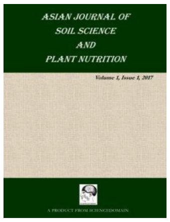

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Effect of Non-classic Nitrogen Fertilizers on Soil Chemical Properties, Growth of Zucchini Plants and N_2O Emission

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The climate is changing and communities all over the world are affected. Changing climate cause severe negative impacts on the natural resources and consequently on food and livelihood security. As such societies need to learn to cope with the changes predicted, warmer temperatures, drier soils, changes in weather extremes and rising sea levels. Therefore, a field experiment was conducted during two successive summer seasons of 2014 and 2015 under open field conditions at Dokki protected cultivation experimental site, Agricultural Research Center (ARC), Egypt. The study aimed to test the effect of non-classic N fertilizer forms such as coffee husk (organic waste), humic acid (organic acid) and proline (amino acid) as compared to ammonium nitrate (traditional mineral fertilizer), through ground and foliar applications, on some soil chemical properties of the studied clay soil i.e. pH, EC_e, chemically available concentrations of N, P and K. Growth and yield parameters of zucchini plants were also studied. In addition, greenhouse gases (GHGs) emission from different applied nitrogen fertilization types and its

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impact on warming and air pollution were calculated. Data revealed that the treatment of coffee husk can replace, at least partially, the traditional mineral fertilizer; can increase soil fertility, growth and yield of growing plants, and reduce GHGs emission. Also, proline and humic acid can consider as activator of phytohormones and growth substances, and recover the effect due to unfavorable climatic conditions or stresses. Obtained values of CO_2 emission varied between 984 for coffee husk treatment and 1629 kg ha^{-1} for mineral fertilizer treatment which increased air pollution.

Keywords: Mineral fertilizer; organic waste; organic acid; amino acid; clay soil; zucchini plants.

1. INTRODUCTION

Zucchini (*Cucurbita pepo*) is one of the popular vegetable crops in Egypt. It is cultivated mainly during autumn and spring seasons, but also can be cultivated in summer season, as it is rich in some nutrients and bioactive compounds contents such as phenolics, flavonoids, vitamins, amino acids, carbohydrates and minerals (especially K), and it is low in energy content (about 17 Kcal/100 g of fresh pumpkin) and has large amount of fiber [1]. It needs like all crops for N, as an essential nutrient, to give vigor growth and better yield, especially in the physiological stages.

The efficient use of nutrients within crop production systems, especially for N, has been in focus for several decades. Millar et al. [2] established that increasing nitrogen fertilizer application rates increased the N_2O flux from soil, and Robertson et al. [3] found that mineral fertilization is the largest source of atmospheric N_2O emission. Nitrous oxide has important effects both on the climate system and on stratospheric ozone [4]. It is a potent greenhouse gases (GHGs) produced in the soil predominantly by the microbial processes of nitrification (ammonia oxidation) and denitrification (nitrate reduction). Management practices that can influence emissions of N_2O from agricultural soils include fertilizer N (rate, type, timing and application method), crop, tillage, residue management, and irrigation [5].

Application of manures to soil can provide potential benefits including improving the fertility, structure, water holding capacity, increasing soil organic matter and reducing the amount of mineral fertilizer needed for crop production [6]. Disadvantages of using manures include the hard and high cost of handling and distribution associated with the large amount of manure required for obtaining sufficient quantities of nutrients for vegetables. Using of fresh manure may also introduce new weeds into fields previously free of that species since certain weed seeds maintain their viability after passage through animals.

So, we need to use another types of fertilizers as a source of N and known as friendly with the environment. Coffee husk is a low cost material contains various functional groups such as phosphate, carboxyl, amine and amides, rich in N and is available in the coffee factories [7]. Also, humic acid is not a fertilizer as it does not directly provide nutrients to plants, but is a compliment to fertilizer. El-Bassiouny et al. [8] reported that humic acids help in saving irrigation water, decreasing fertilizer dose of N, P and K, and at the same time increasing wheat productivity grown under newly reclaimed sandy soil. Abd-Elkader and Abd-Elrahman [9] mentioned that addition of organic acids i.e. humic and fulvic acids to the soil decreased soil pH and increased soil organic matter and nutrient content, as well as increased yield and nutrient uptake of growing plants. In addition, proline amino acid was greatly synthesized within plant tissues more than other amino acids in grown plants and fruits for the protection against stresses. Ahmed et al. [10] indicated that foliar proline amino acid at 100 ppm, on sweet pepper plants, gave the highest values of vegetative growth and yield per plant. Darwesh et al. [11] studied the effect of different types of amino acids i.e. glutamine, asparagines, arginine, proline, tyrosine and tryptophan at weekly foliar spraying rates 100, 200 and 300 mg L^{-1}, on the growth parameters and some of chemical contents in the date palm. They found that highly stimulated growth of plantlets in the green house was achieved by different amino acids, especially tryptophan and proline at the rate 300 mg L^{-1}.

Thus, the main objective of this study is to evaluate the effect of different non-classic forms of N fertilizer (coffee husk, humic acid, proline) on some soil chemical properties, growth and yield of zucchini plants, compared to mineral fertilizer (ammonium nitrate) as control, and also to determine GHG emission from different applied nitrogen fertilization types and its impact on warming and air pollution.

2. MATERIALS AND METHODS

The current study was carried out during two successive summer seasons of 2014 and 2015 under open field conditions at Dokki protected cultivation experimental site, Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Giza governorate, Egypt. The climatic data at Dokki site during the summer seasons of 2014 and 2015 were shown in Fig. 1.

2.1 The Field Experiment

A field experiment was conducted in clay soil, *Vertic Torrifluvents*; some physical and chemical characteristics of the studied soil before cultivation are presented in Table 1. Soil physical and chemical properties were determined by the methods described by [12,13].

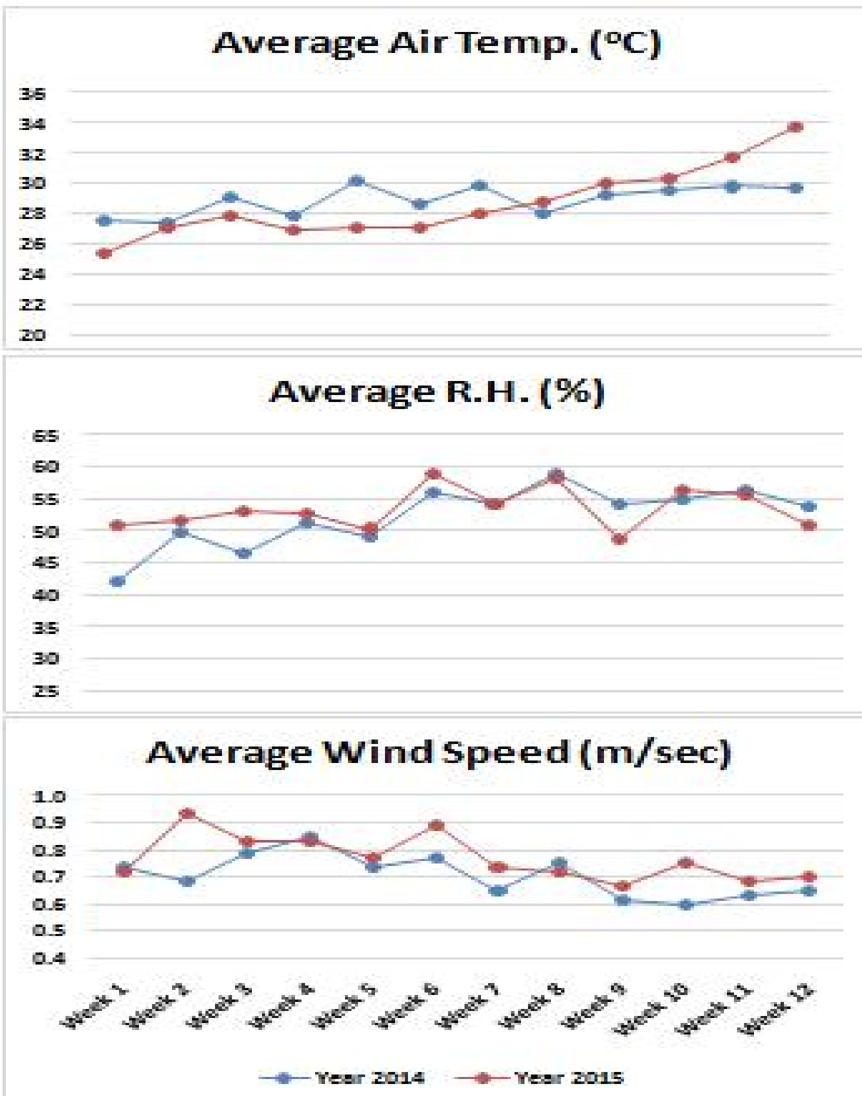

Fig. 1. The climatic data at Dokki site during the summer seasons of 2014 and 2015 (from June to August)

Table 1. Some physical and chemical properties of the studied soil (0-20 cm)

Particle size distribution, %		Soluble cations, mmol$_c$ L^{-1}	
Sand	17.3	Ca^{2+}	7.00
Silt	30.0	Mg^{2+}	5.60
Clay	52.7	Na^{+}	1.50
Textural class	Clay	K^{+}	0.90
$CaCO_3$, g kg^{-1}	60.0	Soluble anions, mmol$_c$ L^{-1}	
OM, g kg^{-1}	10.2	CO_3^{2-}	n.d*
CEC, cmol$_c$ kg^{-1}	49.1	HCO_3^{-}	4.50
pH (1:2.5)	7.53	Cl^{-}	3.11
EC$_e$, dS m^{-1}	0.87	SO_4^{2-}	2.18

**n.d means not detected*

Seeds of zucchini (*Cucurbita pepo* var. *Rossina,* cv. Hybrid F1) were sown in rows (6 m in the row length × 30 cm in the row width and 30 cm in distance between plants) on 26th and 24th June 2014 and 2015, respectively. Five treatments were applied as follows:

1- Control (without any treatments).
2- NH_4NO_3 (0.60 ton/ha/5 cm, as a recommended rate for zucchini plants according to extensions of the Egyptian Ministry of Agriculture).
3- Coffee husk at a rate of 3.24 ton/ha/5 cm, according to [14].
4- Humic acid (5 cm^3 L^{-1} as foliar application, as a recommended rate for irrigating vegetables according to extensions of the producer company) + ½ NH_4NO_3 (as ground application).
5- Proline at a rate of 0.15 g L^{-1} as foliar application, according to [10,11] + ½ NH_4NO_3 (as ground application).

Ammonium nitrate and coffee husk treatments were applied to the soil after seeds germination and mixed thoroughly with the upper 5 cm layer. Coffee husk was collected from Nescafé Company at the 10th of Ramadan City, washed using distilled water, dried at 60°C for 24 h, ground and sieved to get the particle size range of 125-250 µm which was used for this study. Humic acid and proline were added as foliar application after 3 weeks from cultivation for one time. Humic acid produced from FAM Company for agricultural development (Grandy solution). The used treatments were subjected to some chemical analyses as shown in Table 2. The recommended dose of ordinary superphosphate was added at a rate of 36 kg P_2O_5/ha, before plant cultivation. Potassium sulfate was added at a rate of 110 kg K_2O/ha, after 30 days from plant cultivation.

At the end of harvest (51 days from sowing), the plant samples were collected to determine growth parameters as follows: Plant height (cm), stem diameter (mm), number of leaves/plant, total chlorophyll of the fourth mature leaf from outside was measured using Minolta chlorophyll meter Spad-501, total fresh and dry weights (g/plant), fruit fresh and dry weights (g). For mineral analysis of fruits (N, P and K %), three plant samples of each plot were dried at 70°C in an air forced oven for 48 h. Dried leaves and fruits were digested by H_2SO_4/H_2O_2 mixture according to the method described by [13]. Total nitrogen was determined using Kjeldahl method according to the procedure described by [15]. Phosphorus content was determined using Spectrophotometer according to [16], potassium content being determined using Flame photometer as described by [17].

Soil samples were collected after plant harvest at depth of 0-20 cm. The collected samples were air dried, crushed, sieved through a 2 mm sieve and stored for chemical properties determination.

Table 2. Some chemical properties of the used materials

Treatment	pH (1:2.5)	EC$_e$, dS m^{-1}	%N	%P	%K
NH_4NO_3	5.47	n.d	33.5	n.d	n.d
Coffee husk	5.98	1.27	1.95	0.71	0.16
Humic acid	6.91	0.26	0.98	0.40	0.19
Proline	6.74	0.41	28.0	n.d	0.0002

n.d means not detected

2.2 The Greenhouse Gas Emissions from Nitrogen Fertilization

The aerobic microbial oxidation of ammonia to nitrate is called nitrification, while denitrification is the reduction of nitrate to gaseous nitrogen (N_2). Both reactions produce the intermediate gaseous nitrous oxide (N_2O) through microbial activities in the soil and eventually this gas is released to the atmosphere. The emission of N_2O from field was estimated according to [2]; the following equation was adopted:

$$N_2O \text{ emission} = [1.47 + (0.01x\ F)] \times N_2O_{MW} \times N_2O_{GWP}$$

Where:

- F : Mass of N applied from synthetic fertilizer, kg N ha^{-1}
- N_2O_{MW} : Ratio of molecular weight of N_2O to 2N, kg N_2O (kg N)$^{-1}$
- N_2O_{GWP}: Global Warming Potential for N_2O, kg CO_2-e (kg N_2O)$^{-1}$

Global Warming Potential (GWP) has been calculated to reflect how long it remains in the atmosphere, on average, and how strongly it absorbs energy. Gases with a higher GWP absorb more energy than gases with a lower GWP, and thus contribute more to global warming. The GWP value of 298 for N_2O used in the protocol (N_2O_{GWP}) is the 100-year value used in the most recent IPCC fourth assessment report according to [18]. The CO_2-e equivalent emission for each gas (CO_2, N_2O and CH_4) were summed together to give total CO_2-e.

2.3 Experimental Design and Statistical Analysis

The experiment was designed in a completely randomized design and each treatment was replicated three times. Data were statistically analyzed using the analysis of variance adopting a SAS software package at P≤0.05 [19].

3. RESULTS AND DISCUSSION

3.1 Soil Chemical Properties

Data in Table 3 show the effect of the studied treatments on some soil chemical properties i.e. pH, EC_e, chemically available N, P and K in the investigated soil after harvesting zucchini plants during the two seasons of 2014 and 2015. Data revealed that coffee husk treatment was superior in decreasing soil pH, followed by the treatment of proline + ½ NH_4NO_3. It may be due to that coffee husk is a byproduct rich in functional groups such as carboxyl and amine [7] which can make reactions by releasing H^+ ions and chelating elements; causing reduction in soil pH. Also, proline as an amino acid can reduce soil pH. The increment in soil EC went hand by hand with the EC of the applied treatments (Table 2). It was obvious that the EC values of the studied soil in the second season were higher than in the first one; may be due to continuous application of the tested treatments beside the agricultural practices. Significant increase of chemically available N in the studied soil due to the applied treatments compared to the control treatment (without N application, depending on the native amount in the soil). In contrast, the chemically available concentration of N that comes from applying N mineral fertilizer was lower than that comes from the applied coffee husk treatment. It may be due to that N from its mineral fertilizer is in soluble form which is more ready to taken up by growing plants and easy to leach from the soil profile, which reflect on decreasing its amount available in the soil; compared to coffee husk treatment which chelates N and reserves it from leaching downwards. Also, the treatment of coffee husk was applied more than 1.25 folds that of the amount of the applied mineral fertilizer in the soil. The chemically available P and K values of the studied soil in the first season went hand by hand with the obtained values for the applied treatments (Table 2). Similar trend was found in the second season.

Simple correlation coefficient (r) between the studied treatments applied as ground application and soil properties attributes (Table 4) show that coffee husk treatment have the highest effect on electrical conductivity of salts and chemically available concentrations of N, P and K in the studied soil (r=0.99). This could explain the higher yield obtained by the treatment of coffee husk compared to the other treatments as will be mention later. The results of correlation coefficient analysis show that, there is a significant negative correlation between pH and soil EC and its content of chemically available N, P and K. Saline soils have large amount of salts those increase EC values above 4 dS m^{-1} and decrease pH lower than 8.5, confirming the negative correlation between soil pH and EC. Positive correlation was found between EC and chemically available concentrations of N and K in the studied soil, while negative correlation was found between EC and chemically available P. Negative correlation was found between chemically available N and P, while the opposite between chemically available N and K. A strong positive correlation was found between chemically available concentration of P and K in the studied soil. Adekunle et al. [20] studied the relationship between macronutrients in Nigerian soil cultivated with two different plants and confirmed these results.

3.2 Zucchini Plants

Data in Table 5 show the effect of the applied non-classic fertilizers on some vegetative growth measurements of zucchini plants, compared to the applied N mineral fertilizer and control without N addition, during the summer seasons of 2014 and 2015. Data revealed that the treatment of coffee husk gave, generally, the highest significant values of stem diameter, chlorophyll content and total plant fresh and dry weights, followed by the treatment of foliar application of proline combined with ½ NH_4NO_3 as ground application which gave, also, the highest plant height and No. of leaves per plant. The lowest values were obtained in case of control treatment. Oraby [14] studied the effect of some treatments such as bentonite, zeolite, tea dust, coffee husk, eggshell, sawdust, rock phosphate and gypsum on wheat plants grown on contaminated soil with heavy metals, and reported that the treatment of bentonite gave the highest value of dry shoot followed by coffee husk treatment. Proline considered a precursor or an activator of phytohormones and growth substances. In addition, it can prevent and recover the effect due to high temperature or other unfavorable climatic conditions during the growing season; help to increase chlorophyll concentration in the plant leading to higher degree of photosynthesis and make the crop lush green. Moreover, this result agreed with those obtained by [21] on faba bean. The treatment of foliar application of humic acid combined with ½ NH_4NO_3 came in the third order; the role of humic acid in activating and enhancing the plant growth was obvious compared to the application of mineral fertilizer alone. These results agreed with those obtained by [8,9] on wheat plants.

Data in Table 6 show the effect of the studied different sources of N fertilizer on yield and quality parameters of zucchini plants grown on clay soil during the summer seasons of 2014 and 2015. Data revealed that the treatment of coffee husk gave the highest values of yield and its dry matter content, also, its concentration of N, P and K. This can be explained by the fact that coffee husk is rich in the studied elements and can adsorb and chelate elements by its functional groups and can increase soil flocculation which enhance plant growth and increase the yield. The treatment of coffee husk gave the highest value of water content resulted from the difference between total fruits fresh and dry weights (467 g/plant), followed by proline + ½ NH_4NO_3 treatment that gave 415 g/plant. The treatment of foliar application of proline combined with ½ NH_4NO_3 as ground application came in

Table 3. Effect of the studied treatments on some soil chemical properties of the investigated soil after plant harvest during the two seasons of 2014 and 2015

Treatment	pH (1:2.5)	EC_e, $dS\ m^{-1}$	Chemically available macronutrients, %		
			N	P	K
First season (2014)					
Control	7.33	1.26	0.12	0.19	0.17
NH_4NO_3	7.27	1.64	0.57	0.28	0.21
Coffee husk	6.98	2.02	0.70	0.29	0.24
Humic acid + ½ NH_4NO_3	7.30	1.43	0.32	0.27	0.26
Proline + ½ NH_4NO_3	7.07	1.38	0.49	0.24	0.22
$LSD_{0.05}$	**0.07**	**0.09**	**0.07**	**0.02**	**0.01**
Second season (2015)					
Control	7.41	1.13	0.10	0.21	0.19
NH_4NO_3	7.24	1.69	0.56	0.26	0.20
Coffee husk	6.21	2.11	0.72	0.27	0.25
Humic acid + ½ NH_4NO_3	7.36	1.45	0.36	0.28	0.27
Proline + ½ NH_4NO_3	7.00	1.41	0.47	0.23	0.20
$LSD_{0.05}$	**0.06**	**0.12**	**0.08**	**0.01**	**0.01**

Table 4. Simple correlation coefficient between the applied treatments as ground application and soil properties attributes

Correlation matrix	pH	EC	N	P	K
		Mineral fertilizer			
pH	-				
EC	-0.954	-			
N	-0.980	0.930	-		
P	-0.982	-0.980	-0.980	-	
K	-0.982	0.958	0.985	0.995	-
		Coffee husk			
pH	-				
EC	-0.997	-			
N	-0.988	0.986	-		
P	-0.998	-0.998	-0.998	-	
K	-0.994	0.989	0.998	0.998	-

Table 5. Effect of the studied treatments on the vegetative growth measurements of zucchini plants during the two seasons of 2014 and 2015

Treatment	Plant height, cm	Stem diameter, mm	No. of leaves/ plant	Chlorophyll content in leaves, SPAAD	Total plant fresh weight, g	Total plant dry weight, g
			First season (2014)			
Control	33.1	1.73	23.3	42.2	1365	317
NH_4NO_3	46.6	2.07	30.5	46.7	1790	391
Coffee husk	55.3	3.06	34.5	56.4	3590	816
Humic acid + ½ NH_4NO_3	54.2	2.66	33.7	45.4	2425	516
Proline + ½ NH_4NO_3	60.3	2.87	35.5	49.6	3375	750
$LSD_{0.05}$	**4.00**	**0.61**	**1.10**	**0.70**	**35.7**	**22.5**
			Second season (2015)			
Control	31.7	1.70	22.5	40.1	1271	319
NH_4NO_3	45.4	2.15	30.0	46.1	1762	376
Coffee husk	55.9	3.12	33.5	57.6	3707	833
Humic acid + ½ NH_4NO_3	48.4	2.72	29.7	43.0	2282	518
Proline + ½ NH_4NO_3	60.9	2.95	36.3	50.5	3338	675
$LSD_{0.05}$	**2.98**	**0.65**	**1.16**	**1.08**	**40.3**	**26.7**

the second order for giving high fruits quality, followed by humic acid + ½ NH_4NO_3 treatment, with similar trend as previously mentioned in Table 4 for plant growth measurements. Proline and humic acid are known to be very effective chelating agents for macro and micronutrients [22].

3.3 Greenhouse Gas Emission from Nitrogen Fertilization and Impacts on Warming, Pollution and Plant Yield

The results presented in Table 7 show that CO_2 emissions from different applied N types increased with applying the traditional N mineral fertilizer. Values of CO_2 emission varied between 984 for coffee husk treatment and 1629 kg ha^{-1} for mineral fertilizer treatment and thus increase air pollution. The results obtained from the treatments of proline and humic acid combined with ground application of half amount of the applied mineral fertilizer confirmed the risk that proper achieved if the farmers continue apply mineral fertilizers to the cultivated soil with full rate. In Egypt, many researchers studied how to adapt or mitigate climate change impacts; reduce greenhouse gas emission from N fertilizers by decreasing rates without affecting the yield and quality, or finding other sources by recycling wastes which are rich in N [23,24,25]. Changes in the application of fertilizer type had a lower impact on emissions than on changes in the yield; besides recycling wastes and solve another critical environmental problem.

Table 6. Effect of the studied treatments on fruits fresh and dry weights, and elemental content of zucchini fruits during the two seasons of 2014 and 2015

Treatment	Fruits fresh weight, g/plant	Fruits dry weight, g/plant	N	P	K
				%	
First season (2014)					
Control	246	62.6	2.64	2.29	0.77
NH_4NO_3	287	71.4	3.92	3.10	1.28
Coffee husk	542	75.2	4.29	3.90	1.85
Humic acid + ½ NH_4NO_3	364	73.4	4.20	3.50	1.35
Proline + ½ NH_4NO_3	489	73.8	4.43	3.20	1.33
$LSD_{0.05}$	**36.1**	**1.07**	**0.13**	**0.10**	**0.05**
Second season (2015)					
Control	241	60.3	2.61	2.35	0.78
NH_4NO_3	273	72.7	3.45	3.06	1.25
Coffee husk	535	73.5	4.34	3.43	1.81
Humic acid + ½ NH_4NO_3	359	72.6	4.35	3.61	1.34
Proline + ½ NH_4NO_3	492	76.2	4.45	3.19	1.40
$LSD_{0.05}$	**30.6**	**0.64**	**0.10**	**0.12**	**0.06**

Table 7. Greenhouse gas emission (CO_2 equivalent) from nitrogen fertilization as affected by total yield per hectare

Treatment	Total yield, kg ha^{-1}	CO_2 eq kg ha^{-1}	CO_2 eq g/kg yield
NH_4NO_3	26877	1629	60.6
Coffee husk	54617	984	18.0
Humic acid + ½ NH_4NO_3	36707	1160	31.6
Proline + ½ NH_4NO_3	49327	1158	23.5

4. CONCLUSION

It could be concluded that, not only the traditional mineral fertilizers can achieve the goal of obtaining maximum yield, but also the non classic forms can achieve that. In addition, it can cause lower impact on emissions besides recycling wastes. The treatment of coffee husk was superior in increasing soil fertility, improving the studied soil chemical properties, giving the highest values of zucchini plants growth and yield, and reducing GHG emissions compared to the other studied treatments. Also, proline and humic acid came in the second and third order respectively, these considered as activator of phytohormones and growth substances, and protect against stresses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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