ANTIOXIDANT ACTIVITY AND YIELD OF ORGANIC SPINACH (AMARANTHUS SPP.) IN RESPONSE TO DIFFERENT NITROGEN LEVELS

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ABSTRACT

One of the main reasons for the preferences toward organically produced food is due to health awareness, especially the nutritional values (antioxidant) provided by organic produce. Based on the previous study, antioxidant level is reported to have an association with nitrogen (N) uptake by plants. However, the information on the effect of different level of N input (%) on antioxidant activity in vegetable is still limited, especially in organic vegetables. Therefore, glasshouse experiments were conducted to determine the effect of different treatment of N level (%) namely T1: Control (0% N), T2: 2% N, T3: 4% N, T4: 6% N, T5: 10% N, T6:

15% N, T7: 20% N on antioxidant characteristics in organic spinach as test crop. The results revealed that antioxidant activity in spinach was enhanced under lower level (%) of N. Statistically, treatment with 4% N (T3) was found to be significantly ($P<0.05$) highest activity of antioxidant with 61.89 mg AA/ g DW sample compared to spinach grown under 10 – 20% N. The yield obtained under 4% N (T3) was also comparable with spinach treated under 15% N (T6). The negative correlation ($r = -0.192$) between antioxidant activity and N uptake implies that a lower uptake of N can induce plant antioxidant activity. These findings bring to the conclusion that 4-6% of N from organic sources, was identified as an effective amount to increase the antioxidant characteristic of spinach farmed under the organic system, and other similar leafy vegetables with no significant loss in plant growth and yield.

Keywords: Antioxidant, N uptake, organic vegetables, spinach.

1. INTRODUCTION

The consumption of organic food is currently increasing due to rising health consciousness alongside the growth of organic agriculture. Purchasing organic food is significantly influenced by the perception of its significant nutritional values and health benefits. Other than that, organic vegetables are free from genetically modified organism (GMO) and growth hormones. According to Lawrence et al. (2018), people with allergies to certain foods, chemicals and preservatives often found that their symptoms become lesser or disappeared when they consume only organic foods [1].

Among all the benefits offered by organic food, the nutrient contents in organic vegetables are the topmost priority for consumers in choosing organic produce. Their perception had been supported by several findings showing that organic vegetables have higher level of nutrient content such as Vitamin C, certain minerals and antioxidants compared to conventionally grown vegetables. Axel et al. (2017) reported that nutrients content differs between organic and conventional crops with modestly higher phenolic compounds in organic fruit and vegetables. In the same study, they also found that there was likely lower cadmium (Cd) content in organic cereal crops [2].

However recently, the higher levels of phytonutrients represent a powerful argument in elevating the health benefits of organic food. Even though that there were several reviews showing that organic produce contains more nutrients, however there are still some arguments and debate on the reported studies. The opposing viewpoints keep raising the question 'is organic food healthier than commercially grown food?' Labelling food as organic has become questionable whether organic foods are significantly healthier than their conventional counterparts. Therefore, in relation to the arguments, this statement has become an important issue for the organic researchers and players to do further research and always communicate their findings to justify consumers demand on more evidence of the health benefits of organic food.

The value of nutrients depend on several factors to produce higher nutrient content in organic food [3]. As reported in several findings before [6, 7, 8 and 9], some plants were seen to have higher level of phytochemicals and antioxidant compounds if some stress due to lower input of fertilization occur during its development. However, these findings are still not clear on the effective amount of N need to be taken up by plants in order to obtain a peak antioxidant level especially in organic plants. Hence, proper soil N management for high antioxidant level is necessary as such research in this aspect will ascertain and further strengthen the basic findings on organic produce in terms of N requirement and their nutrient contents for everyone's benefit.

Since there is still gaps in the understanding of N uptake in relation to antioxidant characteristics in leafy vegetables, thus the investigation on this aspect becomes necessary to be carried in order to have a better understanding regarding this issue and to confirm either the findings is in agreement or contradictory with the previous studies as reviewed before.

Hence, in this study, the effect of different level of N on the increment of antioxidant characteristics in organic spinach will be studied since N management under organic farming is very important. Previously, several studies on nutrient management for organic vegetables cultivation had been carried out, however study on the effect of different N level to plants antioxidant activity has not been studied yet for organic farming especially in Malaysia. Thus, in this research, the focus will be emphasized on the effect of different level of N application (input) in order to upgrade the nutrient content of organic produce.

2. MATERIALS AND METHODS

Planting preparation and Experimental design

This study was conducted for two cycles under a rain shelter at the integrated organic farm, located in Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Serdang station. In this study, green spinach (Amaranthus spp.), the rounded leaf variety was selected as test crop. Green spinach was direct seeded in pots containing about

10 kg mineral soils as media (sandy-clay type). Spinach seeds were thinned to 10 plants/pot after approximately a week. Fertilization, watering and other crop care were followed according to the standard agronomic practices. The experiment was arranged in Randomized Completely Block Design (RCBD) with 7 treatments and 5 replications totalling 35 pots.

Treatment and sample collection

Seven types of treatment were conducted as comparison treatment to see their effects on antioxidant activity of planted spinach. Treatments consist of control and six (6) types of treatments were differentiated from each other in the level of nitrogen (N) percentage (%) with uniform application rate of 2 tonne/ hectare (t/ha). Details of the treatments are as follow:

Treatment 1 (T1) = N input level (0%) Treatment 2 (T2) = N input level (2%) Treatment 3 (T3) = N input level (4%) Treatment 4 (T4) = N input level (6%) Treatment 5 (T5) = N input level (10%) Treatment 6 (T6) = N input level (15%) Treatment 7 (T7) = N input level (20%)

All treatments were applied at 14-15 days after seeding (DAS) at rates of 8.0 g/pot. Plants sampling was done at harvesting (30-DAS) for yield data collection and further analysis on plant antioxidant activity.

Plant drying and extraction

Fresh edible part (above ground level) of harvested spinaches (30-DAS) were dried in drying oven at 40oC for 3 days before grinding into finely powdered form. One-gram (g) of powdered plant sample was extracted with 70% (v/v) methanol in the ratio of 1:20 (sample weight: volume of solvent). Then, the sample mixtures were shaken overnight in closed conical flask. After 24 hours, the sample mixtures were transferred into centrifuge tubes and centrifuged for three times. Each time, the sample mixtures was centrifuged at 10,000 rpm for 10 minutes. Then, the supernatant was collected into screwed-cap sample bottles. The deposited samples in the centrifuge tubes were then added with another 20 mL of 70% (v/v) methanol and then centrifuged again at 10,000 rpm for 10 minutes. This extraction was assisted with ultrasound (sonicates) for 30 minutes. This process was repeated for three times. Then, the collected supernatant of spinach (extracted plant sample) was kept at 4 o C as sample stock solution for further assay analyses. This extraction method was done by referring to [4] with slight modifications on the extraction process by increasing the number of centrifuging processes into three times in order to increase the yield extracts.

Assessment of plant antioxidant (DPPH Assay)

Antioxidant activity of the plant samples was assayed using stable free radical namely 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The principle of this assay is to measure the capacity of the antioxidant to scavenge the free radicals (DPPH) present in the sample. This DPPH radical-

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scavenging activity was determined using the method described by [5] with some modifications. Prior to antioxidant measurement, the methanolic DPPH reagent was prepared first by dissolving the 0.00789 g of powdered DPPH (Aldrich) into 20 mL of 100% methanol. As precaution, the reagent bottle

Statistical analysis

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

3 RESULTS AND DISCUSSION

Plant antioxidant

The mean value results of antioxidant activity of spinach planted under this study were presented in Table 1. The antioxidant activity was expressed in ascorbic acid (AA) equivalent to the dry matter weight of plant sample (mg AA/g DW sample). The results showed that T3 (4% N) had significantly (P<0.05) highest activity of antioxidant with the value of 61.89 mg AA/g DW sample compared to T1 (control) and T4 (6% N). In comparison with spinach grown under higher N input between 10 – 20% of N (T5-T7), the antioxidant activities resulted in lower values between 39-49 mg AA/ g DW sample

Table 1: Mean results of antioxidant activity and yield of spinach planted under different level (%) of N input needs to be wrapped with aluminium foil and freshly

Treatment	*N uptake (NU)*	*Antioxidant activity (DPPH)*	*Yield*
	(mg/ kg DW)	*(mg AA/g DW sample)*	*(g/ pot)*
T1	85.20b *	20.07b	98.60c
T2	81.34b	22.03ab	111.20c
T3	176.86a	61.89a	215.60ab
T4	172.80a	18.65b	185.83ab
T5	157.45ab	39.45ab	181.80ab
T6	194.44a	29.02ab	234.00a
T7	153.17ab	49.48ab	174.35b

*Mean values with the same letter in the column are significantly different by Tukey's Studentized Range (HSD) test at P<0.05

Prepared for every measurement to avoid oxidation as it is light sensitive. For each measurement, the sample stock solution was diluted to a series of dilution with 70% (v/v) methanol. An aliquot of each dilution was mixed with methanolic DPPH reagent and mixed thoroughly. Then, the mixture was kept in the dark for 30 minutes before measuring with Gen5 BioTek spectrometer at 517 nm. All measurements were carried out in triplicates. The standard (positive control) used

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was L-ascorbic acid (HmbG). Results obtained in IC50 value (mg/mL) were then converted into ascorbic acid equivalent (AA Eq.) and antioxidant activity of the sample was expressed as (mg AA/ g DW sample).

Amount of antioxidant in spinach that was found to be significantly higher at the lower level of N input (4% N) as shown in Figure 1 was in line with the hypothesis of this study; N stress (low N) in leafy vegetable able to improve plant antioxidant level. This finding is also in agreement with several previous studies [6, 7, 8 and 9]. The explanation made by [3] from his finding was used as a basis to justify this high antioxidant production in low N inputs (4% N) from this study. It was explained that when the plant is experiencing N deficiency, it will automatically form a mechanism of internal protection to ensure the growth and survival of the plant. The formation of this mechanism at the same time increases the phytonutrient (antioxidant) content of the plant itself [8] also explained the same mechanism whereby the deprivation of N in plants due to lower N input had induced their antioxidant activity to the higher level as their defence mechanism and survival to keep growing.

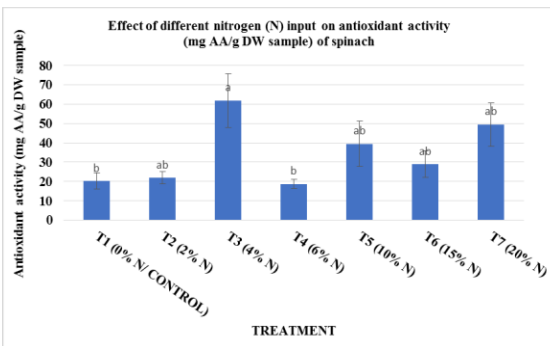

Figure 1: Mean values of plant antioxidant (mg AA/ g DW sample) treated with different level (%) of N input

Other than the explanation done by [3], [10] implied that the lower antioxidant value at higher N input was due to the application of N input that exceeded the crop needs. This excess of N has caused the accumulation in plant tissue which consequently produced the reactive oxygen species (ROS). According to [11], ROS are produced as a normal product of plant cellular metabolism to regulate the various environmental stress due to abiotic and biotic factors. Under normal growth condition, the ROS produced is low, however, in response to various environmental stresses, ROS are drastically increased in plants disturbing the normal balance. The excessive production of ROS causing progressive oxidative damage and ultimately cell death. In relation to this study; the activation of antioxidant in spinach planted under higher N input was speculated to be inadequate to detoxify the excess ROS generated in plants with excess N, which eventually degenerated the antioxidant activity of the spinach.

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Pearson correlation between N uptake, yield and antioxidant activity because of different N input

Table 2 shows the results obtained from Pearson correlation between various variables in the field and laboratory assessment. In order to compare the relationship between N uptake (NU) by spinach with the fresh yield produced (FW) from this study, the comparison then was correlated and it resulted in statistically significant ($P<0.05$) positive correlation ($r = 0.486$) between both parameters. This result indicated that higher N intake by spinach is important for crop growth that gives positive effects to the crop yield performance.

Table 2: Pearson correlation between parameters of N-uptake, yield and antioxidant activity of spinach because of different level (%) of N input

Parameters	*1*	*2*	*3*
1. N-uptake	1.000		
2. Yield (FW)	0.486*	1.000	
3. Antioxidant activity (AOA)	-0.192	-0.119	1.000

Meanwhile, the correlation between N uptakes (NU) on antioxidant activity (AOA) of spinach was done in order to evaluate the relationship of both parameters in relation to this study. The correlation developed diagnosed that there was insignificant ($P>0.05$) negative correlation between NU and AOA analysed using DPPH assay. The negative correlation coefficient ($r = -0.192$) between those two variables was considered as weak. However, the trend of this negative relationship between N uptake in spinach and its antioxidant activity were in line with the hypothesis of this study. It showed that higher antioxidant activity occurs at lower N uptake by spinach. This finding was found to be in agreement with several similar studies done by other researchers and their co-workers [1 and 8]. They explained that plant with lower amount of N can induce plant antioxidant activities as their defence mechanism. However, all the finding justifications used under this study were referring to studies under conventional farming methods. The justifications are relevant though due to the application mechanisms that are similar for plant grown under both inorganic and organic farming systems. In addition, this research used findings from conventional system as a basic reference because up to our best knowledge, there has not been any current findings under organic farming system in Malaysia reported, in relation to the effect of different N inputs (in term of percentage) on plant antioxidant activity.

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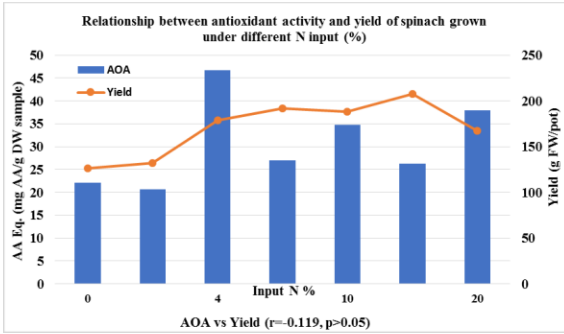

Figure 2: Relationship between antioxidant activity and yield of spinach treated under different level (%) of N input

For the determination of effective amount of N input, it was done by correlating the N input percentage (%) with the antioxidant activity and yield performance of the spinach. Based on the weak insignificant (P>0.05) negative correlation (r = - 0.119), it suggests that N input (%) between the range of 4-6 % produced the highest antioxidant activity with comparable yield (FW) was the effective amount of N input (%) for organic farming system. Referring to Figure 2, at 10% of N input, the correlation between antioxidant activity and yield was also found as the promising relationship (higher antioxidant activity with higher yield), however, the amount of 10% was considered high for organic input which is non cost effective compared to 4-6% N input. That recommended amount of N (4-6%) was found not only able to increase the antioxidant characteristic, but the yield produced is also comparable to the yield obtained under higher N input (15% N) without retarding the plant qualities.

4. CONCLUSION

In relation to this study, the factor of nutrient stress was studied in order to assess the effect of different N input to the activity of antioxidant in leafy vegetable (spinach) and the result obtained would serve as the basis for effective N management in enhancing antioxidant characteristic in organic spinach. The findings from this study concluded that N at a lower amount (ranged between 4-6%) was significantly effective in increasing the antioxidant activity in spinach without retarding the crop yield. These significant results indicate that lower N input exhibited positive response on the improvement of antioxidant activity in spinach, making it a more valuable food serving.

REFERENCES

[1] Lawrence Robinson, J. Segal, and R. Segal, "Organic Foods : What You Need to Know - The Benefits and Basics of Organic Food and How to Keep It." 2018.

[2] A. Mie, H. R. Andersen, S. Gunnarsson, J. Kahl, and E. Kesse-guyot, "Human health implications of organic food and organic agriculture : a comprehensive review," Environ. Heal., vol. 16, no. 111, pp. 1–22, 2017.

[3] S. Heaton, "Antioxidants & Organic Food," no. 1–3. Clean Food Organic, Australia, 2008.

[4] Antonios Chrysargyris, E. Nikolaidou, A. Stamatakis, and N. Tzortzakis, "Vegetative, physiological, nutritional and antioxidant behavior of spearmint (Mentha spicata L.) in response to different nitrogen supply in hydrophonics," J. Appl. Res. Med. Aromat. Plants, vol. 6, pp. 52–61, 2017.

[5] S. A. Akter and J. . M., Eun, "Solvent effects on antioxidant properties of persimmon (Diospyros kaki L. cv. Daebong) seeds," Int. J. Food Sci. Tech., vol. 45, pp. 2258–2264, 2010.

[6] V. Worthington, "Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains," J. Altern. Complement. Med., vol. 7, no. 2, pp. 161–173, 2001.

[7] G. E. Lester and R. A. Saftner, "Organically versus Conventionally Grown Produce : Common Production Inputs , Nutritional Quality

, and Nitrogen Delivery between the Two Systems," J. Agric. Food Chem., vol. 59, no. 19, pp. 10401–10406, 2011.

[8] H. Yasuor, A. Ben-Gal, U. Yermiyahu, E. Beit- Yannai, and S. Cohen, "Nitrogen management of greenhouse pepper production: Agronomic, nutritional, and environmental implications," HortScience, vol. 48, no. 10, pp. 1241–1249,2013.

[9] R. Koyama, H. Itoh, S. Kimura, A. Morioka, and Y. Uno, "Augmentation of antioxidant constituents by drought stress to roots in leafy vegetables," Horttechnology, vol. 22, no. 1, pp. 121–125, 2012.

[10] L. Kong, Y. Xie, L. Hu, J. Si, and Z. Wang, "Excessive nitrogen application dampens antioxidant capacity and grain filling in wheat as revealed by metabolic and physiological analyses," Sci. Rep., vol. 7, pp. 1–14, 2017.

[11] P. Sharma, A. B. Jha, R. S. Dubey, and M. Pessarakli, "Reactive Oxygen Species , Oxidative Damage , and Antioxidative Defense Mechanism in Plants under Stressful Conditions," vol. 2012, pp. 1–27, 2012.