UNIVERSITI PUTRA MALAYSIA

GROWTH AND YIELD QUALITY OF ORGANIC SPINACH (Amaranthus spp.) IN RESPONSE TO DIFFERENT NITROGEN FERTILIZER SOURCES AND RATES

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By

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The use of different organic nitrogen (N) fertilizers were found to give different results in terms of soil fertility, growth and nutrient content of plants. A test by [1] on onions planted on a growth media mix treated with amino acid significantly had ($P < 0.05$) the highest Ca, N and leaf chlorophyll contents. As well as significantly ($P < 0.05$) increased anthocyanin content, the overall plant growth was improved by the amino acid treatment. As reported by [2], positive improvement was observed on acidic soils treated with biochar in the form of a significant corn crop yield effect. Therefore, to study the effect of different inputs of nitrogen on organic leafy vegetables, a greenhouse experiment was conducted on spinach to compare the effect of different treatments of organic N (% of organic N), namely T1: 0% N (Control without any fertilizer), T2: 2% N (Current practice control with 2% N from biochar), T3: 4% N (2% N from biochar + 2% N from fish amino acid/FAA), and T4: 6% N (2% N from biochar + 4% N from FAA) on soil chemical properties, plant physiology and plant phytochemical content (antioxidant, total phenolic and flavonoid content). The research trials were carried out at the integrated organic farming, MARDI Serdang in two cycles. Treatments were applied 14 days after seeding (DAS) at a uniform rate of 8 g/pot (equivalent to 2 t/ha). The results obtained revealed that treatment with T3 (2% N from biochar + 2% N from FAA) had significantly ($P < 0.05$) improved the soil CEC, C, available-P and exchangeable-Ca. Meanwhile, the soil pH was found to remain stable from the initial seeding (0-day) until the harvesting day (35-DAS) under the current practice using 2% N from biochar (T2). Improved nitrogen uptake by spinach was also observed under T3. The plant height and fresh weight of spinach differed significantly ($P < 0.05$) under T3. Spinach analysed using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) statistically showed that the treatment with 4% N (T3) was found to have significantly ($P < 0.05$) the highest activity of antioxidant with 61.89 mg AA/ DW g per sample compared to spinach grown under both control treatments and 6% N input (T4). These findings bring to the conclusion that 4% of N

from organic sources has been identified as an effective amount to improve the antioxidant activity of spinach, other similar leafy vegetables, farmed under the organic system with no significant loss in plant growth and yield. The way forward for this research is to expand the investigation on the other sources of organic materials and to measure the mineralized nitrogen and other secondary metabolites to strengthen the finding and potential benefits, especially for our local organic agriculture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERTUMBUHAN DAN KUALITI HASIL BAYAM ORGANIK (*Amaranthus* spp.) TERHADAP PERBEZAAN KADAR DAN SUMBER BAJA

Oleh

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Penggunaan baja organik nitrogen (N) yang berbeza didapati memberikan hasil yang berbeza dari segi kesuburan tanah, pertumbuhan dan kandungan nutrisi tanaman. Kajian oleh [1] pada bawang yang ditanam menggunakan campuran media yang yang dirawat dengan amino asid secara signifikan ($P < 0.05$) telah meningkatkan kandungan Ca, N dan klorofil daun. Kandungan antosianin dan pertumbuhan keseluruhan tanaman juga telah meningkat secara signifikan ($P < 0.05$) dengan rawatan asid amino. Seperti yang telah dilaporkan oleh [2], peningkatan positif telah diperhatikan pada tanah berasid yang telah dirawat dengan arang-bio, di mana kesan yang signifikan telah ditunjukkan pada hasil tanaman jagung. Oleh itu, bagi mengkaji kesan input nitrogen yang berbeza pada sayuran berdaun yang ditanam secara organik, percubaan rumah hijau dilakukan pada bayam untuk membandingkan kesan rawatan yang berbeza tahap kandungan organik N (% organik N) iaitu T1: 0% N (Kawalan tanpa baja), T2: 2% N (Kawalan dari amalan semasa dengan 2% N dari arang-bio), T3: 4% N (2% N dari arang-bio + 2% N asid amino dari sumber ikan / FAA), T4: 6% N (2% N dari arang-bio + 4% N dari FAA) pada sifat kimia tanah, fisiologi tanaman dan kandungan fitokimia tanaman (antioksida, jumlah kandungan fenolik dan flavonoid). Percubaan lapangan telah dilakukan di ladang organik bersepadu, MARDI Serdang dalam dua kitaran penanaman. Rawatan diberikan pada hari ke-14 hari selepas pembenihan (14-DAS) pada kadar seragam iaitu 8 g / pot tanaman (kadar setara dengan 2 t / ha). Hasil yang diperoleh telah menunjukkan bahawa rawatan di bawah T3 (2% N dari arang-bio + 2% N dari FAA) secara signifikan ($P < 0.05$) telah meningkatkan nilai CEC, C, P-tersedia dan kebolehtukaran-Ca tanah. Sementara pH tanah didapati tetap stabil dari awal (0-hari) hingga hari penuaian (35-DAS) di bawah rawatan amalan semasa yang menggunakan 2% N dari biochar (T2). Pengambilan nitrogen yang lebih tinggi oleh bayam juga diperhatikan di bawah rawatan T3. Ketinggian tanaman dan berat basah bayam juga berbeza dengan ketara ($P < 0.05$) di bawah rawatan T3. Bayam yang telah dianalisa menggunakan 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) secara statistik menunjukkan, rawatan dengan 4% N

(T3) mempunyai nilai aktiviti antioksida yang tertinggi dan signifikan ($P < 0.05$) iaitu sebanyak 61.89 mg sampel AA / g DW (g berat kering) berbanding bayam yang ditanam menggunakan kedua-dua rawatan kawalan dan 6% N input (T4). Penemuan ini membawa kepada kesimpulan bahawa 4% N dari sumber organik, dikenal pasti sebagai jumlah yang berkesan untuk meningkatkan aktiviti antioksidan bayam yang ditanam di bawah sistem organik, dan sayur-sayuran berdaun lain yang seumpamanya tanpa pengurangan ketara pada pertumbuhan dan hasil tanaman. Untuk penyelidikan masa hadapan, dicadangkan supaya bidang penyelidikan ini diluaskan pada sumber bahan organik yang lain dan turut menilai kandungan nitrogen yang diurai di dalam tanah serta menganalisa kandungan metabolit sekunder yang lain bagi memperkukuhkan lagi penemuan dan melihat faedah yang berpotensi dalam memberi manfaat terutama bagi pertanian organik tempatan di negara kita.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
AA Eq.	Ascorbic Acid Equivalent
Al	Aluminium
ALAM	Automated Leaf Area Meter
$AlCl_3$	Aluminium Chloride
ANOVA	Analysis of Variance
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
CETDEM	Centre for Environment, Technology and Development Centre
CHNOS	Carbon, Hydrogen, Nitrogen, Oxygen, Sulphur Analyzer
cm	Centimetre
CoA	Certificate of Analysis
DAS	Day After Seeding
DOA	Department of Agriculture
DPPH	Diphenyl Picrylhydrazyl Hydrate
DW	Dry Weight
EFB	Empty Fruity Bunch
Exc-Ca	Exchangeable Calcium
EPU	Economic Plan Unit
FAA	Fish Amino Acid
FAO	Food and Agriculture Organization
FC	Folin-Ciocalteu

Fe	Iron
Fm	Maximum fluorescence
Fo	Initial fluorescence
Fv	Variable fluorescence
FW	Fresh Weight
GA	Gallic Acid
GMO	Genetically Modified Organisms
g	Gram
H_2O	Water
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
ha	Hectare
IFOAM	International Federation of Organic Agriculture Movements
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IOAS	International Organic Accreditation Service
K	Potassium
KCl	Potassium Chloride
K_2SO_4	Potassium Sulphate
Kg	Kilogram
MARDI	Malaysian Agricultural Research and Development Institute
Mg	Magnesium
MOA	Ministry of Agriculture
MS 1529	Malaysian Standard for Plant-based Organically Produced Foods
mg	milligram

mm	millimetre
mL	millilitre
myOrganic	Malaysian Organic Certificate
µg	Microgram
N	Nitrogen
N	Normality
Na_2CO_3	Natrium Carbonate
$NaNO_2$	Natrium Nitrite
NAP3	Third National Agricultural Policy
NaOH	Natrium Hydroxide
NGO	Non-Governmental Organization
NH_4OAc	Ammonium Acetate
NH_4	Ammonium
NH_4-N	Ammonium-Nitrogen
NH_4F	Ammonium Fluoride
NO_3-N	Nitrate-Nitrogen
NU	Nutrient Uptake
nm	Nanometre
P	Phosphorus
p	Probability
PEA	Plant Efficiency Analyzer
PO_4^{3-}	Phosphate ion
r	Pearson's Correlation Coefficient
RCBD	Randomized Completely Block Design
RCC	Relative Chlorophyll Content

RM	Ringgit Malaysia
ROS	Reactive Oxygen Species
R&D	Research and Development
SD	Standard Deviation
SIRIM	Standard and Industrial Research Institute of Malaysia
SCP	Sustainable Consumption and Production
SWITCH-Asia	Grant programme for Asia funded by the European Union (EU) as part of the global Sustainable Consumption and Production (SCP) programme
TF	Total Flavonoid
TPC	Total Phenolic Content
T	Tonne
t/ha	Tonne/hectare
UN	United Nation
US	United States
USD	United States Dollar
UV-Vis	Ultraviolet Visible Spectrophotometry
V/V	Volume by Volume
WHO	World Health Organization
W/V	Weight by Volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Overview of organic farming

Historically, organic farming is not a new method in agricultural practice in this world. This method has been practiced originally for thousands of years since prehistoric times. Traditional farming is considered organic as practiced before the industrial revolution. The revolution then introduced the inorganic methods in late 1960s known as the green revolution [3]. However, this era of green revolution relies heavily on synthetic fertilizers and pesticides, and subsequently causes harmful side effects especially to the environment. Some of the negative impacts due to heavy reliance on the new agricultural technique are: soil toxicity, loss of soil fertility, soil erosion, diminishing water resources, pollution of underground water, increased incidence of human and livestock diseases, global warming and emergence of poisonous weeds and pests that are difficult to control [4]. Due to these disastrous results, especially in the environmental issues and food safety scandals, a movement towards organic farming system as an alternative to the inorganic agricultural system had begun in the 1940s as a reaction towards negative effects of rapidly changing farming practices and agriculture methods that rely heavily on these new synthetics [3].

Organic farming or organic agriculture can be defined as an integrated farming system that strives for sustainability, the enhancement of soil fertility and biological diversity whilst, with rare exceptions, prohibiting synthetic pesticides, antibiotics, synthetic fertilizers, genetically modified organisms (GMO) and growth hormones [5]. The aim of organic farming is to balance the interaction between soils, organisms, plants, animals and human in agricultural ecosystem.

This current revival of organic agricultural methods is internationally regulated and legally enforced by many nations, based on large parts on the standard set by the International Federation of Organic Agriculture Movements (IFOAM), an international umbrella organization for organic farming which was established in 1972 [4].

Other than the environmental issues mentioned before, the other major concern towards organically produced food is mainly due to health issues. Non-organically produced food, in brief, contains more traces of artificial chemical fertilizers and pesticide residues compared to organic food. Continuous consumption of these foods may lead to gradual ‘bioaccumulation’ of toxins and unnecessary chemicals in our body. Subsequently, this will lead to various health-related problems like obesity, diabetes, coronary heart disease and cancer [6 and 7]. Due to these problems, consumers are becoming more aware of the effect of food consumption on their health. Awareness on these health issues has become one of the main reasons for consumers

to opt for organic food. Organic foods are clear in its origin and all applied farming systems are regulated and certified free from pesticides and synthetic fertilizers.

Furthermore, organic food is perceived to be safe and superior compared to conventional farming products especially in their nutritious values [3]. One of the most important nutrients referred is the antioxidant content. Antioxidant, in brief, is a molecule that slows or prevents the oxidation of other chemicals. It is found in varying amounts in foods especially in vegetables, fruits, cereals and nuts. Plant antioxidants are vital constituents in foods, promoting both plant and human well-being. They can be categorized based on their activity, either enzymatic or non-enzymatic antioxidants. The enzymatic antioxidants work by breaking down and removing free radicals, while non-enzymatic antioxidants work by interrupting free radical chain reactions [6].

Most of the studies revealed that organic farming methods can increase concentrations of antioxidants in vegetables, fruits, grains and dairy products. Elevated antioxidant levels were found in about 85 percent of the cases studied to date, where the average levels were about 30 percent higher compared to conventionally grown food [7]. The concentrations of a range of antioxidants were found to be substantially higher in organic crops, with those of phenolic acids, flavanones, flavanols and anthocyanins being an estimated 19, 69, 50 and 51% higher than conventional crops, respectively [8]. Based on these reported results, the higher antioxidant activities found in organic crops indicate the high potential of nutritional benefits, where many of these compounds have previously been linked to a reduced risk of chronic diseases, including certain cancers, cardiovascular and neurodegenerative diseases.

This antioxidant level is reported to have an association with nitrogen (N) uptake by plants as well as its (N) role as primary macronutrient for plant growth and development [9]. Besides its role as an important nutrient that affect growth, development, fruit yield and plant quality, N is also an essential mineral for the biosynthesis of amino acids and enzymes, which are principal components of antioxidants [10].

The consistent consumption of organic foods may result in increasing levels of antioxidant recommended for human nutrition. Even though there is no specified recommended daily amount for antioxidants intake, dieticians highly recommend and encourage us to consume a varied diet with at least five servings of fruits and vegetables every day for antioxidant's requirement in our body. These reported estimated concentrations of antioxidants would be in line with the dietary recommendations of antioxidants to be consumed daily, as the amount reported is considered to be equivalent to the number of antioxidants present in one to two portions of fruits and vegetables recommended to be consumed daily [11]. Medical experts also agree that it is best to obtain antioxidants from eating foods rather than from taking supplements.

1.2 Problem statement

Low N content in organic fertilizers as compared to chemical fertilizer

Thus, what is the problem with the current organic farming system? The organic food industry in Malaysia is facing several challenges. Although the demand for organic food in Malaysia is growing, the supply of local organic products is unable to keep up with the increased demand. In addition to the inconsistent supply, the variety of local organic food is also limited. One of the biggest challenges faced in organic farming, especially in Malaysia, is the low nutrient content from organic sources of fertilizer compared to inorganic fertilizers [12]. However, most of the sources for organic fertilizers contain low amount of N compared to inorganic fertilizers. As mentioned by [13], N content (%) in organic nutrient sources ranges between 1.7 – 3.3%. Therefore, this low amount of N has become a problem to organic growers because N supply is restricting organic crop production. As a result, organic growers have put higher rate of application, mostly between 40-50 t/ha of organic fertilizers, in order to provide more N required for optimum crops growth and yield. The rate of application of organic fertilizer is four times higher than the conventional rate. This practice, however, incurs higher expenditure of fertilizer cost. Besides, using this large quantity of organic sources can also cause wastage and will eventually lead to increment in farm operational cost.

For that reason, the questions that arise for this research are: Firstly, on the quantity of N required by organic crops; does a higher N input or by lowering N input actually provide adequate supply needed for crop production. Secondly, is there any effect or potential relationship in the amount of N taken up by plants on the plants nutritional values (antioxidant) and finally, if there is a correlation between N uptake and antioxidant; and if so, what is the trend of the correlation to be used as a basis for the determination of effective amount of N needed in order to achieve higher antioxidant content of plants (organic spinach) without retarding the crop growth and yield.

1.3 Objectives

The aim of this research study is to determine the effective amount of N from organic sources needed in order to increase their antioxidant content without retarding the crop growth and yield. The following three specific objectives have been outlined in order to achieve the research aim:

1. To assess the effect of different organic N application input (% of organic N) on soil chemical properties and plant tissue.
2. To evaluate the effect of nitrogen (N) uptake by plant on growth quality and antioxidant content of organic spinach.
3. To determine the effective organic N application input (% of organic N) for higher antioxidant content in organic spinach planted on mineral soil.

1.4 Significance of study

The demand for organic produce especially in Malaysia is projected to grow more than 12.4% a year with the financial value of more than RM20 million a year [14]. However, there is still a problem in terms of expensive organic produce prices, catering only for a niche market. The high price is closely related to high operating costs of the farm, mainly contributed by high usage and subsequently, the cost of organic fertilizer [3].

The findings from [15, 16 and 17] revealed that N stress (N deprivation) in crop can induce high plants antioxidants activity; this study hypothesizes that giving N at lower and higher input will result in different amount of plant antioxidant level.

If the hypothesis proves that lower N input produces higher antioxidant plants, therefore a decrease in N supply for improving antioxidant capacity could be used as a strategic tool to enhance both the quality and profitability of organic crops. In addition to that understanding, it is indicative that the problem of low N in organic farming will become an opportunity, as it will turn into an added benefit to the improvement of crops quality especially the nutritional value (antioxidant). In addition, it will greatly benefit the organic farming industry in cost saving by solving the problem of high organic fertilizers consumption. As N management is one of the most important and significant factors, this research is focusing on the determination of an effective amount of N (%) required to increase antioxidant characteristic in organic vegetables with sustainable productivity.

Thus, it is hoped that the success of this study can enable organic growers to reduce their farm operational costs and eventually motivates more farmers to grow organically. Therefore, when the supply can meet the market demand, the price of organic produce can be reduced, and thus it will become affordable for the masses.

Overall, the outcomes from this study include strategies to increase the plants nutritional content especially the antioxidant level, thereby solving the problem of large input of organic fertilizers and reducing costs in organic farming management, which justifies the significance of this study.

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LIST OF PUBLICATION

Publication in journal

Faridah, M., Roslan, I, Arina Shairah, A.S. and Razali, M. 2019. Antioxidant activity and yield of organic spinach (Amaranthus Spp.) in response to different nitrogen levels. International Journal of Agriculture, Environment and Bioresearch (submitted).

Publication in proceeding of conferences and seminars

Faridah, M., Roslan, I., Arina Shairah, A.S. and Razali, M. 2019. Does different nitrogen (N) input affect yield and phytochemical content of organic leafy vegetables. Poster presentation in South East Asia Vegetables (SEAVEG) Symposium, Melaka. 9-11 July 2019.

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