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EFFECT OF DIFFERENT RATES OF ORGANIC NITROGEN INPUTS ON PLANT TOTAL NUTRIENT, UPTAKE, AND GROWTH PERFORMANCE OF LEAFY VEGETABLE

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ABSTRACT

In organic farming, nitrogen (N) is one of the most crucial factors. Other than the limited source of N for organic fertilizer as the key factor for growth-limiting nutrients in organic farming, the cost of fertilization is also affected by different N application rates in organic farming running. Therefore, in this study, emphasis was given to the effect of different rates of N in organic farming on the plant total nutrient, uptake, and growth performance of leafy vegetables. A study was conducted in a glasshouse at an integrated organic farming research area in the Malaysian Agricultural Research and Development Institute (MARDI) at Serdang, Selangor for two consecutive cycles. The experimental treatments were focused on the different rates of N inputs (12, 24, and 36 kg/ha of N from organic sources) with soil alone as a control. The treatments were applied 14 days after seeding (14-DAS) using green spinach as a test crop. The study result found that a 24-63 kg/ha rate of organic N input had improved N and P uptake in spinach (leafy vegetables), as well as their growth performances. Study findings have shown that the range of application rate of organic N between 24 to 36 kg/ha, promotes nutrient uptake in plants and can be scaled up to farm level to sustain organic system productivity.

Keywords: Organic N input, nutrients uptake, growth performance, leafy vegetables.

1. INTRODUCTION

The concept of nutrient management in organic agriculture is viewed differently than in conventional agriculture. The organic farming system emphasizes nutrient recycling to sustain the nutrient supplies to crop plants [1]. Nevertheless, for optimum growth and yield of organic produce, a sufficient amount of nutrients is required. Conventional produce also has the same requirements. However, under the organic farming system, the amount of nutrient requirement is not the same as in conventional farms. The amount of input applied in organic farming tends to reduce once the agricultural ecosystem has been established. By that time, the crop growth and performance will be synergistically supported by this complete ecosystem, and they will not rely fully on the fertilizer input application only [2]. The ultimate aim of organic agriculture is to supply the soil with nutrients but not directly feed the plants with soluble nutrients [3].

Despite that, managing nutrient uptake in relation to nitrogen (N) input is crucial, especially in organic farming systems since nutrient management through fertilization is an important and costly cultural practice, especially for organic vegetable growers. According to regulations, organic growers are limited to organic sources of N or those derived from natural processes only.

However, most of the organic sources contain a low amount of N compared to inorganic sources.

Generally, N is supplied in organic farming through biological fixation or organic sources input. For instance, in temperate countries like Europe, N is supplied through the inclusion of legumes such as red or white clover in grass mixtures. In Malaysia, N is mainly supplied by animal manure, and it is highly recommended to use processed and composted ones to reduce the potential health and environmental risk of applying raw manure. These manures are excellent organic nutrient sources for organic vegetable cultivation. On average, the amount of N ranges from 1- 4% [4]. Besides animal manure, plant biomass can be used as organic nutrient sources, for example, composted empty fruit bunches (EFB), green manure, and leguminous cover crops. Most of these plant-based compost contains between 1 – 3% of N.

Due to the limited source of organic N availability and a costly nutrient to manage, it becomes necessary to find out an optimum rate of organic N input for the effective management of fertilizer and nutrient uptake for plant growth under the organic agricultural system. Hence, to investigate the effect of organic N on plant physiology and nutritional quality, this component of the study was conducted to find out how different rates of N input affect nutrient uptake and crop growth performance under an organic agricultural system.

2.MATERIALS AND METHODS

2.1 Planting preparation and experimental design

This study was conducted for two cycles (cycles 1 and 2) under a rain shelter at the integrated organic farm, located at Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Serdang Station. In this study, green spinach (*Amaranthus* spp.), the rounded leaf variety was selected as the test crop. Green spinach was direct seeded in pots containing about 10 kg of mineral soils as media (sandy-clay type; 53.38% sand, 37% clay, and 9.57% silt). Spinach seeds were thinned to 7 plants/pot after approximately a week. Crop care throughout the planting period was done according to the standard agronomic practices; Composts were applied onto all pots at 160 g/ pot as a basal application before planting spinach. Fertilization (treatments) were applied manually for 14 days after seeding (14-DAS) on T2, T3, and T4 only, according to their specified rate of treatment. Watering was delivered thrice per day by an automatic system using a sprinkler, while pest and disease control was managed by using the close-netted structure throughout the experiment. The experiment was arranged in Randomized Completely Block Design (RCBD) with 4 treatments and 5 replications with each replication having 7 plants totaling 140 plants for every cycle of planting.

2.2 Treatment application

Treatments consist of a control which is T2 (12 kg N/ha) as current practice under the organic farming system and three (3) different rates of nitrogen (N) from organic sources were conducted to see their effects on nutrient uptake and plant growth. Detail of treatments: T1 (0 kg N/ha); T2 (12 kg N/ha); T3 (24 kg N/ha) and: T4 (36 kg N/ha). Sources of organic N for T2 are from biochar with enriched-N, while T3 and T4 contain enriched-N biochar and added fish amino acid (FAA). The selection of 12, 24, and 36 kg of N/ha as the application rate was referring to the current practice and the maximum level permitted for the organic farming system in Malaysia [5]. The organic source of N is from commercial organic fertilizer and Fish Amino Acid (FAA).

2.3 Leaf sampling and analysis

The whole plant parts of the spinach in each treatment were sampled at harvest (35-DAS) for the analysis. Dried plant samples (leave part) were ground to a fine powder for plant nutrient content analysis (N, P, K, Ca, and Mg). Thirty milligrams (30 mg) of dried plant samples and 30 mg of tungsten powder were weighed and wrapped into tin foil square and placed in the sampler for plant N content using CHNOS analyzer (ELEMENTAR Vario Macro Modules 11.44-5201). While for plant macronutrients (P, K, Ca, and Mg), plant samples were digested and determined using inductively coupled plasma-optical emission spectrometry (ICP-OES 7300 DV Perkin Elmer). The measurement of nutrient uptake by spinach was done by the calculation of percentage (%) of nutrients (for this study focusing on N and P) by dry weight (DW) of plant samples and normalized on an mg/kg DW basis.

2.4 Assessment of plant growth

Plant growth assessment was done on harvesting day (35-DAS). Five parameters were measured to see the effect of different rates of organic N inputs on plant growth, namely the plant height, number of leaves, total leaf area, fresh weight (yield), and dry weight of harvested spinach.

2.4.1 Plant height

Plant heights of spinach were measured from the base (soil level) to the top of each plant using a measuring tape and expressed in centimeters (cm).

2.4.2 Number of leaves

The number of leaves was determined by counting manually. Leaf number was the total number of leaves from stems and shoots.

2.4.3 Total leaf area

The total leaf area was determined using Automated Leaf Area Meter, ALAM (LI-3100, LICOR Inc., U.S.A.) on the harvested spinach (35-DAS). All leaves of each plant were separated from stems before being inserted on the conveyer of ALAM. The measured total leaf area was expressed in square centimeters (cm^2).

2.4.4 Fresh and dried weight

Harvested spinaches (35-DAS) were measured for fresh and dried weight. The fresh weight of spinach was measured immediately after harvesting using a digital balance (SQW-3, SmartWEIGH, Malaysia). Then, they were dried to constant weight at 80 ºC for 72 h in a drying oven (Model 100-800, Memmert, Germany) for the dry weight determination. The dried weight was measured using a semi-micro analytical digital balance (GR-200, A&D Company Limited, Japan). The fresh and dried weights of the plant were expressed in grams (g).

2.5 Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA). Treatment means were compared using Tukey's HSD test at a significance level of P<0.05 using SAS version 9.4. Pearson correlation analysis was carried out to correlate the nitrogen (N) uptake with the crop yield and antioxidant activity.

3.RESULTS AND DISCUSSION

3.1 Total nutrient content

The mean values of total nutrient content (total N, P, K, Ca, and Mg) in spinach at harvesting day (35-DAS) are presented in Table 1. The results revealed that the treatment effect only showed a significant difference in total N content. The significant difference ($P < 0.05$) for N content in leaves of spinach was observed on 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 ($P > 0.05$) between all the treatments used on harvesting day (35-DAS).

Table 1: Mean results of the total nutrient content of spinach treated under different rates of organic N (% N) input at 35-DAS

Treatment	*Nitrogen (N)*	*Phosphorus (P)*	*Potassium (K)*	*Calcium (Ca)*	*Magnesium (Mg)*
	(%)	------------------------ *(mg/kg)*			----------------------
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}
Mean	5.05	50.03	726.38	65.90	60.00
HSD ($P \leq 0.05$)	0.006	0.09	0.71	0.68	0.46
CV (%)	14.77	16.58	7.69	21.06	30.47

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Values (mean ± SD) 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.

Results from this study showed that the total N content of spinach leave was significantly ($P < 0.05$) influenced by the different rates of N applied as an organic fertilizer input (Figure 1). The addition of 2 and 4% of FAA with biochar distinctly increased the N content in spinach' leave by about 37 to 42% respectively compared to the current practice (T2) which received only biochar with enriched N as fertilizer input.

Initially, this result was expected to increase linearly with an increased application rate of N input. However, the analysis revealed that T1 with no input of N at all during the planting period showed higher N content compared to current practice (T2). This result is most likely due to the use of compost in the early stages of soil preparation before planting (basal treatment). The decomposition of the applied compost has increased the N content in the spinach leaves up to the comparable level with spinach that has been given with N input. A similar result was also observed from [6], their study of the moringa seedlings which only treated with compost applied four weeks before planting gave higher N content in leaves of moringa with 4.13% compared to moringa treated with NPK which N content of only 3.28%.

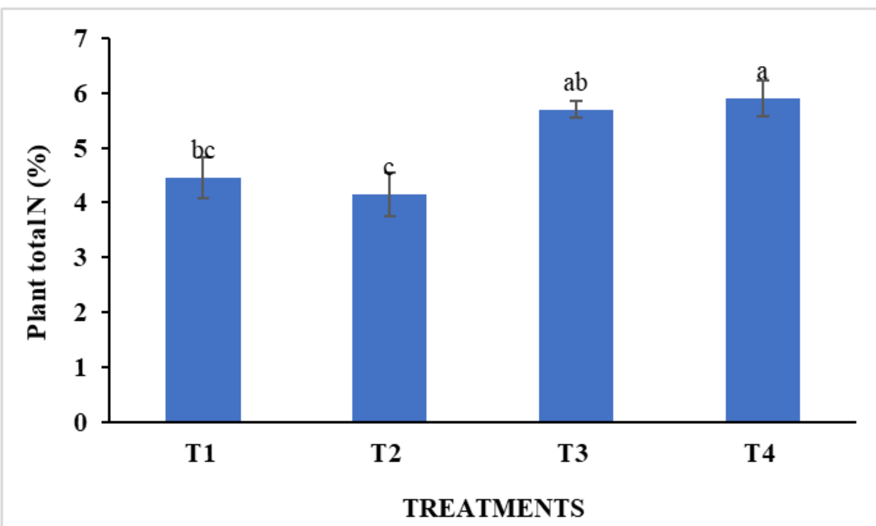

Figure 1: Mean values of total N in spinach treated with different rates of organic N input (kg N/ha) at 35-DAS. Means with different letters indicate a significant difference between treatments by Tukey's test at $P \leq 0.05$.

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 [7], this effect is associated with the function of organic acids resulting from the catabolism of amino acids which has led to more efficient uptake of N by spinach. In relation to this study, the effect on higher nutrient uptake has been proven by the significantly higher uptake of N by spinach

(Table 2) under T3 and T4 with respectively 176.86 and 172.80 mg/ kg DW of spinach. Apart from the effect of the presence of organic acids, there are also other studies for instance [8], [9] reported that the high N content is due to the possibility of direct acquisition of organic N from amino acids in the FAA or any input from the organic source which contain amino acids. The results of their study have shown that spinach is one of the crops that has such capabilities besides bak choy (*Brassica rapa*), carrot, and upland rice.

Table 2: Mean values of N and P uptake in spinach planted under different rates of organic N input

Treatment	*N uptake*	*P uptake*
	(mg/kg DW)	
T1 (0% N)	85.20 ± 42.82 [b]	5.33 ± 1.16 [a]
T2 (2% N)	81.34 ± 33.14 [b]	5.63 ± 0.83 [a]
T3 (4% N)	176.86 ± 42.77 [a]	4.80 ± 0.43 [a]
T4 (6% N)	172.80 ± 33.96 [a]	4.25 ± 0.71 [a]
Mean	129.05	5.00
HSD $_{(P \leq 0.05)}$	0.002	0.09
CV (%)	29.51	16.58

Values (mean ± SD) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at P ≤ 0.05. n=5 per treatment.

The values of N uptake by spinach reported by several researchers [10 - 12] are between 129-300 mg/kg DW of spinach. When referring to Table 2, the value of N uptake obtained under T3 and T4 was found to be within that range. While both of the control treatments; T1 and T2, the values were found to be relatively lower than the reported range.

The measurement of the uptake of N by spinach in this study was carried out as it is one of the requirements to explain the effect of different N levels from organic sources on plant growth. Other than that, the result of this measurement will be the basis for determining the effective N application rate (kg/ha of organic N fertilizer) for improved quality of organic spinach in terms of growth and its nutritional value without retarding the yield.

From this measurement, T3 and T4 also exhibited significant increases in the uptake of N by spinach with 176.86 and 172.80 mg/ kg DW of spinach respectively. This has clearly been attributed to the relative amount of N amino acid in FAA liquid containing T3 and T4. However, N taken up by spinach under T4 with the highest rate of N input had shown a slight decrease although the difference is not significant compared to T3, indicating that the addition of FAA-containing amino acids should be in an effective amount only because this effect had been seen by [13]. They found that the increased concentration of amino acids directly decreased the uptake of N by sweet grass *(Anthoxantum odoratum)*. The need to ensure that the FAA concentration is properly measured was agreed upon by [14], who emphasized that the concentration of the applied amino acid is a crucial factor because even at very low concentrations, their effect is very substantial.

3.2 Plant growth

Among all the fertilizer nutrients, N is crucially the most important for plant growth regardless of the organic or conventional farming system. Different levels of N application consequently affect the plant growth performance and production.

Therefore, in this study, the effect of different N rates on growth performance and spinach yield was measured in all treatments. The mean value of results on the growth performance of spinach were presented in Table 3. The parameters of plant height, number of leaves, total leaf area, plant fresh weight (yield), and plant dried weight (plant biomass) were measured at harvest day (35-DAS) indicative of spinach growth performance. Among all the parameters measured, it was found that only plant height and yield (fresh weight) of spinach were significantly affected by the different rates of organic N (% N) in the treatment application. However, there was no statistical difference detected in the number of leaves, total leaf area, and dry weight of the spinach.

Table 3: Mean results of growth performance of spinach treated under different rates of organic N input (% N) at 35-DAS

Treatment	*Plant height*	*Number of leaves/ plants*	*Total leaf area*	*Plant fresh weight*	*Plant dried weight*
	(cm/ plant)		*(cm^2 /plant)*	------- *(g /plant)* -------	
T1 (0% N)	36.64 ± 3.19^b	14 ± 2.91^a	316 30 ± 102.13^a	98.60 ± 32.38^b	26.20 ± 4.60^a
T2 (2% N)	38.94 ± 3.18^{ab}	12 ± 1.2^a	247.83 ± 80.28^a	111.20 ± 14.86^b	26.60 ± 2.88^a
T3 (4% N)	44.77 ± 2.50^a	15 ± 2.57^a	313.36 ± 125.57^a	215.60 ± 32.68^a	37.20 ± 9.9^a
T4 (6% N)	40.87 ± 4.78^{ab}	16 ± 2.84^a	364.78 ± 106.12^a	185.83 ± 16.46^a	31.09 ± 6.54^a

Mean	40.30	14.40	310.57	152.81	30.27
HSD (P ≤ 0.05)	0.03	0.18	0.49	<0.0001	0.09
CV (%)	9.54	18.55	37.60	18.41	22.71

Values (mean ± SD) with the same letter within the same column are not significantly different by Tukey's Studentized Range (HSD) test at P ≤ 0.05. n=5 per treatment.

As for plant height, there was a significant difference ($P < 0.05$) observed between the spinach treated with T3 compared to T1 (control) (Figure 2). T3 resulted in significantly highest plant height at 44.77 cm as compared to T1 with only 36.64 cm. This result indicated that plant height is highly dependent on a sufficient supply of N for their growth and development. This result was also supported by [15] who reported that the application of organic fertilizer in adequate amounts resulted in higher plant height due to its balanced nutrient contents.

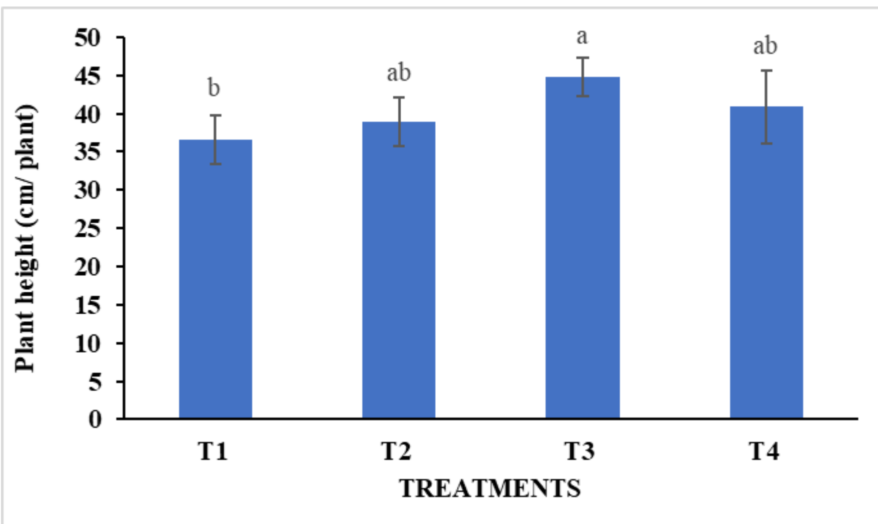

Figure 2: Mean values of plant height of spinach treated with different rates of organic N input (%) at 35-DAS. Means with different letters indicate significant difference between treatments by Tukey's test at P ≤ 0.05.

Apart from the factor of sufficient amount of N input, the synergistic effect due to combination of liquid FAA and enriched biochar also was being a key ingredient to this significant height of spinach. The use of FAA to stimulate plant height has been reported by many researchers. For instance [16 - 18] revealed that application of FAA significantly influenced the height their respective crop study namely spinach, mung bean and green bean. As explained by [19], this effect might be due to higher availability of N from FAA which has led to an increase of the cell division and metabolic activity resulting in higher plant height during the growth stage.

According to [19], there is a corresponding increase in plant fresh weight (FW) as plant height increased because water formed about 60% of the shoot system. In this study, fresh weight of spinach which measured immediately after the harvesting (35-DAS) is recorded as 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). Highly significant yield ($P < 0.0001$) of spinach was obtained on T3 with highest yield of 215.60 g/ plant compared to treatment control with only 98.60 and 111.20 g/ plant on T1 and T2 respectively. However, there was insignificant difference in yield of spinach between T3 and T4 (185.83 g/ plant) (Figure 3).

The lower yield in T1 was expected due to zero application of N during its growth stage. This result is most probably due to the incomplete mineralization of N from organic source. However, the mineralized N was not measured in this study. This estimation was supported by [20] which emphasized that organic manure applied must be mineralized before being utilized by crops. It is of great importance to play a key role in the transfer of N from soil/ fertilizer to plant.

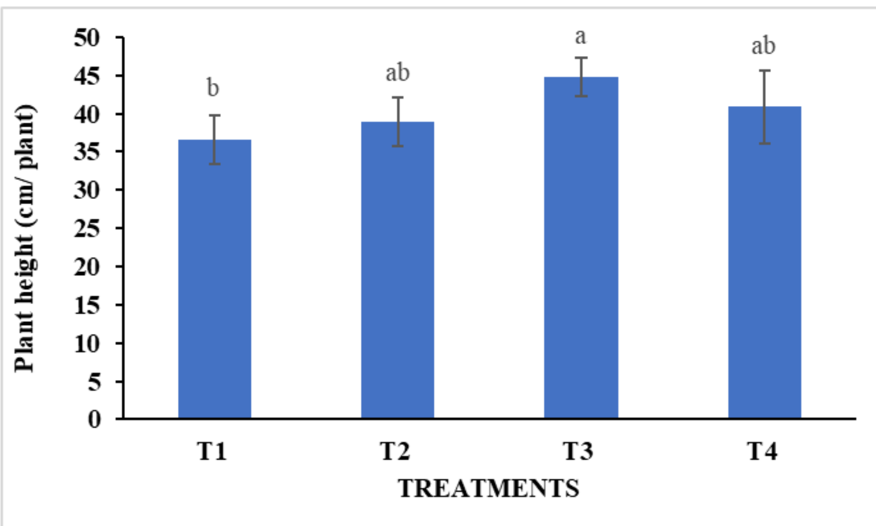

Figure 3: Effect of different rates of N input on plant fresh weight (yield) at 35-DAS. Means with different letters indicate significant difference between treatments by Tukey's test at $P \leq 0.05$.

4.CONCLUSION

It is undeniable that N is one the most important growth factors in controlling yield and plant quality, however the application of N is necessary in the appropriate amount so that it does not affect the crop quality and production and consequently the operational cost of the planting.

In the plant physiological component in this study, it concluded that N with 24 kg/ha (T3) was observed to have significant effect on total N uptake in the spinach as shown in the plant height and the fresh weight as well. This study showed that the use of organic N input at rate of 24 kg

N/ha was also able to increase the physiological properties of spinach in term of plant height and its yield performance.

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