EFFECT OF CHICKEN MANURE BIOCHAR AND WATER STRESS ON ROOT CHARACTERISTICS AND YIELD OF PURSLANE *(Portulaca oleracea* L.*)*

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ABSTRACT

Purslane (*Portulaca oleracea* L.), aside from being known as a weed, is a healthy food that has been highly beneficial to humans as a nutritious vegetable and folk medicine for centuries, and recognized by the WHO as one of the most used medicinal plants. In this study the root characteristics and yield of purslane with biochar application and water stress was analyzed. With 45 polybags used during the study, biochar was added to the planting medium two weeks before planting at rates of 0 t/ha, 10 t/ha and 20 t/ha. One-week old purslane seedlings were exposed to drought conditions by giving different watering intervals of 1 day, 2 days and 3 days. After harvest, the fresh and dry weight of purslane was taken, while the root characteristics, such as total root length, root surface area and root/shoot ratio were taken by WinRHIZO software, as well as soil pH, total nitrogen and total available phosphorus as the soil chemical properties. For plant growth parameters, Treatment B10 (10t/ha biochar rate) showed the highest value of plant fresh weight (105.78 g) and plant dry matter (9.12 g). While Treatment 1D (1 day watering interval) showed the highest value of plant fresh weight of 115.85 g and plant dry matter of 10.30 g. As for root characteristics, Treatment B10 resulted in the highest value of root surface area, total root length and root/shoot ratio with 676.30 cm^2, 2941 cm and 0.19, respectively. For root surface area, Treatment 1D resulted in the highest value with 743.18 cm^2, Treatment 3D (3 days watering interval) showed the highest total root length (3399 cm) and Treatment 2D resulted in the highest root/shoot ratio (0.20). Soil chemical properties such as pH and total nitrogen were greatly affected by water stress compared to biochar rate, except for total available phosphorus. However, the results showed that there was no significant interaction between the biochar rate and watering intervals. This study concludes that, biochar had no significant effects on both plant growth and root characteristics due to the short period of application before planting, therefore less nutrients were released into the soil.

KESAN BIOCHAR DAN KETEGASAN AIR TERHADAP CIRI-CIRI AKAR DAN HASIL TANAMAN PURSLANE

ABSTRAK

Tanaman purslane (Portulaca oleracea L.), selain dikenali sebagai rumpai, ia adalah makanan yang sihat yang memberi banyak manfaat kepada manusia sebagai sayur-sayuran dan ubat-ubatan selama berabad, dan diiktiraf oleh WHO sebagai salah satu tumbuhan ubatan yang paling banyak digunakan. Dalam kajian ini, ciri-ciri akar dan hasil tanaman purslane dengan aplikasi biochar dan kuantiti air dianalisis. Dengan menggunakan 45 pasu semasa kajian, biochar telah ditambah kepada medium penanaman dua minggu sebelum menanam pada kadar 0 t/ha, 10 t/ha dan 20 t/ha. Anak pokok purslane berumur satu minggu telah diberi tekanan kemarau dengan memberikan hari penyiraman berbeza pada selang 1 hari, selang 2 hari dan selang 3 hari. Selepas menuai, berat basah dan kering telah diambil, manakala ciri-ciri akar seperti jumlah panjang akar, kawasan permukaan akar dan nisbah akar/pucuk telah diambil menggunakan perisian WinRHIZO, serta pH tanah, jumlah nitrogen dan jumlah fosforus sebagai sifat-sifat kimia tanah. Bagi parameter pertumbuhan pokok, Rawatan B10 (10t/ha biochar) menunjukkan nilai tertinggi bagi berat segar (105.78 g) dan berat kering (9.12 g). Manakala Rawatan 1D (selang 1 hari) menunjukkan nilai tertinggi bagi berat segar sebanyak 115.85 g serta berat kering sebanyak 10.30 g. Bagi ciri-ciri akar, Rawatan B10 mencatatkan nilai tertinggi bagi kawasan permukaan akar, jumlah panjang akar dan nisbah akar/pucuk yang masing-masing bernilai 676.30 cm^2, 2941 cm dan 0.19. Bagi kawasan permukaan akar, Rawatan 1D mencatatkan nilai tertinggi sebanyak 743.18 cm^2, Rawatan 3D (selang 3 hari) menunjukkan nilai tertinggi bagi jumlah panjang akar (3399 cm) dan Rawatan 2D mencatatkan nilai nisbah akar/pucuk tertinggi (0.20). Ciri-ciri kimia tanah seperti pH dan jumlah nitrogen telah dipengaruhi oleh ketegasan air, kecuali jumlah fosforus. Walau bagaimanapun, keputusan menunjukkan bahawa tidak ada interaksi yang bererti diantara kadar biochar dan selang penyiraman. Kajian ini menyimpulkan bahawa, biochar tidak mempunyai kesan yang besar ke atas kedua-dua pertumbuhan tumbuhan dan ciri-ciri akar kerana tempoh aplikasi adalah singkat sebelum menanam, oleh itu tidak banyak nutrien telah dibebaskan ke dalam tanah.

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ANOVA	Analysis of Variance
Ca	Calcium
CABI	Centre for Agriculture and Bioscience International
CAM	Crassulacean Acid Metabolism
CEC	Cation Exchange Capacity
CHN	Carbon Hydrogen Nitrogen
CRD	Completely Randomized Design
DAS	Days After Sowing
DOM	Dissolved Organic Matter
FSA	Faculty of Sustainable Agriculture
K	Potassium
LOI	Loss-on-ignition
Mg	Magnesium
N	Nitrogen
OB	Olive tree pruning biochar
P	Phosphorus
PB	Pine woodchip biochar
PEPC	Phosphoenolpyruvate carboxylase
RHB	Rice husk biochar
UMS	Universiti Malaysia Sabah
WB	Wood biochar

CHAPTER 1

INTRODUCTION

1.1 Background

The genus Portulaca, which belongs to the Portulacaceae and sub family Portulacarioidae, has more than 120 species grown worldwide. *Portulaca oleracea,* or sometimes referred to as "pigweed", is widely considered as a weed due to its invasive characteristics. Purslane is not only cultivated, it also grows wild in fields and disturbed soils, as well as in open grassland and bushland, up to an altitude of 2,400 meters above sea level (Grubben and Denton, 2004). Purslane is a very good source of alpha-linolenic acid. Alpha-linolenic acid is an omega-3 fatty acid which plays an important role in human growth and development and in preventing diseases (Kamal Uddin *et al.,* 2014). Hence, many Asian and European regions grow purslane as a staple leafy vegetable. Other than human consumption, purslane has long been used for animal feed in Thailand (Siriamornpun and Suttajit, 2010). Seeds take 7 to 10 days to germinate at temperatures ranging between 20 and 32 degrees Celsius. Harvesting it can start 3-4 weeks after sowing, and 2-3 cuts at 2-3 week intervals are possible in commercial production (Grubben and Denton, 2004).

Water stress is the main abiotic stress that is perceived as a limiting factor in crop production worldwide compared to other environmental stresses. Water stress is a result of water deficit, such as a drought or high soil salinity. Every year, water stress on arable plants in different parts of the world disrupts agricultural activities and food supply (Ismail and Hasegawa, 2012). Water is an integral part of the plant body that plays a key role in growth initiation, maintenance of the developmental process of plant life and hence has a critical function in crop production (Aslam *et al.,* 2013). Scarcity of water results in developmental and morphological changes such as growth inhibition and reproductive failure, reduction of water content and turgor, stomatal closure, limits gaseous exchange, reduction in transpiration and reduction in carbon assimilation rates. Therefore, drought stress affects crop yield at all stages of growth. The roots of plants are important for anchor and the uptake of nutrients and water by plant for various physiological, biochemical and other plant processes. Usually the growth of plant roots is influenced by genetic characteristics of the plant and various environmental factors such as soil type and properties, and climate. Soil conditions or health can be improved by amending the soil with either chemical or organic materials. However, the use of organic amendments such as biochar is more favoured as a sustainable agronomic practice.

Biochar is a term used to describe a carbon-rich product which is added to the soil, produced through a process called pyrolysis. Biochar can be created from wood, plant materials and manure (International Biochar Initiative, 2015). It has potential as an important tool for improving food security and diversity of agricultural land in areas with depleted soils, scarce organic resources and inadequate water supply and limited chemical fertilizers. Biochar also improves the quality and quantity of water and increases the retention of soil nutrients and agricultural chemicals for use by plants and crops. In the soil, biochar provides a good habitat for soil microbes such as bacteria that help in recycling of nutrients so that nutrients can be absorbed by plants.

Justification

Purslane has been highly beneficial to humans as a nutritious vegetable and folk medicine, for centuries, despite also being known as an invasive weed. Scientific analysis of its chemical components has shown that this common weed has uncommon nutritional value, making it one of the potentially important foods for the future (Kamal Uddin *et al.,* 2013). The usefulness of biochar for soil improvement and the environment has been recognized since Terra Preta or "black earth" was discovered and it is now used worldwide. According to Zheng *et al.,* (2010) adding biochar to fertile soils can reduce the need to use of chemical fertilizers.

Most farmers in Malaysia still do not know the other potentials of purslane because the uses are limited to folk medicines and as a herb and decorative plant. Hence, this study was done to find out how the purslane plant, including the roots, responds to biochar amendment and water stress. This study can contribute to increasing the production and economic value of purslane since it has potential to be widely used in agriculture, such as living mulch and animal feed. This study can also help towards the effective utilization of biochar in agriculture. Also, biochar can be used to help combat climate change by holding carbon to soil and displacing fossil fuel use (International Biochar Initiative, 2015).

1.2 Objectives

The objectives of this study were:

i. To evaluate the effects of biochar and water stress on the root characteristics of purslane.
ii. To evaluate the effect of biochar and water stress on yield of purslane.

1.3 Hypotheses

H_0: The use of biochar and drought stress has no significant effect on root characteristics of purslane.

H_A: The use of biochar and drought stress has significant effect on root characteristics of purslane.

H_0: The use of biochar and drought stress has no significant effect on yield of purslane.

H_A: The use of biochar and drought stress has significant effect on yield of purslane.

CHAPTER 2

LITERATURE REVIEW

2.1 Purslane

Purslane, or its botanical name *Portulaca oleracea L.* is a succulent annual plant which belongs to the Portulacaceae family has worldwide distribution. The seeds of purslane are able to remain viable in soil for up to 40 years. Aside from being a noxious weed, purslane is also known as healthy food that contains a whole range of health-boosting nutrients, including higher omega-3 fatty acids, antioxidant vitamins and minerals, making it listed in the World Health Organization (WHO) as one of the most used medicinal plants and given the term 'Global Panacea' (Samy *et al.,* 2005). Many cultures embrace this plant as food including Europe, the Middle East, Asia and Mexico. Purslane is likely native to North Africa, the Middle East and the Indian subcontinent. It has been grown for more than 4,000 years as food and traditional medicine in China and parts of India.

Purslane is now naturalized in most parts of the world, both tropical and temperate region. It can be found in flower beds, cultivated fields, and roadsides or other disturbed or waste places. Also, there are some studies carried out to evaluate the potential of purslane as living mulch in broccoli production. Worldwide, purslane is also known by several names as shown in Table 2.1.

Table 2.1: Other names of *Portulaca oleracea* by language and country

Name	Language/Country
Purslane	English
Krokot	Indonesia, Malaysia
Портулак	Russia
Persleyn	Philippines
Mǎ chǐ xiàn	Chinese
Genda pala	Sri Lanka
Lunia	Hindi
Pourpier	France
Suberi-hiyu	Japan

Other names of *Portulaca oleracea* according to language and country

Source: Anonymous, 2005

2.1.2 Morphology

According to Grubben *et al.*, (2004), purslane is an annual herb which has a stem with many branches; glabrous; up to 35 cm long; often red or brown in colour, with short internodes. The leaf are alternate and simple with a hairy ocrea up to 3mm long, often silvery-white in colour, irregularly fringed and has short petiole. The leaf blade is very small, linear to obovate-elliptical, 1-2 cm x 2-5 mm in size, rather thick and leathery and has dark green colour turning red. While, the inflorescence has a congested raceme with short branches and has 1-5 flowered clusters. The flowers are bisexual with perianth of 2 mm long, greenish with 4 lanceolate-elliptical lobes of 1.5 mm long, the outer pair keeled, white to pale pink coloured; have 5-8 stamens and ovary is superior with 1-celled styles, free and 3 mm long. The fruit has a trigonous nut up to 2 mm long, shiny black and smooth textured. The roots are rather thick with many fibrous secondary roots. For efficient absorption, the root system may reach 153 cm in diameter. In addition, it produces adventitious roots that emerge only from the cut or broken surface of stem fragments (Mitich 1997).

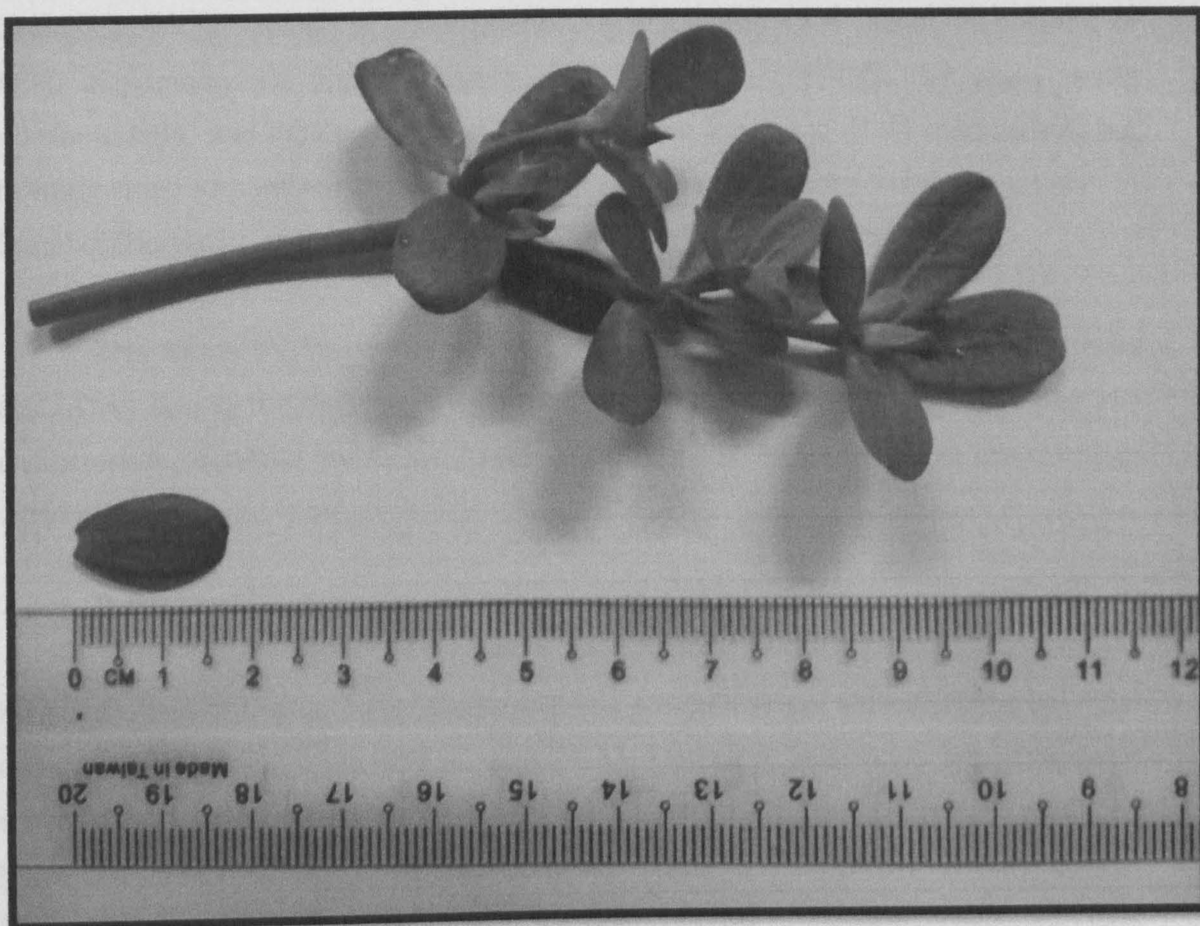

Figure 2.1 Purslane (*Portulaca oleracea*) without its flower

2.1.3 Physiology

Portulaca oleracea is mostly an annual, but it may be perennial in the tropics. It grows rapidly in open, disturbed soils. Purslane produces flowers, fruits and seeds within 6 weeks of germination. It has a wide tolerance of photoperiod, light intensity, temperature, soil type and moisture. This species is self-compatible which means that it is able to breed by its own pollen (CABI, 2015).

Most plants have evolved three photosynthetic pathways known as C3, C4, or Crassulacean Acid Metabolism (CAM). Purslane has the ability to adapt to a broad range of environmental conditions due, in large part, to its unique photosynthetic metabolism. Under sufficient water supply, purslane utilizes C4 photosynthetic metabolism. However, purslane will shift to a CAM-like photosynthetic metabolism under water stressed conditions, with nocturnal acid accumulation in the leaves and reduced CO_2 uptake. Phosphoenolpyruvate carboxylase (PEPC) catalyzes the initial step in photosynthetic fixation of atmospheric CO_2 in C4 and CAM plants. Regulation of PEPC is primarily via phosphorylation by PEPC kinase (PEPCK). For well-watered purslane, PEPC and PEPCK transcript abundance are indicative of C4 metabolism, but in water stressed purslane, PEPCK transcripts accumulate at night, suggesting a shift in the phosphorylation pattern of PEPC to CAM-like metabolism (Proctor, 2013).

This allows the leaves to trap carbon dioxide at night and convert it to malic acid. Then during the daytime, the malic acid is converted into glucose. Due to this characteristic, purslane leaves will taste different in the morning and in the evening, when it has 10 times more malic acid (Blair, 2014).

2.1.4 Nutritional values

Purslane is one of the leafy vegetables containing high levels of vitamin E and essential omega-3 acids called alpha-linoleic acid (ALA). Omega-3 fatty acids are considered essential fatty acids. They are necessary for human health, but cannot be produced inside the body and have to be obtained from foods (Ehrlich, 2015). These essential fatty acids play key roles in maintaining the heart and enriching brain health. Purslane is high in vitamins A and C which are known for their antioxidant powers. It is also low in calories and fat, but this weed does contain high amounts of dietary minerals such as iron, magnesium, calcium, potassium and manganese (Melgren, 2010). The nutritional values per 100 g purslane are shown in Table 2.2.

Table 2.2 Composition of nutrients per 100 g of raw purslane

Composition	Raw purslane (100 g)	
Energy	20 kcal	
Carbohydrates	3.39 g	
Fat	0.36 g	
Protein	2.03 g	
Vitamins		
Vitamin A	1320 IU	
Thiamine (B_1)	0.047 mg	4%
Riboflavin (B_2)	0.112 mg	9%
Niacin (B_3)	0.48 mg	3%
Vitamin B_6	0.073 mg	6%
Folate (B9)	12 μg	3%
Vitamin C	21 mg	25%
Vitamin E	12.2 mg	81%
Minerals		
Calcium	65 mg	7%
Iron	1.99 mg	15%
Magnesium	68 mg (19%)	19%
Manganese	0.303 mg	14%
Phosphorus	44 mg	6%
Potassium	494 mg	11%
Zinc	0.17 mg	2%
Water constituent	92.86 g	

Source: USDA, 2006

2.2 Water and Agriculture

Today, agriculture accounts for around 70% of water used in the world. Water resource is derived primarily from rain and is drained via ditches, streams and stored in dams, ponds, swamps and lakes. Water is a major constituent of living organisms, and comprises about 80 - 90% of fresh weight of herbaceous plants and over 50% of woody plants (Rashidi and Seyfi, 2007). The functions of water in plants include the transport of nutrients and organic compounds. The material is transported in the form of nutrients from the soil and organic materials from photosynthesis and other cells; is the basis for biochemical reactions such as photosynthesis; is a buffer in the regulation of plant body heat; and the main constituent of protoplasm (Anonymous, 2003). In agriculture, water is needed for irrigation and watering. For example, a crop that requires irrigation is wetland rice where without water stagnation, it will not survive hence, affecting rice production. Also, water is essential for livestock to determine the performance of animals. For example, a laying hen that gets insufficient water will be affected in terms of laying performance, because water is vital in egg formation. Likewise, a dairy cow that has insufficient water will produce less milk, because water is the highest constituent in fresh cow milk (Akinbobola, 2015).

2.2.1 Effect of Water Deficit on Plants

Drought is defined as an extended period of unusually dry weather when there is not enough rain (Rodgers, 2013). It is a major threat which limits crop production as the growth and development of plants are severely affected by water stress. To date, the effects of drought stress on plants have been studied in many plants, including the effects on crop yield. Drought stress affects crop yield by reducing grain yield and all yield components, but also affects seed quality such as germination and vigour tests (Ahmad *et al.,* 2011). According to Tahar *et al.,* (2010) the different treatments of water stress led to a decline in the shoot and root dry weights, as well as total dry weight per plant.

Plant growth under drought is affected by altered physiological processes such as photosynthesis, transpiration and hormonal regulation. Photosynthesis is a biochemical process of nutrient establishment, such as carbohydrates, from water and carbon dioxide with the help of light energy. Photosynthesis will only occur in the presence of chlorophyll, a pigment that serves as a catcher and converts sunlight energy into chemical energy bound in carbohydrate molecules. The amount of water, sunlight and carbon dioxide available to the plant directly influences the amount of food a plant can produce for itself. In recent research using *Solanum scabrum* Mill., the leaf photosynthetic rate was tremendously reduced by decrease in stomatal conductance under stress conditions, resulting in decreased leaf, stem and root growth (Assaha *et al.,* 2016).

Transpiration is the process by which moisture is carried through plants from roots to small pores on the underside of leaves, where it changes to vapour and is released to the atmosphere (Anonymous, 2016). Transpiration serves as transport of minerals, maintaining optimum turgidity and eliminates a large amount of heat from the leaves. Minerals absorbed into the roots move up the plant through the xylem. It also helps in the sorption of minerals from the soil and transports it through the system. Under conditions of water deficit, the stomatal pores of many mesophytes will close, therefore reducing transpiration significantly.

Hormones play a special role in plant reaction to water stress conditions. Abscisic acid (ABA) is the main hormone correlated to water stress. Plants under drought conditions substantially increase in the levels of ABA in shoots and roots (Nan *et al.,* 2002; Wang *et al.,* 2008; Leach *et al.,* 2011).

2.2.2 Effect of Water Deficit on Root Characteristics

As a plant organ, roots function as absorber of water, mineral salts and oxygen from the soil and are also an anchor for the establishment of the plant. Because the root system greatly affects the absorption of nutrients and water that plants need for photosynthesis, thus it increased plant productivity (Zulkifli, 2016).

Besides the reduction in dry weight, drought stress treatment showed a notable effect on the root/shoot ratio. The increase in root/shoot ratio was a result of a greater reduction in aboveground biomass rather than an increase in root biomass, because under drought stress the absolute root dry weight was not greater than that under well-watered treatment (Xu *et al.,* 2015). A study on root physiological traits and root biomass of *Reaumuria soongorica* by Shan *et al.,* (2015) showed that the seedlings retained higher root activity in serious drought stress by enhancing root respiration. Aslam *et al.,* (2013) observed that drought resistant maize had the maximum root fresh weight, compared to less drought resistant varieties.

2.2.3 Effect on Excessive Water on Plants

Too much water can bring damaging consequences. It can impact crop growth in both the short and long term. Waterlogging in crop plants results in oxygen deprivation as the excess water does not react chemically with the plant. Plants need oxygen for cell division, growth and the uptake and transport of nutrients. Since oxygen diffuses through undisturbed water much more slowly than in a well-drained soil, oxygen requirements rapidly exceed that which is available in the event of excess water (Ransom, 2013). Excess water promotes pathogen growth especially fungi. Fungi grow best in warm (70 to 90° F) conditions under limited light and lots of moisture. Large populations of fungi will attack plants stressed by excessive water. Bacterial and viral disease can also take advantage of these plants as a host to spread diseases. On the bottom parts of the plant, the wet roots succumb to root rot mainly caused by pathogen attack (Sprague, 2015). In terms of root growth, waterlogging impedes the oxidative breakdown of ethylene which influences root growth and functions such as root extension, while carbon dioxide in the soil can severely damage roots of certain species (Jackson, 2013).

2.3 Biochar and Their Properties

In recent years, health and quality of the soil have become a major concern in the agricultural field with increased efforts to reduce the use of chemicals which bring adverse impacts to the environment. Thus, many studies have been carried out on biochar to evaluate its potential as a soil amendment. The term biochar was first coined by Peter Read, referring to any charcoal that is used for amending soil (Read, 2009). Biochar comes from the combustion of organic materials from agricultural residues, such as grass, wood, rice husk, palm oil or animal manures, and is produced through burning under less oxygen, a process known as pyrolysis. However, this process differs from the formation of charcoal, because biochar is produced for the purpose of applying it to the soil.

Its history dates back 2,000 years to a civilization in the Amazon Basin where a carbon rich, highly fertile soil known as "Terra Preta" or black earth was discovered and analyzed, revealing high concentrations of charcoal and organic matter, such as animal and plant residues (Biochar Now, 2016). Terra Preta soils contain high concentrations of nitrogen, phosphorus, potassium, calcium, and stable organic matter (Glaser *et al.,* 2001). The formation of this rich soil comes from the "slash-and-char" process practiced by the ancient Amazonians. In this process, plant material or crop remains were cut, burned, and buried to smolder, which eventually produced char, now commonly referred to as "biochar" or "agrichar" (Anonymous, 2016). The addition of biochar into the soil shows various of benefits in terms of soil quality including improving the cation exchange capacity (CEC); reducing soil acidity; improving soil structure; improving water holding capacity; and improving the efficiency of fertilization.

2.3.1 Types of Biochar

Biochar can be produced from various sources of biomass such as rice husks, wood remains, nut shells, manure and crop residues. The commonly used biochar includes that from rice husk, wood, empty fruit bunch and livestock manure.

Uzoma *et al.,* (2010) reported that application of cow manure biochar to sandy soil is not only beneficial for crop growth but it also significantly improved the physico-chemical properties of the coarse soil. A field experiment by Varela *et al.,* (2013) stated that wood biochar (WB) added to soil increased the plant weight of water spinach by increasing the root size and leaf width, while rice husk biochar (RHB) added to soil increased the plant weight of water spinach by increasing the stem size and leaf length.

2.4 Effect of Biochar on Soil Properties

The amendment of biochar to the soil results in changes to the physical properties, chemical properties and biological properties of the soil. However the changes in soil properties depend on the types of biochar, application rate and the soil type itself.

2.4.1 Soil Water Retention

Soil water is the primary component of the soil in relation to plant growth. If the soil moisture content is optimum for plant growth, plants can readily absorb soil water. Soil is able to hold water due to their colloidal properties and aggregation qualities. The water is held on the colloids surface and other particles and in the pores (Anonymous,

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