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EFFECT OF DIFFERENT NITROGEN PERCENTAGE INPUT ON SOIL FERTILITY, PLANT NUTRIENTS AND YIELD OF ORGANIC SPINACH (*Amaranthus* spp.)

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ABSTRACT

Nitrogen (N) management is important to assess the N effect on soil and plant under the organic farming system. With this in mind, a glasshouse experiment was conducted to determine the effect of various N inputs on selected soil chemical properties, nutrient content and yield of spinach (*Amaranthus* spp). The experimental treatments were focused on the different percentages of N input (2, 4 and 6% of N from organic sources) with soil alone as a control. The treatments were applied 14 days after seeding (14-DAS) at a recommended rate of 2 t/ha. The study result found that 4-6% of N input (with T3 and T4) had improved selected soil parameters such as pH, CEC, total N, available-P and exchangeable-Ca. Further, total N content in spinach (cultivated organically), as well as spinach yield, were significant. Study findings have shown that within 4% to 6% of N input promotes soil quality, total nutrients in spinach (leafy vegetables), and can be scaled up to farm level to sustain organic system productivity.

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INTRODUCTION

Nitrogen plays a major role (nutrient) for plant growth and development (Yanez-Mansilla et al., 2015; Bernal et al., 2017) either in organic or conventional farming system. Further, N can be a limiting factor for other plant essential nutrient uptake as well. Managing soil fertility in relation to nitrogen (N) input is crucial, especially in organic farming system. According to regulation, organic farmers are limited only to utilize N from organic sources (from natural processes). However, most of the organic sources contain low amount of N (Mikkelsen et al., 2008) compared to inorganic sources, plus limited availability of organic sources. Such limitations became a main constraint in organic farming's system especially, N input (source).

Manures from livestock are often used to supply organic N. Besides, plant biomass can also be used as a source of organic N and nutrient sources for vegetable farming. One such example is empty oil palm fruit bunches (EFB) turned into compost. Other study (Yasuor et al., 2013), stated legumes can be used as green-manure. Most of these plant-based composts often contain between 1-3% of N (Vimala, 2005). However, the plant-based composts response often varies highly based on soil type and vegetable grown. Based on Kala et al. (2011) and Sheikh and Ishak (2016), the variation likely due to the effect of carbon and nitrogen (C/N) ratios. The authors stated, unstabilized organic fertilizer with high C/N ratio immobilize significant amount of N, making it unavailable for plant utilization and resulting in deficiency problems. In such cases, the organic growers are encouraged to find out the C/N ratios of their organic material sources and, nutrient contents. Reasonably sound well, however least practical.

Nitrogen applied to soil, whether through chemical fertilizer or organic sources or even in-combination of both, N fundamentally subjected to losses through; volatilization and leaching, besides uptake by plant. Optimal N uptake and retention is favored, and based on Singh (2018), optimal use of N by crops shown no negative effect on organic matter and microbial activity in the soil. On the other hand, excessive N fertilization (or known as luxury application) can cause eutrophication and adverse nitrate pollution in ground or surface water (Khan et al., 2018). Furthermore, (Ma et al., 2018; Qaswar et al., 2019) stated, luxury N application may lead to low C ratio compared to N ratio, thus inhibit soil microbial function. Thus, selection of organic source (to partially supply N) for leafy vegetables is paramount. In this study, two types of materials were selected and used as organic source of N fertilizer namely, fish amino acid (FAA) and biochar. FAA is generally produced by fermenting fresh fish by-products (bones, head, skin and other tankage parts) with locally-available brown sugar. The fermentation process takes about 2 to 6 months to produce FAA. When completely fermented, FAA has a sweet, slightly fishy odour and with 3-5% of N content (Weinert et al., 2014). FAA in liquid form can be applied as foliar or soil drenching. Meanwhile, biochar is commercially produced by biomass pyrolysis (between 600^{o}C to 830^{o}C), and biomass ideally from organic based-waste. Biochar usually has lower N content (1.8 – 2.01% N) compared to FAA (Aini et al., 2005; Manickam et al., 2015).

Spinach (*Amaranthus* spp.) is a type of leafy green vegetable commonly cultivated around the globe, used widely in food industry and home-kitchen. In Malaysia, Department of Agriculture (DOA), records the total spinach production in year 2018 about 75,220 metric tons (mt) roughly. Forty six percent of total vegetable production in Malaysia, with net worth of USD 283,000 (DOA, 2018). Directly, this figure contributes to income generation and increase in socioeconomic condition of the farmers community. Price increase in N fertilizer (commercially available organic fertilizer) may occur, as it is a trade-market. In the process, farmer's communities are equally affected. Thus, alternative source of N is necessary in two-fold; first to farm in a sustainable manner and, second to reduce potential environmental pollution.

Past studies (Overeem, 2015; Seaman et al., 2016; Russelle et al., 2017) used N rate (kg/ha) as a factor, with minimal emphasis on N% (percentage). In organic farming system, N% is a key and crucial factor. It is so because, different N% means different price and grade of the organic fertilizer, either readily-available or self-produced at farm. Therefore, the study objective is to determine the effect of the various N% input from organic sources on soil chemical properties, plant

MATERIALS AND METHODS

Experimental setup

This study was conducted at an integrated organic farm, located in Malaysian Agricultural Research and Development Institute (MARDI Serdang), Malaysia. Test crop was green spinach (*Amaranthus* spp.) with rounded leaf type. The spinach was planted by direct seeding in 10 kg soil/pot as grow media (sandy-clay soil type). Experimental set up was arranged in

Randomized Completely Block Design (RCBD) with 4 treatments and 5 replications. Each replication had 7 plants, totalling 140 plants. Details of the treatments are shown in Table 1.

Treatments were applied after 14 days of seeding (14-DAS) at a rate of 14 g/pot. Soils (0-20 cm) were sampled at initial (before) planting (at 0 day), middle cropping (20-DAS) and at harvesting (35-DAS), in order to see the soil chemical changes throughout the cropping season.

Assessment of soil chemical properties

Collected soil samples were air dried, grounded with agate mortar and passed through 2mm sieve and were subjected for selected chemical analyses as follows:

Soil pH: One (1) gram of air-dried soil sample was mixed with 25 mL of distilled water for the determination of soil pH in water (H_2O) with sample to water ratio of 1:2.5. The mixture was then stirred and left stoppered overnight. The pH value of the suspension was determined using a calibrated pH meter (Mettler Toledo, Delta 320).

Soil Exchangeable Cations (K, Ca, Mg) and Cation Exchange Capacity (CEC): Exchangeable cations (K^+, Ca^{2+}, Mg^{2+}) were determined by leaching method; dissociation of exchangeable cations with ammonium acetate (1 N NH_4OAc) adjusted to pH 7.0. Exchangeable cations (K, Ca, Mg) were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES 7300 DV Perkin Elmer). The determination of cation exchange capacity (CEC) was done by washing the absorbed NH_4^+ with 100 mL of methylated spirit to remove excess NH_4^+ which was then consequently replaced by 100 mL of 1N K_2SO_4. Cation exchange capacity was determined by analyzing the displaced NH_4^+ with titration method using an auto titrator (Metrohm titrator potentiometer).

Soil Total Carbon (C) and Total Nitrogen (N): Sixty (60) milligrams (mg) of air-dried 2-mm sieved soil samples and tungsten powder were weighed and wrapped in tin foil squares and placed in the sampler for C, and N determination using CHNOS analyzer (ELEMENTAR Vario Macro Modules 11.44-5201). The tungsten powder was used to remove the alkali sulphur.

Soil Available-Phosphorus (P): Available phosphorus in the soil was determined using Bray and Kurtz No.1 extraction method (Bray and Kurtz, 1945). Two (2) gram of air dried 2-mm sieved soil samples were placed into glass tubes and 20 mL extraction solution of 0.03 N NH_4F and 0.1 N HCl were added. The glass tube was shaken for 45 seconds and the soil extracts were filtered using Whatman filter paper No.42. The extractants were then filtered and measured using UV-Vis Spectrophotometer (Shimadzu UV-1700 Double Beam Scanning UV-Vis Spectrophotometer).

Assessment of plant nutrient status

The dried plant samples then were ground to a fine powder for plant total nutrient analysis (N, P, K, Ca and Mg). Total plant nutrients were analysed as follow:

Total Nitrogen (N): Thirty (30) milligrams (mg) of dried plant samples and tungsten powder were weighed and wrapped in tin foil squares and placed in the sampler for N determination using CHNOS analyzer (ELEMENTAR Vario Macro Modules 11.44-5201). The tungsten powder was used to remove the alkali sulphur.

Plant Macronutrient (P, K, Ca, Mg): One (1) gram of dried plant samples were digested using concentrated nitric acid (HNO_3 65%) and left overnight. The samples were digested at 110^oC on digestion block for about 4-5 hours. The samples were then cooled aside before adding another 5 mL of concentrated hydrochloric acid (HCl 37%) and the digestion was continued for another 4-5 hours. The clear and transparent solution samples obtained were cooled down before being filtered into 100-mL volumetric flasks. Volume then was marked up with distilled water to the calibration line for determination of plant total nutrients (P, K, Ca and Mg) using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES 7300 DV Perkin Elmer).

Plant yield

Harvested spinaches (35-DAS) were measured for fresh weights. The fresh weight of spinach was measured immediately after harvesting using a digital balance (SQW-3, SmartWEIGH, Malaysia). The fresh weights data were expressed as yield in gram (g).

Statistical analysis

All data were subjected to one-way Analysis of Variance (ANOVA). Treatment means were compared using Tukey's Studentized Range (HSD) test at significance level $p < 0.05$ using SAS version 9.4. (SAS Institute Inc. 2013. SAS ® 9.4 Statements: Reference. Cary, NC, USA)

RESULTS AND DISCUSSION

Effect of Different N (%) Input on Soil Chemical Properties

The results of soil chemical properties from initial (0 day) until harvesting day (35-DAS) are shown in Table 2 until Table 4. Soil chemical properties showed variation throughout the cropping period, likely due to different N % (treatment) as application input, based on spinach. During the middle of cropping (20-DAS), soil chemical properties namely CEC, N, C available-P and exchangeable-Ca were significantly affected by the treatment's application. Soil CEC showed significant differences ($p < 0.05$) in T3 (4% N) compared to T2 (2% N) and T1 (0% N) (Table 2). Soil C and N content were significantly different in all treated soils (T2, T3 and T4) compared to the untreated one (T1) (Table 3). Meanwhile, soil available-P and exchangeable-Ca differed significantly ($p < 0.0001$) under T3 and T4 compared to T1 and T2 (Table 3 and Table 4, respectively). However, the treatment effect showed insignificant differences on soil pH, C, exchangeable-K and -Mg during 20-DAS of planting. Meanwhile, at the harvesting stage (35-DAS), pH of soil showed significant difference in T2 compared to T3 and T4 (Table 2). The highest level of N input with 6% of N (T4) under this study had significant effect ($p < 0.05$) on the soil CEC and available-P during the harvesting day (35-DAS). The concentration of soil available-P showed an increasing trend with the level of N input during the 20-DAS. Towards the harvesting day (35-DAS), the soil available-P concentration remained significantly increased in T4 (6% N) application (Table 3). Soil exchangeable cations (K, Ca, Mg) under all treatments during 20-DAS and 35-DAS are shown in Table 4. About a week after the treatment application (20-DAS), T3 (4% N) and T4 (6% N) showed a significant increase in exchangeable-Ca of the soil, with the highest for T4 (12.80 $cmol_c$/kg).

Table 1. Treatment application with different of N input percentage (%)

Treatment	Label	N input level (%)	Source of N input
Treatment 1	T1	0 (Control)	-
Treatment 2	T2	2	Biochar and Fish Amino Acid (FAA)
Treatment 3	T3	4	
Treatment 4	T4	6	

Table 2. Soil pH and CEC throughout the planting period

Planting stage	Treatment	pH (H_2O)	CEC ($cmol_c$/kg soil)
Before planting (0-Day)	-	6.51 ± 0.02	6.29 ± 0.64
------------- **After treatment** -------------			
Middle of planting (20-DAS)	T1 (0% N)	6.47 ± 0.57^{a}	11.06 ± 0.87^{c}
	T2 (2% N)	6.31 ± 0.42^{a}	11.43 ± 1.22^{bc}
	T3 (4% N)	6.13 ± 0.28^{a}	15.00 ± 1.05^{a}
	T4 (6% N)	6.36 ±0.15^{a}	13.52 ± 1.86^{ab}
	p-value	0.311	0.001
At harvesting (35-DAS)	T1 (0% N)	6.02 ± 0.53^{ab}	8.38 ± 0.60^{b}
	T2 (2% N)	6.18 ± 0.42^{a}	9.66 ± 0.85^{ab}
	T3 (4% N)	5.42 ± 0.39^{b}	10.02 ± 1.21^{ab}
	T4 (6% N)	5.41 ± 0.27^{b}	10.49 ± 1.12^{a}
	p-value	0.01	0.02

Values (mean ± SE) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at $p \leq 0.05$. $n = 5$ per treatment

Table 3. Soil C, N and available-P throughout the planting period

Treatment		Total carbon (C) (%)	Total nitrogen (N)	Available-P (ug/g soil)
Before planting (0-Day)	-	1.58 ± 0.02	0.19 ± 0.01	31.33 ± 2.08
--------- **After treatment** ---------				
Middle of planting (20-DAS)	T1 (0% N)	5.47 ± 0.53[a]	0.45 ± 0.04[b]	238.02 ± 29.44[b]
	T2 (2% N)	6.04 ± 0.76[a]	0.46 ± 0.05[ab]	246.58 ± 22.91[b]
	T3 (4% N)	6.71 ± 1.36[a]	0.56 ± 0.07[ab]	425.83 ± 54.59[a]
	T4 (6% N)	5.34 ± 0.97[a]	0.57 ± 0.08[a]	473.96 ± 27.00[a]
	p-value	0.123	0.015	<0.0001
At harvesting (35-DAS)	T1 (0% N/)	4.18 ± 0.64[a]	0.32 ± 0.03[a]	239.75 ± 37.39[c]
	T2 (2% N)	3.43 ± 0.55[a]	0.37 ± 0.08[a]	267.54 ± 48.25[bc]
	T3 (4% N)	4.20 ± 1.36[a]	0.35 ± 0.11[a]	356.84 ± 62.45[b]
	T4 (6% N)	3.70 ± 1.08[a]	0.44 ± 0.05[a]	512.46 ± 87.16[a]
	p-value	0.230	0.127	<0.0001

Values (mean ± SE) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at $p \leq 0.05$. $n = 5$ per treatment

Table 4. Soil exchangeable cations (K, Ca and Mg) throughout the planting period

Planting stage	Treatment	Exchangeable cations ($cmol_c$/ kg)		
		K	**Ca**	**Mg**
Before planting (0-Day)	-	1.14 ± 0.02	1.36 ± 0.02	0.29 ± 0.01
---------- **After treatment** ----------				
Middle of planting (20-DAS)	T1 (0% N)	4.36 ± 3.03[a]	8.56 ± 0.68[b]	5.41 ± 1.15[a]
	T2 (2% N)	5.58 ± 2.25[a]	9.51 ± 1.11[b]	5.77 ± 1.12[a]
	T3 (4% N)	5.91 ± 2.56[a]	12.11 ± 1.10[a]	5.76 ± 1.12[a]
	T4 (6% N)	4.74 ± 0.76[a]	12.80 ± 0.92[a]	6.05 ± 1.45[a]
	p-value	0.561	<0.0001	0.893
At harvesting (35-DAS)	T1 (0% N/)	3.53 ± 2.13[a]	6.23 ± 0.78[a]	3.97 ± 0.82[a]
	T2 (2% N)	3.67 ± 1.85[a]	5.22 ± 0.67[a]	2.91 ± 0.57[a]
	T3 (4% N)	4.03 ± 2.82[a]	7.04 ± 2.58[a]	3.53 ± 1.47[a]
	T4 (6% N)	2.31 ± 1.61[a]	6.32 ± 1.77[a]	3.27 ± 1.45[a]
	p-value	0.411	0.360	0.486

Values (mean ± SE) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at $p \leq 0.05$. $n = 5$ per treatment.

Table 5. Total nutrient content of spinach treated under different percentage of N (% N) input at 35-DAS

Treatment	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
	(%)	------------------ (ppm) -----------------			
T1 (0% N)	4.46 ± 0.85[bc]	53.29 ± 11.58[a]	702.90 ± 76.70[a]	62.83 ± 7.77[a]	63.22 ± 16.47[a]
T2 (2% N)	4.16 ± 0.88[c]	56.28 ± 8.26[a]	738.62 ± 57.94[a]	68.74 ± 14.46[a]	69.58 ± 19.70[a]
T3 (4% N)	5.70 ± 0.32[ab]	48.02 ± 4.27[a]	739.55 ± 77.43[a]	70.61 ± 17.94[a]	53.99 ± 10.66[a]
T4 (6% N)	5.91 ± 0.73[a]	42.52 ± 7.12[a]	724.44 ± 46.18[a]	61.44 ± 13.42[a]	53.17 ± 20.62[a]
p-value	0.006	0.095	0.709	0.679	0.460

Values (mean ± SE) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at $p \leq 0.05$. $n = 5$ per treatment

Effect of different N inputs on plant nutrient status

The mean values of total nutrient content (total N, P, K, Ca and Mg) in the spinach leaves on harvesting day (35-DAS) are presented in Table 5. The results revealed that the treatment effect showed a significant difference only in total N content. The significant difference ($p < 0.05$) for N content in spinach leaves was observed for T4 (6% N) with 5.91% of N and the lowest was on T2 with 4.16%. As for total P, K, Ca and Mg content in plant leaves, the differences were not significant ($p > 0.05$) on harvesting day (35-DAS) between all the treatments used.

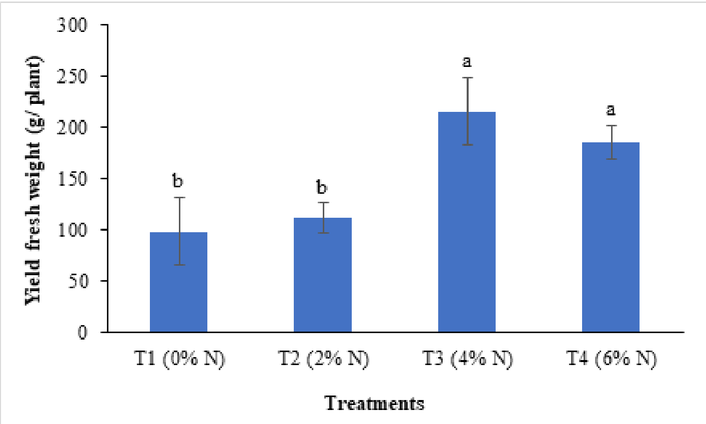

Figure 1. Effect of different percentage of N inputs (% N) on plant fresh weight (yield) at 35-DAS. Means with different letters indicate significant difference between treatments by Tukey's test at $p \leq 0.05$ ($p = <0.0001$)

Plant yield

In this study, the fresh weight of spinach which was measured immediately after the harvesting (35-DAS) and recorded as spinach yield. The yield performances of spinach in this study were shown through mean values of total fresh weight (FW) of spinach during harvesting day (35-DAS). A highly significant yield ($p < 0.0001$) of spinach was obtained on T3 with the highest yield of 215.60 g/plant compared to the treatment control at only 98.60 and 111.20 g/plant on T1 and T2 respectively. However, there was no significant difference in the yield of spinach between T3 and T4 (185.83 g/plant) (Figure 1).

DISCUSSION

Effect of different N inputs on soil chemical properties

Since the introduction of organic farming system, the use of organic sources as organic soil-treatment gain acceptance among farmers, especially organic farmers. These organic treatment (or some refer it as, amendments) are known to improve soil fertility status, naturally. Positive effect of organic treatment on soil chemical properties (CEC, C, N, available-P, exchangeable cations) were recorded through this study.

Significant differences ($p < 0.05$) that were observed in T3 (4% N) and T4 (6% N) on CEC, N, available-P and exchangeable-Ca in soil can be attributed to positive-synergy interaction between biochar and fish amino acid (FAA). This is supported by Pavlikova et al*.* (2017) who stated that biochar (a carbon rich material), acts as microbial catalyst. Adsorptive capacity (i.e. surface attachment of microbes/ nutrient) enhances the benefits of the FAA by their breakdown and readily available for plant uptake and/ or cations to adhere. This synergism acts as an effective organic fertilizer.

In line with the above, the effect of adsorption and decomposition process of biochar and FAA contributes well towards soil CEC. At 20-DAS, both treatments using biochar and FAA; T3 (2% biochar + 2% FAA) and T4 (2% biochar + 4% FAA) had significantly improved their initial value of CEC (6.29 $cmol_c$/kg soil) to higher CEC value with 15.00 $cmol_c$/kg soil on T3 and 13.52 $cmol_c$/kg soil on T4 respectively. This indicates, nutrient retention capacity of the soil has been increased by one-fold, the least, which is sustainable.

On the other hand, soil pH presented a decrease value from seeding to harvesting (35 DAS), on all treatments. Such a decrease is expected, due to nutrients removal through plant intake, as stated by Neina (2019). It is a general fact, that soil nutrient becomes much mobile and available for crop uptake at pH between pH 5.5 to 6.5. After spinach harvest, lowest mean soil pH (T4) was about pH 5.47 ± 0.27, which is still within lower limit of pH for nutrient mobility. Meanwhile, under T2 (2% N + biochar + FAA), only small changes (0.38 unit) on soil pH (Table 2). Brendova et al (2015), stated that biochar capacity to retain nutrients (cations etc.) strongly influence changes in soil pH. Biochar is a stable, carbon-rich material with higher surface area for any interactions once applied into soil. As such, these characteristics combined with FAA, further improve the organic treatments (T2-T4) to support spinach growth.

All treatments recorded increase in N content in soil, which is in line with N addition from biochar and FAA. For T1, with no fertilizer input at all, the increase of N content in soil was assumed to come from the soil growing media itself. Whereas, T2 – T4 had depended on N input from organic fertilizer which is biochar and FAA for its nutrient supply. Biochar had an advantage of slow releasing properties which helped in reducing the problem of nutrient being leached out. This characteristic also safeguarded the plant from receiving excess amount of all nutrients at one go, which could cause problems of plant scorch (burn). Throughout the planting period of spinach, soil N content and soil available-P increase proportionately with N% input (Table 3), to the highest level with 0.57% of N (from initial, 0.19% N) and 473.96 ug/g of soil available-P (from initial, 31.33 ug/g) during 20 DAS. Therefore, application of solid-based organic fertilizer is practical under organic farming, because first, it adsorb-retain N then, secondly release N when required by plant. Gaskell and Smith (2007) noted a similar finding as stated. FAA (liquid fertilizer) on the other hand, has relatively rapid N mineralization. This facilitates to accelerate N availability from the solid organic fertilizer (for this study, biochar) into soil. Hartz et al. (2006) stated that organic liquid fertilizer can function similarly to conventional N fertilizers.

The concentration of soil available-P noted to continual increment from 20 DAS until the harvesting day (35 DAS) in T4. This result indicated the accumulation of available-P in soil, which indirectly explained the lower uptake of P in spinach under T4 (Table 5). This similar trend also observed by Khan et al. (2019). For earlier study, Klein and Johnson (1954) had related the low solubility of phosphate ion (PO_4^{3-}) in organic acid condition (amino acid in FAA). Further stated, that negative effect on the uptake of P if amino acids (found in the FAA) are used in excessive doses (> 0.2% in hydroponic mixtures). Currently, there was no references doses for FAA application on the soil. Thus, the FAA doses applied (10 – 40 mL/ 10 kg soil) in this study were calculated based on recommendation of N for spinach. Based on the doses applied, the available-P (in soil) under this study were recorded to increase up to 512.5 ug/g soil, except for T3 that recorded lower value.

Besides phosphorus (P) increment, exchangeable-Ca (soil) also increase with N% used. Han et al. (2020) stated that organic treatment significantly increased the exchangeable-Ca and -Mg in the soil after their application. In comparison with this study, the exchangeable-Mg (soil) also showed the highest content even though the difference between other treatments, statistically recorded not significant. The principal mechanism involved in this increment of exchangeable cations as suggested by Opala et al. (2020) is based on the interaction between the greatest strength of organic acids which were produced from the decomposition of organic fertilizers used to significantly influence the mobility of base cations (focusing on exchangeable-Ca and -Mg in relation to this study) into the soil layer. However, at harvesting stage (35-DAS), it showed a decline in all exchangeable cations (K, Ca, Mg) content in all types of treatment. The results showed no significant differences between all the treatments used. As described earlier, this nutrient decrement is expected due to the nutrient uptake by plants, with no addition of chemical fertilizers through the experimental period. Noteworthy that, sandy clay soil often has limited nutrient retention, due to large soil macropores. However, biochar which is a solid based material, with higher surface area, able to reduce the macropores, and hence increase micropores in soil. Micropores are well aerated pores within the soil, which can minimize N loss (volatilization or leaching) and able to retain soil nutrient for extended time until vegetables uptake.

Effect of different N Inputs on plant nutrient status

Initial hypothesis was linear increment of plant total nutrients with increment of N input. This hypothesis has been answered by the higher N content (5.7 – 5.9% N) in spinach treated under T3 (4% N input) and T4 (6% N input). The presence of amino acid in FAA from both treatments (T3 and T4) was found to have a significant effect on the N content in the spinach leaves. According to a study conducted by Lopez-Bucio et al. (2000), this effect is associated with the function of organic acids, resulted from the catabolism of amino acids which led to increase efficiency in uptake of N by spinach. Apart from that, Yamagata et al. (2001) and Nasholm et al. (2009), reported that high N content is possible due to direct acquisition of organic N from amino acids in the FAA or any input from organic sources which contain amino acids. Both authors agreed that, spinach is one of the crops that has such capabilities besides bak choy (*Brassica rapa*), carrot and upland rice.

Plant yield

Yield of spinach was reported to be significantly influenced by the combined application of biochar and FAA. Highest plant yield was observed on equal combination of biochar and FAA, which is under T3 with 2% biochar and 2% FAA. From this study, this combination seems to be effective in yield performance. However, further study needs to be done to determine the most effective combination since statistically there was no significant difference in yield between T3 (equal combination) and T4 (2% biochar and 4% FAA). The lower yield in T1 was as expected due to no application of N during its growth stage. However, T2 received 2% of N from biochar application, recorded low yield, probably due to incomplete mineralization of N from organic sources. However, the mineralized N was not measured in this study. This supposition was supported by Ren et al., (2014) who emphasized that organic manure applied must be mineralized before being utilized by crops. It plays a key role in the transfer of N either from soil or fertilizer to plant.

CONCLUSION

Soil treatment with 4% N and 6% N inputs (T3 and T4 respectively) showed favorable result for soil quality parameters (pH, total N and exchangeable Ca) under sandy clay soil. Besides, treatments T3 and T4 also have significant effect on plant total N as well as the yield. Sandy clay soil often has limited nutrient retention, due to higher soil porosity, by which macropores are relatively more than micropores. Biochar likely were able to improve (lower) soil porosity as well. Biochar combined with FAA (as organic source of N) on the percentages obtained from this study was found to be helpful and can support leafy vegetable (i.e. green spinach) N requirement. Further, study data can be scaled up to farm level for sustainable organic system.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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