefit-cost ratio (3.26) was obtained at the treatment of 5 ton/ha WCF.

Through the implementation of the experiment and the obtained results, the use of worm castings fertilizer with the amount of 5 tons/ha created the total revenue and benefit-cost ratio. However, the effect of these organic fertilizers on basil (*Ocimum basilicum* var. *pilosum*) should be continued on a larger area and with different crops for more comprehensive view of each type of fertilizer.

Conflict of interest

The authors declare no conflict of interest.

Table 4. Effect sources and application levels of organic fertilizers on economic efficiency of basil

Treatment		Actual yield	Total revenue	Cost	Total profit	Real rate of return
Types of fertilizers	Applied rates (ton/ha)	(ton/ha/3 harvests)	(VND/ha/3 harvests)			
Cow manure fertilizer	5	20.4	510,000,000	199,930,000	310,070,000	1.55
	10	23.7	592,500,000	259,930,000	332,570,000	1.28
	15	28.3	707,500,000	319,930,000	387,570,000	1.21
	20	30.3	757,500,000	379,930,000	377,570,000	0.99
	25	33.5	837,500,000	439,930,000	397,570,000	0.90
Worm castings fertilizer	5	28.1	702,500,000	164,930,000	537,570,000	3.26
	10	29.7	742,500,000	189,930,000	552,570,000	2.91
	15	32.8	820,000,000	214,930,000	605,070,000	2.82
	20	36.6	915,000,000	239,930,000	675,070,000	2.81
	25	40.1	1,002,500,000	264,930,000	737,570,000	2.78
Komix organic fertilizer	5	24.9	622,500,000	168,430,000	454,070,000	2.70
	10	26.7	667,500,000	196,930,000	470,570,000	2.39
	15	27.7	692,500,000	225,430,000	467,070,000	2.07
	20	32.4	810,000,000	253,930,000	556,070,000	2.19
	25	33.7	842,500,000	282,430,000	560,070,000	1.98

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