EFFECT OF ORGANIC SOIL AMENDMENTS ON GROWTH, PHYTOCHEMICAL CONTENT AND ANTIMICROBIAL ACTIVITY OF SABAH SNAKE GRASS (*Clinacanthus nutans*)

TEH NING WEI

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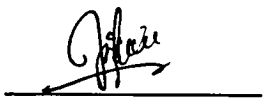

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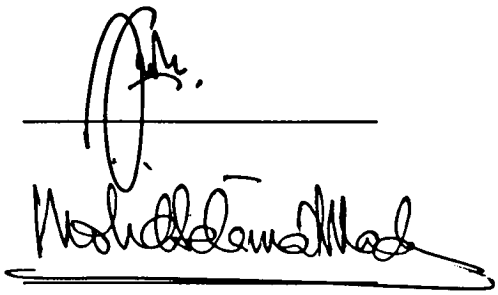

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ABSTRACT

Clinacanthus nutans has been used to treat various diseases in Asian countries. The leaves of *C. nutans* contain high level of antioxidant activity which is important in the protective against oxidative damage of human body. The study was conducted under the rainshelter and laboratory of Faculty of Sustainable Agriculture, Universiti Malaysia Sabah to determine the effect of organic soil amendments on growth, phytochemical content and antimicrobial activity of Sabah Snake Grass (*Clinacanthus nutans*). This research was conducted using Completely Randomized Design (CRD). Six treatments which are rice husk, rice bran, cocopeat, empty fruit bunch (EFB), spent mushroom substrate (SMS) and a control with five replicates for each treatment make a total of 30 samples. The results were statistical analysed using SPSS® version 21 software package. One-way Analysis of Variance (ANOVA) followed by Tukey test was carried out to determine the significance between means. The values were considered to be significantly different when $P<0.05$. The growth development of *C. nutans* showed significant differences ($P<0.05$) in plant height. The plant height of *C. nutans* was found to be highest that grow in the EFB amendment (45.06 ± 11.01 cm). The highest total phenolic content of *C. nutans* was recorded in the rice husk treatment (10.55 ± 1.68 mg GAE/g), while the highest total flavonoid content of *C. nutans* was recorded in the SMS treatment (11.75 ± 2.84 mg QE/g). The planting of *C. nutans* in SMS amendment recorded the highest DPPH radical scavenging activity with IC_{50} of 6914.32 µg/mL. It was observed that only *C. nutans* that treated with rice bran amendment exhibit antimicrobial activity against *Escherichia coli* (6.17 ± 0.29 mm). Next, *C. nutans* that planted in control treatment showed the highest antimicrobial activity against *Staphylococcus aureus* (9.67 ± 1.53 mm) followed by rice husk (8.33 ± 0.58 mm), and cocopeat amendment (8.00 ± 1.00 mm). SMS and rice husk amendments are potential for the improvement of total phenolic and total flavonoid content to enhance human health. Thus, different application rate of SMS and rice husk amendments in the growing media can be conducted to improve the phytochemical content and maximum yield production of *C. nutans*.

Key words: Organic amendments; spent mushroom substrate; rice husk; antioxidant; phenolic; flavonoid; antimicrobial; *Clinacanthus nutans*

KESAN PENAMBAH TANAH ORGANIK TERHADAP PERTUMBUHAN, KANDUNGAN FITOKIMIA DAN AKTIVITI ANTIMIKROB BELALAI GAJAH (Clinacanthus nutans)

ABSTRAK

Clinacanthus nutans digunakan untuk merawat pelbagai penyakit di negara Asia. Clinacanthus nutans mengandungi tahap aktiviti antioksidan yang tinggi dalam perlindungan terhadap kerosakan oksidatif dalam tubuh badan manusia. Kajian ini telah dijalankan di struktur perlindungan hujan dan makmal Fakulti Pertanian Lestari, Universiti Malaysia Sabah untuk mengkaji kesan penambah tanah organik terhadap pertumbuhan, kandungan fitokimia dan aktiviti antimikrob daun Belalai Gajah (Clinacanthus nutans). Kajian ini telah dianalisi menggunakan reka bentuk rawak lengkap. Sebanyak enam rawatan seperti sekam padi, dedak padi, sabut kelapa, tandan buah kosong kelapa sawit, sisa substrat cendawan dan kawalan dengan lima replikasi akan menghasilkan sebanyak 30 sampel. Keputusan kajian telah dianalisis statistic dengan menggunakan SPSS® versi 21 pakej perisian. ANOVA sehala diikuti Tukey ujian telah dijalankan untuk menentukan signifikan antara bererti. Nilai akan dipertimbangkan sebagai perbezaan yang signifikan semasa P<0.05. Pertumbuhan ketinggian belalai gajah, berat basah dan kering daun telah menunjukkan signifikan antara bererti (P<0.05). Ketinggian C. nutans yang paling tinggi adalah tumbuh dalam tanah dengan penambah tandan buah kosong kelapa sawit (45.06 ± 11.01 sm). Jumlah kandungan fenolik daun C. nutans didapati tertinggi pada penambah sekam padi (10.55 ± 1.68 mg GAE/g), manakala jumlah kandungan flavonoid yang tertinggi adalah C. nutans yang ditanam dengan penambah sisa substrat cendawan (11.75 ± 2.84 mg QE/g). Clinacanthus nutans yang ditanam dengan penambah sisa substrat cendawan menghasilkan antioksidan aktiviti tertinggi dengan jumlah IC_{50} 6914.32 µg/mL*. Kajian ini mendapati bahawa C. nutans yang ditanam dengan penambah dedak padi mempunyai antimikrob aktiviti terhadap Escherichia coli (6.17 ± 0.29 mm). Penanaman C. nutans dalam kawalan rawatan menunjukkan antimikrob aktiviti tertinggi terhadap Staphylococcus aureus (9.67 ± 1.53 mm) diikuti dengan penambah sekam padi (8.33 ± 0.58 mm) dan penambah sabut kelapa (8.00 ± 1.00 mm). Penambah sisa substrat cendawan dan sekam padi berpotensi untuk meningkatkan kandungan fenolik dan flavonoid dalam C. nutans bagi kesihatan manusia. Oleh itu, kadar pertambahan sisa substrat cendawan dan sekam padi yang berbeza dalam media boleh dijalankan untuk menambah kandungan fitokimia dan meningkatkan penghasilan C. nutans.*

Kata kunci: Penambah organik; sisa substrat cendawan; sekam padi; antioksidan; fenolik; flavonoid; antimikrob; Clinacanthus nutans

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

®	Registered
Ac	Absorbance value for control
ANOVA	Analysis of variance
As	Absorbance value for sample
CRD	Completely Randomised Design
DPPH	2, 2-diphenyl-1-picrylhydrazil
EC	Electrical conductivity
EFB	Empty fruit bunch
FRSA	Free radical scavenging activity
FSA	Faculty of Sustainable Agriculture
GAE	Gallic acid equivalents
H_0	Null hypothesis
H_A	Alternative hypothesis
HSV	Herpes simplex virus
IC_{50}	DPPH scavenging capacity
LDL	Low density lipoprotein
LSD	Least Significant Difference
M	Molarity
MIC	Minimum inhibitory concentration
mS	Millisiemen
$NaNO_3$	Sodium nitatre
nm	Nanometre
P	Phosphorus
POME	Palm oil mill effluent
QE	Quercetin equivalents
SMS	Spent mushroom substrate
SPSS	Statistical Package for the Social Sciences
VZV	Varicella-zoster virus

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

Today, medicinal plants have gained the attention of the world for its pharmaceutical properties which can solve the health care problems. Medicinal plants are cultivated throughout the world especially in China, India, Japan, Pakistan, Sri Lanka, and Thailand (Mukhtar *et al.*, 2008) which are used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO, 2008). Medicinal plants contain important bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, and phenolic compounds (Edeoga *et al.*, 2005). Herbs have been made into the first Entry Point Project (EPP1) by the government due to their high market potential. There are eleven herbs (Tongkat Ali, Kacip Fatimah, Misai Kucing, Hempedu Bumi, Dukung Anak, mengkudu, roselle, ginger, Mas Cotek, Belalai Gajah and pegaga) being studied and developed under the 10th Malaysia Plan (Daily Express, 2013).

Clinacanthus nutans (Burm.f.) Lindau (family Acanthaceae) is a small shrub herb which is native to tropical Asia, particularly in Malaysia and Thailand. It is locally known as Sabah Snake Grass or Belalai Gajah in Malaysia and well known with its medicinal properties which have been used to cure cancer (Roosita *et al.*, 2008). The characteristics of *C. nutans* leaves are pale green in colour with the arrangement of paired opposite, and narrowly elliptic-oblong in shape. The stem is small, soft, thin, and slightly curve. The fresh leaves have been consumed as herbal tea in Malaysia (Shim *et al.*, 2013).

Clinacanthus nutans has been traditionally used in Thailand as a medicine to treat skin rashes, insect and snake bite, herpes simplex virus (HSV), and varicella-

zoster virus (VZV) lesions. It contains antiviral chlorophyll a and chlorophyll b compound which can be used to cure herpes infections (Sakdarat *et al.*, 2006). The 80 % ethanol extract of *C. nutans* showed anti-inflammatory, anti-dengue virus and immune-modulating activity (Tu *et al.*, 2014). The chloroform extract of *C. nutans* leaves possess antioxidant and antiproleferative properties. It has the ability of scavenging free radical and inhibiting the cultured growth of cancer cell lines due to its potent constituents (Yong *et al.*, 2013). The previous academic research by Gunasekaran (2014) stated that *C. nutans* is one of the herbs which need to be included in medicinal research due to its nutritional values and various diseases curing ability. It is helpful in treating various diseases such as diabetes, fractures, and kidney problems. He also found out that it is scientifically considered as a potent anti-cancer herb.

Organic soil amendments is the plants or plant products that occur naturally which help to improve soil structure, quality, and restore soil organic matter. The soil quality that influences by the organic soil amendments will affect the crop production and plant health. Besides that, organic soil amendments can also increase plant growth and crop yield (Bonilla *et al.*, 2012). The use of organic soil amendments such as peat and bark is significantly improved soil physical properties and increase the growth and yield of blueberries when grown in a fertile mineral soil (Haynes and Swift, 1986).

Antioxidant is important to protect our cells from the damage of free radicals by slowing or preventing the oxidation of other molecules. Once the oxidation reactions is starting and producing free radicals, chain reactions will begin to damage cells (Hamid *et al.*, 2010). Beta-carotene, lycopene, vitamins C, E, and A are the examples of antioxidants (Sies, 1997). There is increasing interest in the study of antioxidants for its use in pharmacology. Furthermore, it plays important role in maintaining health and preventing chronic diseases such as cancer and heart disease.

Antimicrobial agent has the ability to inhibit or kill the growth of microorganism. The rich sources of antimicrobial agents and powerful drugs of medicinal plants are important to improve the human life quality because the drugs can control harmful microorganisms which help in the treatment of infections (Bhalodia and Shukla, 2016).

1.2 Justification

There is little study on the effect of organic soil amendments on phytochemical content and antioxidant activity of *C. nutans*. Considering *C. nutans* as the popular medicinal plant with high market value in Malaysia, this research was conducted in Faculty of Sustainable Agriculture to evaluate the phytochemical content and antioxidant activity of *C. nutans* leaves by applying organic soil amendments.

Clinacanthus nutans contains high level of antioxidant which has the properties that prevent cell degeneration caused by harmful free radicals in human body. Moreover, the rich sources of antimicrobial agents of *C. nutans* enhance human health from treatment of infections by controlling harmful microorganisms. *C. nutans* has high nutritional values and capable of treating various diseases. However, there is no similar research on the effect of organic soil amendments on phytochemical content, antioxidant activity and antimicrobial activity of *C. nutans* leaves.

Thus, this study may contribute more information on the phytochemical content antioxidant activity and antimicrobial activity of *C. nutans* leaves besides as a reference for improving the efficacy of *C. nutans* and widely used as medicinal plant for human health.

1.3 Objectives

The objectives of this study were

i. To determine the effect of organic soil amendments on growth of Sabah Snake Grass (*Clinacanthus nutans*).
ii. To determine the effect of organic soil amendments on phytochemical content of Sabah Snake Grass (*Clinacanthus nutans*).
iii. To determine the effect of organic soil amendments on antimicrobial activity of Sabah Snake Grass (*Clinacanthus nutans*).

1.4 Hypothesis

Hypothesis for objective (i):

H_0: There is no significant difference between the growth of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

H_A: There is a significant difference between the growth of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

Hypothesis for objective (ii):

H_0: There is no significant difference between the phytochemical content of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

H_A: There is a significant difference between the phytochemical content of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

Hypothesis for objective (iii):

H_0: There is no significant difference between the antimicrobial activity of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

H_A: There is a significant difference between the antimicrobial activity of Sabah Snake Grass (*C. nutans*) by using organic soil amendments.

CHAPTER 2

LITERATURE REVIEW

2.1 *Clinacanthus nutans*

Clinacanthus nutans (Burm. f.) Lindau is a medicinal plant which belongs to family Acanthaceae. Acanthaceae is a dicotyledonous flowering plant which comprises of 250 genera and about 2500 species. Acanthaceae family mostly are tropical herbs, shrubs, and some are epiphytes. The common name *C. nutans* is Sabah Snake Grass in English, Belalai Gajah in Malay, and Ezuihua in Mandarin. The fresh leaf of *C. nutans* is green in colour, while the powder form of *C. nutans* is green to dark green in colour. It has slight acrid smell with light bitter taste (Globinmed, 2015).

Table 2.1 Scientific classification of *Clinacanthus nutans*

Rank	Taxon
Kingdom	Plantae
Division	Mannoliophyta
Class	Magnoliosida
Order	Lamiales
Family	Acanthaceae
Subfamily	Acanthoideae
Tribe	Justicieae
Genre	*Clinacanthus*
Species	*Clinacanthus nutans*

Source: The Taxonomicon, 2016

2.2 Botanical Descriptions of *Clinacanthus nutans*

Clinacanthus nutans is a one meter perennial shrub which has pubescent branche. Its stem is cylindrical, striate, and glabrescent. The 2.5-13.0 cm long and 0.5-1.5 cm wide of leaves are green in colour, simple, opposite, narrowly elliptic-oblong or lanceolate. Figure 2.1B showed the shape of leaf blade is lanceolate-ovate, lanceolate or linear-lanceolate, while the shape of leaf base is cuncate and obtuse rounded. Its petiole is sulcate and bifariously pubescent with the measurement of 0.3-2.0 cm (Alam *et al.*, 2016).

The flower of *C. nutans* in Figure 2.1C shows that it is covered with 5-alpha cymules at the top of branches and branchlets which is sordidly yellow or greenish yellow in colour. The flower has about 1.0 cm long of calyx with grandular-pubescent. There is stamen exerted from the throat of dull red with green base of corolla. In addition, there are two cells are being compressed in the ovary with two ovules each. It has filiform with shortly bidentate of style and oblong capsule that basally wrapped into 4-seeded short (Alam *et al.*, 2016). The morphological features of *C. nutans* are illustrated in Figure 2.1.

Figure 2.1 (A) Matured plants, (B) leaves, and (C) flower of *C. nutans*
Source: Globinmed, 2015

2.3 Properties and Characteristics of *Clinacanthus nutans*

The total ash of *C. nutans* is not more than 21%, the loss of drying is less than 14%, and the acid-insoluble ash is less than 4%. Furthermore, the alcohol-soluble extract, water-soluble extract, and ether-soluble extract of *C. nutans* is more than 27%, 26%, and 1% respectively (Globinmed, 2015).

The safety tests of *C. nutans* also have been carried out. The results showed that *C. nutans* contains low heavy metals which is safe for consumption. The arsenic, mercury, lead, and cadmium of *C. nutans* is less than 5.0 mg/kg, 0.5mg/kg, 10.0mg/kg, and 0.3 mg/kg respectively (Globinmed, 2015).

2.4 Chemical Constituents of *Clinacanthus nutans*

The ethanolic extract of the aerial parts of *C. nutans* has isolate four new sulphur containing compounds named clinamides A-C and 2-*cis*-entadamide A with three known compounds which are entadamide A, entadamide C, and trans-3-methylsulfinyl-2-propenol. The physical and spectroscopic data of the known compounds were compared to those reported in the literature for identification (Tu *et al.*, 2014). Antiviral chlorophyll a and chlorophyll b related eight compounds for herpes infections health care that isolated from the chloroform extract of the *C. nutans* leaves have been discovered. The eight compounds are 13^2-hydroxy-(13^2-S)-chlorophyll b, 13^2-hydroxy-(13^2-R)-chlorophyll b, 13^2-hydroxy-(13^2-S)-phaeophytin b, 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin a, 13^2-hydroxy-(13^2-R)-phaeophytin a, purpurin 18 phytyl ester and phaeophorbide-a (Sakdarat *et al.*, 2006).

The *n*-BuOH and water soluble fractions of the methanolic extract of *C. nutans* in Thailand has isolate six-known *C*-glycosyl flavones such as vitexin, isovitexin, schaftoside, isomollupentin 7-O bglucopyranoside, orientin and isoorientin. There are five glucosides isolated from the *n*-BuOH soluble fractions of methanolic extract of *C. nutans* stems and leaves (Teshima *et al.*, 1988).

2.5 Medicinal Uses of *Clinacanthus nutans*

Clinacanthus nutans has been used traditionally in different regions of Asia. The alcoholic extract of *C. nutans* fresh leaves was used to treat skin rashes, snake and insect bite, herpes simplex virus (HSV), and varicella-zoster virus (VZV) lesions in Thailand (Sakdarat *et al.*, 2006). In addition, *C. nutans* has the mode of action of anti-cell lysis property which is used for the treatment of scorpion bites and nettle rash. Furthermore, Sangkitpporn *et al.* (1995) reported that *C. nutans* products are used to replace tropical acyclovir, an anti-viral drug to treat herpes simplex and herpes zoster in hospitals. The extract from *C. nutans* has the ability to fight with HSV and varicella zoster virus (VZV) (Thawaranantha *et al.*, 1992).

In Malaysia, *C. nutans* is consumed as herbal tea by boiling its fresh leaves with water. *C. nutans* also can be consumed by mixing it with apple juice, sugarcane, and green tea as fresh drink. Moreover, the leaves are also consumed as raw material. Nowadays, *C. nutans* has been processed and marketed into different products that in the form of herbal tea, capsules, tablets, and concentrated plant extracts. However, the lack of these products information and promotion initiative caused its low popularity in the northern region of Peninsular Malaysia.

Furthermore, *C. nutans* has been traditionally used to treat dysentery in Indonesia. This whole plant has been used for the treatment of inflammatory conditions such as hematoma, contusion, strains and sprains of injuries and rheumatism in China. Furthermore, it is also used as the function of controlling menstrual, relieving pain, anemia, jaundice, and setting of fractured bones by the Chinese healers (Andrea *et al.*, 2007).

According to P'ng *et al.* (2013), *C. nutans* has been for the treatment of diabetes mellitus, fever, diarrhea and dysuria. Besides that, it has the ability of heat and stasis reducing effects, cleanse liver and gallbladder effects, and regulate menstruation. The anti-inflammatory of skin and insect bites has been treated by using the tropical cream or lotion which extracts from *C. nutans* (Satayavivad *et al.*, 1996).

Recently, *C. nutans* gets high attention for its nourishing and antioxidant properties. Its leaves extract become the primary sources of complementary and

alternative healthcare or as economical in-house regimens for cancer patients (P'ng *et al.*, 2013). After consumed *C. nutans* leaves for a period of time, some cancer patients claimed that they have recovered from the illness. The phytochemical constituents from the chloroform extract of *C. nutans* leaves able in scavenging free radical and inhibiting the growth of cultured cell lines. It may not be a strong anticancer regimen, but it could be used as an alternate adjunctive or chemopreventive regimen for cancer patients (Yong *et al.*, 2013).

2.6 Biological and Pharmacological Study

2.6.1 Anti-inflammatory Activity

Clinacanthus nutans was used as anti-inflammatory agents to treat insect bites, allergy responses, herpes simplex and VZV lesions. The study of Tu *et al.* (2014) showed that 80% ethanol extract of aerial part of *C. nutans* has anti-inflammatory activity in bioactive screening. The results showed that ethanolic extract at 10 mg/mL produced inhibition effect at 68.33 %.

2.6.2 Anti-dengue Activity

In bioactive screening, the 80 % ethanol extract of aerial part of *C. nutans* showed anti-dengue activity. The extract at IC_{50} 31.04 µg/mL had displayed moderate anti-dengue virus activity (Tu *et al.*, 2014).

2.6.3 Anti-herpes Simplex Viral Activity Assay

A research was studied by Sakdarat *et al.* (2009) to discover the inhibitory activities against HSV-1F in pre-viral entry step. The chloroform extract of *C. nutans* leaves has isolate three chlorophyll derivatives by using chromatographic techniques and bioactivity-guided fractionation to obtain three pure compounds that related to chlorophyll a and chlorophyll b such as 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin a and 13^2-hydroxy-(13^2-R)-phaeophytin a. These compounds have anti-herpes simplex activity by exhibiting anti-HSV-1F activity at subtoxic concentrations.

2.7 Organic Soil Amendments

Organic soil amendments has the ability to improve soil structure and quality, aeration, water holding capacity, nutrient holding capacity, and restore soil organic matter. Naturally occur plants products (peat moss), by-products of processing plants or mill (sawdust, cedar chips, bark, rice husk), and waste disposal plants (compost, processed sewage sludge, biosolids) are the examples of organic soil amendments. Today, compost and animal manure (chicken and cow manure) are the most popular organic soil amendments due to the source availability and economically affordable. However, peat moss is the most preferable organic amendment in the professional agriculture (Beat *et al.*, 2012). The crop production, yield and plant health will be affected by the improvement of soil quality through organic soil amendments. Furthermore, it enhance against soil-borne pathogens through suppressive organic amendments (Bonilla *et al.*, 2012).

Both sewage sludge and urban solid waste at concentrations of 45 t ha^{-1} and 135 t ha^{-1} have been used as organic soil amendments to determine the phytochemicals of rocket leaves (*Eruca sativa*). The yield of *E. sativa* was increased 5.5 times after the addition of sewage sludge compared to control and urban solid waste. Besides that, both sewage sludge and urban solid waste increased the water content of *E. sativa* from 82.5% in control leaves to 89.5% and 88.6% respectively. The total flavonoid content of *E. sativa* decreased significantly after the addition of organic soil amendments. The lowest dose of amendments produced higher total flavonoid content compared to the highest dose (Selma *et al.*, 2010).

The addition of seaweeds, farmyard manure, wheat bran, coconut coir, and garden clippings as organic soil amendments increased the cowpea plant growth and nutrient uptake compared to the use of chemical fertilizer and control. The root length of cowpea plants is enhanced by coconut coir, farmyard manure, and garden clippings. However, the use of chemical fertilizer showed negative effects on both plants root length and shoot length. The fresh weight of cowpea plants increased significantly after the application of coconut coir, farmyard manure, and wheat bran, while the dry weight of plants is enhanced by the garden clippings. Coconut coir and farmyard manure increased the growth of cowpea plants by 44 % and 52 % respectively (Badar *et al.*, 2015).

Evaluation of phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) by using organic fertilizer (chicken manure) and inorganic fertilizer (NPK green) has been conducted. It was observed that chicken manure increased the total phenolic, flavonoid and antioxidant activity of *L. pumila* compared to NPK green. The highest total phenolic, flavonoid and antioxidant activity was recorded at the rate of 90 kg N/ha. The nitrate content is lowest with chicken manure fertilization. The study revealed that high N supply will decrease the antioxidant activity of *L. pumila* (Ibrahim *et al.*, 2013).

2.7.1 Rice Husk

Rice husk is the left over product from the rice milling process. There are 75-90 % of organic matter and mineral components present in rice husk. The ash of rice husk is 87-97 % silica which has high external surface area, highly porous, and light weight (Mohanta *et al.*, 2012). The soil properties, soil pH, organic carbon, and available nutrients can be improved and enhanced by the addition of rice husk (Milla *et al.*, 2013).

The impacts of composted rice husk on the growth and biochemical parameters of sunflower plants was studied by Badar and Qureshi (2014). Rice husk that composted with *T. hamatum* (JUF1), bradyrhizobium *sp-II* (JUR2) alone, and JUF1 in combination with *Rhizobium sp-I* (JUR1) is an effective way to increase shoot and root lengths, total chlorophyll, carbohydrate, crude protein, and mineral (nitrogen and phosphorus) content of sunflower plants.

A previous study conducted by Varela *et al.* (2013) was to determine the growth rate of water spinach using rice husk biochar and wood biochar. The results showed that rice husk biochar increased the stem size and leaf length due to the high silicon and potassium content besides it can also increase the available phosphorus, enhance soil pH, and improve soil properties. The stem size and root size are proportional to the water holding capacity/silt ratio and soil organic matter/organic carbon ratio respectively (Milla *et al.*, 2013).

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