INTEGRATION OF BIOCHAR WITH CHEMICAL FERTILIZER FOR IMPROVING YIELD AND QUALITY OF TULSI (*Ocimum sanctum* L.)

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Integration of biochar with chemical fertilizer for improving yield and quality of tulsi (*Ocimum sanctum* L.)

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A B S T R A C T

A pot culture experiment on "Integration of biochar with chemical fertilizer for improving yield and quality of Tulsi (*Ocimum sanctum* L.)" was carried out in the net house, ICAR- DMAPR, Boriavi during *kharif* season of year 2020.

The experiment was laid out on completely randomized design. The pot culture experiment was conducted by integrating the biochar prepared from tulsi (*Ocimum sanctum* L.) distillation waste and chemical fertilizers (CF). The biochar were characterized and then applied in different combinations along with chemical fertilizer to evaluate as nutrient source under pot culture experiment. The treatment comprised of seven levels of inputs *viz.*, T_1: Control, T_2: Biochar 5 t ha^{-1}, T_3: 100 % RDF, T_4: Biochar 2.5 t ha^{-1} + 50 % RDF, T_5: Biochar 2.5 t ha^{-1} + 100 % RDF, T_6: Biochar 5 t ha^{-1} + 50 % RDF and T_7: Biochar 5 t ha^{-1} + 100 % RDF.

All the growth parameters *viz.*, plant height, number of branches and yield parameters *viz.*, total fresh biomass, fresh leaf yield, total dry biomass, dry leaf yield, L: S ratio, total chlorophyll content and oil yield showed significantly ($P = 0.05$) higher in the treatment receiving biochar 5 t ha^{-1} + 100 % RDF treatment when compared to other treatments.

The bioactive compound of tulsi significantly improved with biochar application. Essential oil content (%) in the herbage was significantly (P = 0.05) higher in the treatment receiving biochar 5 t $ha^{-1}$and biochar 2.5 t ha^{-1} + 50 % RDF treatment. Total phenol content, total flavonoid content and anti-oxidant potential was found higher in the treatment receiving biochar 5 t ha^{-1}, biochar 5 t ha^{-1} + 50 % RDF and biochar 2.5 t ha^{-1} + 50 % RDF respectively.

Plant N, P and K (%) content was significantly (P = 0.05) higher in the treatment receiving biochar 5 t ha^{-1} + 100 % RDF treatment.

After harvesting, soil sample were analyzed to study the influence of biochar and chemical fertilizer on soil properties.

Application of biochar 2.5 t ha^{-1} + 100 % RDF recorded significantly (P = 0.05) higher soil pH (7.81). While treatment receiving biochar 5 t ha^{-1} + 100 % RDF recorded higher soil EC (0.41 dSm^{-1}). Soil organic carbon was recorded significantly (P = 0.05) higher (3.36 g kg^{-1}) in the treatment receiving biochar @ 5 t ha^{-1}+ 100 % RDF. Application of biochar (5 t ha^{-1}) showed significantly (P = 0.05) higher values

for soil CEC (21.6 cmol(p+)kg^{-1}). Significantly (*P = 0.05*) higher mineral (NH_4^+ + NO_3^-) N (46.67mg kg^{-1}), available P (24.77 mg kg^{-1}) and available K (116.80 mg kg^{-1}) was observed in the treatment receiving biochar 5 t ha^{-1}+ 100 % RDF. Soil biological properties such as microbial biomass carbon (215.13 mg kg^{-1}), dehydrogenase activity (44.55 µTPF g^{-1} h^{-1}) and soil respiration (3.91 mg CO_2-C kg soil^{-1} day^{-1}) were found significantly higher (*P = 0.05*) in the treatment receiving biochar 5 t ha^{-1} + 100 % RDF.

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C E R T I F I C A T E

This is to certify that the thesis entitled **"Integration of biochar with chemical fertilizer for improving yield and quality of tulsi (*Ocimum sanctum* L.)**" submitted by **PRAVEEN SINGH (Reg. No. 2010119078)** in partial fulfillment of the requirements for the award of the degree of **MASTER IN SCIENCE (Agriculture)** in **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY** of the Anand Agricultural University is a record of bonafide research work carried out by him under my personal guidance and supervision. The thesis has not previously formed the basis for award of any degree, diploma or other similar title.

Place: Anand **(B. B. BASAK)**
Date: / /2021 **Major Guide**

DECLARATION

This is to declare that the whole of the research work reported here in the thesis entitled **"Integration of biochar with chemical fertilizer for improving yield and quality of tulsi (*Ocimum sanctum* L.)"** for the partial fulfillment of the requirement for the degree of **MASTER IN SCIENCE (Agriculture)** in **SOIL SCIENCE & AGRICULTURAL CHEMISTRY** by the undersigned is the result of investigation done by me under the direct guidance and supervision of Dr. B. B. Basak, Scientist (Soil Science), Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand and no part of work has been submitted for any other degree so far.

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LIST OF ABBREVIATIONS

%	Per cent
&	And
/	Or
@	At the rate of
<, >	Less than, More than
=	Equals to
μTPF g^{-1} h^{-1}	Micro Triphenylformazan per gram per hour
^{0}C	Degree Celsius
$^{-1}$	Per
AAU	Anand Agricultural University
BACA	Bansilal Amrutlal College of Agriculture
BD	Bulk Density
C	Carbon
C : N	Carbon nitrogen ratio
C.D.	Critical Difference
CEC	Cation exchange capacity
$CHCl_3$	chloroform
cm	Centimeter
CO_2	Carbon dioxide
CRD	Complete Randomized Design
CV	Coefficient of variance
DAP	Diammonium Phosphate
DAT	Days after transplanting
DMAPR	Directorate of Medical and Aromatic Plant Research
dSm^{-1}	Deci Siemen(s) per meter
EC	Electrical conductivity
et al.	Et allii; and co-workers
FAI	Fertilizer Association of India
FAO	Food and Agricultural Organisation
Fig.	Figure
FTIR	Fourier transformation infrared spectra
FYM	Farm Yard Manure
g	Gram
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
ha^{-1}	Per hectare
HCl	Hydrochloric acid
$HClO_4$	Per chloric acid
HNO_3	Nitric acid
hrs.	Hours
ICAR	Indian Council of Agricultural Research
K	Potassium
K_2O	Potassium oxide
K_2SO_4	Potassium sulphate
KBr	Potassium bromide
KCl	Potassium chloride

Kg	Kilogram
kg ha^{-1}	Kilogram per hectare
kg K_2O ha^{-1}	Kilogram potassium per hectare
kg N ha^{-1}	Kilogram nitrogen per hectare
kg P_2O_5 ha^{-1}	Kilogram phosphorus per hectare
Ltd	Limited
M t	Million tones
m^{-2}	Per square meter
max.	Maximum
Mg	Milligram
mg kg^{-1}	Milligram per kilogram
mg GAE g^{-1}	Milligram gallic acid equivalent per gram
mg QE g^{-1}	Milligram quercetin equivalent per gram
per min.	Minimum
Mm	Millimeter
MOP	Muriate of potash
N	Nitrogen
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4OAc	ammonium acetate
nm	Nanometer
P	Phosphorus
P_2O_5	Phosphorus pentaoxide
pH	Potential of hydrogen ion
$plant^{-1}$	Per plant
RDF	Recommended dose of fertilizer
RH	Relative Humidity
RP	Rock Phosphate
S. No.	Serial number
SEM	Scanning Electron Microscope
S.Em	Standard Error of mean
Sig.	Significant
SOC	Soil Organic Carbon
sp.	Species
SSP	Single Superphosphate Fertilizer
t ha^{-1}	Ton(s) per hectare
TPF	Triphenylformazan
TTC	Triphenyltetrazolium chloride
UV–vis	Ultra violet –visible radiation
viz.,	Namely
w/v	Weight by volume ratio
µm	Micro meter

CONTENTS

1. INTRODUCTION

To meet the rising demand for food, agriculture has become increasingly reliant on fertilizers as a source of plant nutrients in recent decades. Environmentalists and agricultural scientists have learned in recent years that sustained and unbalanced fertilizer use will alter the physical and chemical qualities of the soil, causing pollution and affecting soil biological activity. As a result, more people are becoming aware of the benefits of using biochar to maintain soil fertility and productivity.

The world's demand for major chemical fertilizer nutrients has increased since 2015. Total nutrient demand for N, P_2O_5 and K_2O (in Mt) has been increased from 105.15, 44.48 and 35.43 in 2016 to 111.59, 49.10 and 40.23 respectively in 2020 (FAO, 2019). On the other side the cost of chemical fertilizers has also been increasing with the time (World Bank, 2018). Small and marginal farmers cannot have enough money to buy fertilizer. So, they may be guided to use the locally available low-grade mineral powders. As they are difficult to use as such, the idea is to alter the minerals with biochar and compost which are also prepared from agricultural waste biomass.

The organic materials play an important role to enhance the physical properties of the soil, such as bulk density, improve microbial activities, water absorption and nutrient retention in soil. Increased crop yield is commonly recorded when the biochar is applied to soils, in addition to improved soil health. Many of the reported studies, on the other hand, are highly variable and dependent on a variety of variables, most notably the initial soil properties and conditions, as well as the biochar characteristics. Positive crop and biomass yield was found for biochar produced from wood, paper pulp and poultry waste (Jeffery *et al.*, 2011).

Sustainable crop production requires for soil fertility management. The nutrient use efficiency varies in relation to crop species as well as source of a nutrients and ranges from 20 to 60%, depending on the management practice and the chemical properties of the fertilizer source. Judicious use and over exploitation of chemical fertilizers lead to reduced humus content in various soils throughout the world. Also, to meet the global food demand, it is significant to enrich the organic inputs in addition to composting the agricultural wastes accessibility to the plant

(Mohanty *et al*., 2006). Application of organic inputs such as crop residues, manures, composts, and mulches have frequently been used for restoration as well as improvement of soil fertility. However, rapid organic amendment and their availability are the major challenges to overcome. Thus, there is a need for easily available more stable organic amendment to overcome these challenges.

Biochar has become a popular fertilizer supplement in recent years and it plays a significant role in increasing crop yields. Biochar has been discovered to have a significant impact on soil fertility and crop productivity without creating any risks to the soil. While the word "biochar" is fresh and the biochar has been used in some form or another throughout history, primarily for soil improvement. One of the first historical mentions of 'biochar' and used for soil improvement dates back at least 2000 years (O'Neill *et al*., 2009). The biochar is a solid material made from the thermochemical conversion of biomass in the absence of oxygen (The International Biochar Initiative). Biochar includes stable carbon, which is sequestered for far longer after it is applied to soil than it would be in its original organic carbon form. Biochar is a variable-charge organic material with a large surface area and pore volume that can improve soil water-holding capacity, pH, cation exchange capacity (CEC), surface sorption capacity, nutrient content and base saturation (Liang *et al*., 2006).

Biochar, carbon rich pyrogenic substances considered as a good soil amendment for sustainable crop and environmental management, agricultural wastes, wood waste, forest residues and food waste are all widely used as biochar precursors. Farmers in India are encouraged to grow aromatic crops that produce high-demand essential oils. The conversion of this essential oil distillation waste into a value added soil amendment like biochar will be a promising alternative strategy for their effective management (Saha *et al*., 2019).

Medicinal plants have a significant role in the production of effective herbal medicines. The herbal medications form the backbone of the essential traditional medicinal methods. Recently, there has been a surge in global interest in medicinal plant research. Medicinal herbs utilized in various traditional systems medicine have been shown to have immunological potential against a variety of ailments.

Traditional herbal practitioners use medicinal plants on a daily basis to treat a variety of diseases. In traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of tulsi (Scientific name: *Ocimum sanctum* L.; 2n=32; Family: Lamiaceae / Labiatae; Local name: Sacred basil / Holy basil (English), tulsi (Hindi, Gujarati, Sanskrit). Tulsi (*Ocimum sanctum* L.) plant is erect, branched, slightly hairy and aromatic in nature. It prevents cold, fever and cough etc. Tulsi cultivation is economically profitable.

O. sanctum L., is a straight, herbaceous, much-branched, softly hairy biennial or triennial, which grows to a height of 30-75 cm. Leaves are entire, serrate, pubescent on both sides, flowers purplish or crimson, in racemes, fruits are sub-globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish with small black markings. The species is worshiped by the Hindus and generally grown in yards and temples. Steam distillation of the leaves of this plant yields a bright yellow colour volatile oil with a good odour and a noticeable note of clove oil. The plant contains largely phenols, aldehydes, tannins, saponins and fats. The essential oil components are eugenol (71%), eugenol methyl ether (20%), carvacrol (3%) and minor portions of nerol, caryophyllene, selinene, α-pinene, ß-pinene, camphor, cineole, linalool etc. The plant is used as a pot herb; leaves are used as condiment in salads and other foods (Extension Bulletin, DMAPR, 2014).

Ayurvedic ethical formulations contribute the remaining sum. Cosmetics and aromatherapy are two crucial fields where Indian medicinal plants and extracts, as well as essential oils, can make a global contribution. With global demand for herbal products, increasing at a rate of 7% per year, medicinal and aromatic plants have a large market potential. Tulsi leaves had higher levels of sodium, copper and zinc (Bhowmik *et al*., 2008).

Traditional medicines, mainly plant medicines, are estimated to meet the health needs of approximately 80% of the world's population, according to WHO estimates. Out of these 4.22 lakh, plants species recognized worldwide, about 12.5% are reported to have medicinal or other comparable values (Rao *et al*., 2004). Annually, India’s medicinal plants export is worth of 1250 Cr. and essential oil amounts 260 Cr. Organically grown medicinal and aromatic plants have an excellent global market and India can exploit this market to its advantage (Rao and Rajput, 2005).

Considering the promise of biochar as an effective soil amendment and economic importance of the medicinal herb, tulsi, the present experiment entitled **"Integration of biochar with chemical fertilizer for improving yield and quality of tulsi (*Ocimum sanctum* L.)''** is planned out with the following objectives.

OBJECTIVES

1. To study the nutrient supplying potential of biochar in laboratory condition
2. To evaluate the effect of biochar as source of nutrient on yield and quality of tulsi under pot culture experiment

2. REVIEW OF LITERATURE

Today's agriculture faces the dual challenge of achieving food, fodder, fiber and fuel protection as well as sustainability, with a focus on soil resource restoration, water quality improvement, climate change mitigation and soil and natural resource preservation for long-term usage. A renewed interest in soil health has arisen as a result of the current focus on sustainable agriculture. Soil health focuses on the combination of biological, chemical and physical soil quality indicators that affects farmer profitability and the environment. Sustainable agriculture is founded on the basis of fertile soil, which is an integral component of a healthy climate.

2.1 BIOCHAR PRODUCTION AND CHARACTERIZATION

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2.1 BIOCHAR PRODUCTION AND CHARACTERIZATION

Biochar Production

Fast pyrolysis is now widely recognized as a feasible and viable method for producing renewable liquid fuels, chemicals and by-products. It is also clear that liquid goods outperform gas and heat in terms of storage and transportation. Because of these benefits, quick pyrolysis has received more attention, resulting in major advancements in process development (Bridgwater and Peacocke, 2000).

Antal and Grnli (2003) found that manipulating pressure, moisture content and gas flow allowed biomass carbonization with fixed-carbon yields approaching or exceeding the theoretical limit after just a few tens of minutes of reaction time. A large portion of the heat required to carbonize the feed was released by vigorous, exothermic secondary reactions that minimize the formation of undesirable tars by increasing the rate of carbonization.

During pyrolysis, Mohan *et al.* (2006) looked at the influence of wood composition and structure, heating rate and residence time on the overall reaction rate and volatile yield. Pyrolysis of biomass produces many different products depending on how quickly or slowly it is performed.

According to Brownsort and Peter (2009) high char yields allow for greater net CO_2 benefits, whereas slow or intermediate pyrolysis produced lower electrical production. Quick pyrolysis produced more electricity than direct combustion because of higher liquid and/or gas yields. When the amount of char retained was minimal, both fast pyrolysis and direct combustion produced similar net CO_2.

Biochar has been hailed as an amendment to revitalize the depleted soils by improving soil carbon sequestration, increasing agronomic efficiency and gaining access to potential carbon trading markets, according to Spokas *et al.* (2012). Biochar is a member of the black carbon spectrum with variable properties as a result of processing (e.g., feedstock and pyrolysis co-products). As a consequence, biochar is a broad word that refers to a number of black carbon-based materials. While biochar is black carbon, it is not the same as all black carbon.

Biochar was produced as a bi-product of bioenergy production using pyrolysis. Pyrolysis is the thermochemical decomposition of organic matter in the absence of oxygen at high temperatures (or any halogen). Pyrolysis is a form of thermolysis that occurs when organic materials are exposed to extremely high temperatures (Krishnakumar *et al.*, 2013).

Gai *et al.* (2014) produced biochar form three different agricultural by products: peanut-shell, corn-straw and wheat-straw at different pyrolysis temperature i.e. 400 ˚C, 500 ˚C, 600 ˚C and 700 ˚C. The result revealed that the increasing the pyrolysis temperature from 400 to 700 ˚C, the yields of wheat-straw-BC, corn-straw-BC and peanut-shell-BC samples reduced from 32.4 % – 36.8 % to 22.8 % – 25.8 %.

This is because higher pyrolysis temperatures result in more volatile component losses.

Hasan *et al.* (2019) studied the effect of pyrolysis temperature and time on different properties of Palm kernel shell biochar and they discovered that raising the pyrolysis temperature from 400 ˚C to 500 ˚C reduced the biochar yield from 43.13 % to 30.69 %, with a difference of 12.44 %. If the pyrolysis temperature is 400 ˚C, the pyrolysis time must be increased by 150 %, from 30 to 75 minutes, in order to reduce the biochar yield by 13.3 %. Increasing the time by 150 % for a temperature of 500 ˚C would only result in a 5.19 % decrease in biochar yield.

Biochar Characterization

According to Lima and Marshall (2005), the chemical constituents of biochar were directly influenced by the temperature, time and material kept in a given temperature and heating rate during pyrolysis. They also mentioned that during the heating process, the individual elements may be lost to the atmosphere, fixed into recalcitrant forms or liberated as soluble oxides.

Adsorption, cation exchange power, mobile matter (tars, resins and other short-lived compounds) and the type of organic matter feedstock used are the most important biochar quality indicators. Biochar's adsorption ability decreased over time, while its cation exchange capacity increases (Cheng *et al*., 2008; McLaughlin *et al*., 2009).

Novak *et al*. (2009) noticed that the majority of the carbon in biochar (58 %) was distributed in aromatic structures; with smaller quantities of carbon containing single bonds to oxygen (29 %) and carboxyl (13 %) units but little carbohydrate carbon and that the carbon content of biochar was inversely linked to biochar yield.

Lee *et al*. (2010) studied biochar materials made from corn waste under two pyrolysis conditions: quick pyrolysis at 450 ˚C and gasification at 700 ˚C. The cation exchange capacity (CEC) of the quick pyrolytic biochar was around two times higher than that of the gasification biochar and a regular soil sample.

In a glasshouse experiment, Van *et al*. (2010) examined two biochars derived from the slow pyrolysis of paper mill waste. Both biochars had a wide surface area (115 m^2 g^{-1}) and calcium mineral agglomeration areas, according to the results.

Liming values (33 % to 29 %) and carbon content (50 % and 52 %) of the biochars varied significantly.

Peng *et al*. (2011) evaluated that charring temperature and duration had a significant impact on the chemical, physical, morphological and spectral properties of biochar. Increasing the temperature and duration of charring significantly reduced the biochar yield and volatile matter content while increased C, K and P.

Hossain *et al*. (2011) performed an experiment to see how pyrolysis temperature affects the development of wastewater sludge biochar and to assess the properties required for agronomic applications. They found that increasing the pyrolysis temperature (from 300 ^{o}C to 700 ^{o}C) reduced the yield of biochar. They also discovered that biochar produced at low temperatures was acidic, while biochar produced at high temperatures was alkaline in nature. With increasing temperature, nitrogen concentrations fell while micronutrient concentrations rose. The concentrations of trace metals in wastewater sludge varied with temperature, with biochar being the most enriched. The physicochemical properties of coconut coir biochar was found with pH 9.40, EC 3.25 dSm^{-1}, CEC 32 cmol(p+)kg^{-1}, total organic carbon 276 g kg^{-1}, total nitrogen 8.5 g kg^{-1}, total phosphorous 1.5 g kg^{-1}, and total potassium 5.3 g kg^{-1} (Shenbagavalli and Mahimairaja, 2012).

According to Zhao *et al.* (2013), the carbon content of biomass and biochars varied between 21.6 to 58.0 % and 12.7 to 77.0 %, respectively. As compared to the biomass feedstock, pyrolysis treatment increased C content in biochar by 34.1 % on average. The C content of plant-based biochars was higher than that of waste-based biochars and the H and O contents of food waste biochars were higher. They also found that the majority of organic matter (OM) is released as syngas and pyrolytic oils during the pyrolysis of an organic feedstock, resulting in a relative enrichment of ash. However, depending on the feedstock and pyrolysis conditions, the final concentration and composition of organic matter varies.

Varela *et al.* (2013) measured pH, EC and total carbon in wood biochar and found that they were 7.32, 704 (Sm^{-1}) and 527.4 (mg kg^{-1}), respectively. Jindo *et al.* (2014) characterized four organic waste biochars, namely rice husk, rice straw, apple tree wood and oak tree wood biochars with their pH 6.84, 8.62, 7.02 and 6.43, respectively.

Zhang and Sun (2014) revealed that adding wood biochar to sludge at a concentration of 12 to 18 % (w/w) changed the microstructure of the sludge, resulting creation of channels or voids on the sludge surface, which was thought to help the compost substrate disintegrate and aerobically biodegrade.

Nartey and Zhao (2014) found that the biochar can be prepared from a variety of feedstocks, including sewage sludge, forest residue, organic and agricultural wastes and at a variety of pyrolysis conditions and balances. Physical and chemical methods used to identify the characteristics of biochars demonstrate the basic structure and properties of biochar. Biochar had a lot of surface area, a charged surface and functional groups, so it can adsorb a lot of heavy metals and organic pollutants.

Rehrah *et al*. (2014) made biochar from various by products [pecan shells (PC), peanut shells (PS) and cotton gin (CG) trash and *Panicum virgatum* L.] at various pyrolysis temperatures and residence times evaluated the physico-chemical properties [yield, ash, pH, total surface area (TSA), surface charge (SC) and electrical conductivity (EC)] and elemental composition of the resulting biochar. The biochar yield was depending on the type of the feedstock, pyrolysis condition (N_2 environment), pyrolysis temperatures (300, 500 and 750 °C) and residence periods (8, 16 and 24 h), (4, 8 and 12 h) and (1, 2 and 3 h). Lower biochar recovery, greater TSA, higher pH, minimal SC and higher ash contents obtained in the higher pyrolysis temperatures.

The addition of biochar to composting changes the C/N ratio of the starting substrates since it is a carbon-rich material (Dias *et al.,* 2010). Biochar has a much longer residence period (from decadal to millennial timescales) than its precursor content, demonstrating high recalcitrance to abiotic/biotic degradation (Lehmann and Joseph, 2015).

Biochar can influence the moisture content of compost mixtures by increasing pile porosity. Despite the lack of clear evidence that biochar addition will increase the moisture content or moisture storage ability of compost piles, studies have discovered that biochar amendment soils have a higher moisture storage capacity (Lehmann and Joseph, 2015).

Purakayastha *et al*. (2015) compared maize, pearl millet, rice and wheat biochars, finding that rice and wheat biochars had lower bulk densities (0.46 Mg m^{-3} and 0.48 Mg m^{-3}, respectively) and higher water holding capacity (543 and 561 %, respectively) than maize and pearl millet biochars.

Punnose and Anitha (2016) found that woody biochar had higher porosity (62.9 %), water holding capacity (363.9 %), total carbon (50.98 %) and lower bulk density (0.34 g cm^{-3}) as compared to coconut petiole and leaf waste biochars.

A laboratory study was carried out by Mary *et al.* (2016) to classify three types of biomass wastes, namely carbonized cauliflower leaf (CL), orange peels (OP) and pea pod (PP) at various temperatures. The organic carbon, total surface anions, water holding capacity and mineral content of the PP and CL biochars were higher than the OP biochar for use as a soil amendment.

Lee *et al*. (2016) found that the Peanut hull-derived biochar materials have cation exchange capacity (CEC) values ranging from 6.22 to 66.56 cmol(p^+) kg^{-1}, which are considerably higher than those of southern yellow pine-derived biochar, which are near zero or negative. The highest CEC value was 66.56 cmol(p^+)kg^{-1} for biochar made from peanut hulls using a steam activation process, which was around 5 times higher CEC than the [12.51 cmol(p^+) kg^{-1}] of a reference soil sample.

Sugarcane bagasse biochar was characterized by Figueredo *et al*. (2017), who found that the biochar had a pH of 7.28, a total organic carbon content of 341 g kg^{-1} and a CEC of 10.6 cmol(p^+) kg^{-1}. Castor stalk biochar was characterized by Hilioti *et al.* (2017) and found to have a pH of 9.25, 0.73 % phosphorus and 2.41 % potassium.

Sarfaraz *et al*. (2020) found that the biochars made from animal manure and crop straws in a muffle furnace had different properties, including biochar percentage output. Except for N, which was found to be extremely low in all biochars, carbon and other nutrients (P, K, Ca and Mg) were found to be at higher levels. Biochars had a higher cation exchange capacity, which could help soil retain more nutrients when applied as amendment. The pH and EC of the biochar were also higher, indicating that biochars made at 450 ˚C may help to raise the pH of acidic soil.

A study was conducted by Chatterjee *et al.* (2020) to investigate the impact of different pyrolysis temperatures on biochar physicochemical properties, activation and carbon capture. Sugarcane bagasse, miscanthus, switchgrass and corn stover were used for synthesis biochar at four different temperatures (500, 600, 700 and 800 ˚C). In most of the samples, raising the pyrolysis temperature (500 – 700 ˚C) resulted in higher percent C and percent ash contents with lower percent H, O and N contents.

2.2 BIOCHAR AS A SOIL AMENDMENT – TROPICAL SOIL PERSPECTIVE

2.2.1 Effect of biochar on soil physical properties

Glaser *et al*. (2002) demonstrated that addition of biochar (45 % by volume) was effective in increasing soil water retention by 18 % (Tryon, 1948) in sandy soil. It was also found that charcoal-rich anthrosols with surface areas three times higher than surrounding soils also exhibited 18% higher field capacity.

Gundale and DeLuca (2007) found that biochar had a lower bulk density than mineral soils and therefore its application led to lower the overall bulk density of the soil. However, biochar increased porosity, available soil water content, while decreasing bulk density and soil strength.

The effect of lack locust (*Robinia pseudocacia* L.) biochar on soil hydraulic properties and nutrient retention in a sandy soil was studied by Uzoma *et al.* (2011). When compared to un-amended sandy soil, biochar formed at 500 ˚F and applied at a rate of 20 t ha^{-1} increased usable water capacity by 97 % and saturated water content by 56 %.

An experiment was performed by Mankasingh *et al.* (2011) to investigate the effects of biochar on soil properties revealed that, when biochar was applied at different rates (0, 1.1, 2.2 and 4.0 %), a steady decrease in bulk density was observed from 0.99 g cm^{-3} to 0.89 g cm^{-3}.

Artiola *et al.* (2012) performed a greenhouse experiment with biochar amendments of 2 %, 4 % and 5 % and found that the water keeping ability ranged from 185 to 200 %, indicating that biochar can carry water twice its weight. With the application of 2 % and 4 % biochar the bulk density reduced significantly from 1.62 g cm^{-3} in the control to 1.38 g cm^{-3} and 1.22 g cm^{-3} respectively.

Mapa *et al.* (2012) conducted an experiment and concluded that adding biochar increased the volumetric water content of soil at the field capacity (FC) from

0.16 cm^3cm^{-3} to 0.24 cm^3cm^{-3} when amended with 3 % biochar and from 0.16 cm^3 cm^{-3} to 0.33 cm^3 cm^{-3} when amended with 5 % biochar. It was also observed that field air capacity, a measure of soil aeration, dropped from 0.42 cm^3cm^{-3} in control to 0.33 and 0.22 cm^3cm^{-3} in the 3 % and 5 % biochar treatments, respectively.

An incubation study conducted by Herath *et al*. (2013) reported that after 295 days of studies, the total soil pore volume of an alfisol significantly increased by 10 % with the application of 7.18 t ha^{-1} of corn stover biochar and same rate of application in andisol resulted 21 % increase in soil available water content.

Mukherjee and Lal, (2013) based on their greenhouse experiment in sandy soil, noticed a significant increase in porosity from 56.1 % (control) to 62.1 % with application of eucalyptus wood biochar at 2.27 g kg^{-1} soil.

A long-term experiment conducted by Liang *et al*. (2014) to determine the effect of biochar on soil properties in calcareous soils and revealed that soil pH, was increased by a maximum of 0.35 units after 2 years of biochar application. While after 3 years, soil bulk density decreased significantly from 1.40 to 1.31 g cm^{-1} and the water holding capacity rised by 9 %.

Chaves *et al*. (2018) used poultry litter biochar in a laboratory analysis in an ultisol and found that biochar amended soil (15 t ha^{-1}) had a higher water holding capacity (1.2 %) than control soil.

A field experiment was performed by Adekiya *et al.* (2019) for over two years to investigate the effects of biochar (B) and poultry manure (PM) on soil properties. The result revealed that there was a decrease in bulk density and an increase in porosity and moisture content as compared to the control.

2.2.2 EFFECT OF BIOCHAR ON CHEMICAL AND BIOLOGICAL PROPERTIES

Arocena and Opio (2003) found that ashes have the potential to neutralize acidic soil. Another explanation for the rise in soil pH caused by biochar application is due to biochar has a large surface area and highly porous, which improves the soil's cation exchange capacity (CEC).

Keech *et al*. (2005) studied that biochar had a greater surface area, porosity and a charge that had the potential to increase soil water-holding capacity, cation exchange capacity, surface sorption capacity and base saturation. Because of its larger surface area, higher negative surface charge and higher charge density, biochar has a greater ability to adsorb cations than other soil organic matter (Liang *et al.*, 2006).

Gundale and DeLuca (2007) evaluated that application of biochar increased organic carbon, soil pH, available P, CEC and exchangeable K while decreasing, exchangeable Al and soluble Fe. In a corn trial in Colombia, Rodriguez *et al.* (2009) found that biochar made from sugarcane bagasse raised the pH of the soil from 4.0 – 4.5 to 6.0 – 6.5. Biochar is found primarily in fractions of soil organic matter (SOM) that are contained in small clusters of soil particles, rather than as free organic matter (Liang *et al.,* 2006). Masulili *et al*. (2010) studied that when rice husk biochar at 10 t ha^{-1} was applied to acid sulphate soils, soil pH increased significantly from 3.36 (control) to 4.40.

An incubation experiment conducted by Yaghoubi and Reddy (2011) found that when soil amended with 5 and 20 % of biochar, particularly when 20 % biochar (w/w) is applied to soil, the pH value rised from 5.3 to 7.3, indicating that the pH value rised from acidic to neutral. Yuan *et al*. (2011) found that the alkalinity of biochar was a key factor affecting its liming potential and the higher the alkalinity, the greater the reduction in soil acidity. Biochars improved soil fertility by decreasing soil exchangeable acidity and increasing soil exchangeable base cations and base saturation.

Hossain *et al*. (2011) found a substantial improvement in electrical conductivity (EC) after adding biochar, which may be attributed in part to the fact that their biochar was made from sewage sludge, which is mineral-rich, as compared to biochar made from hardwood.

Nigussie *et al*. (2012) conducted an experiment and found that the highest mean pH values and the highest mean value of EC were found in soils treated with 10 t ha^{-1} biochar, whereas the lowest values were found in control soils (0 t ha^{-1}). Carbonates of alkali and alkaline earth metals, variable quantities of silica, heavy metals, sesquioxides, phosphates and small amounts of organic and inorganic N present in biochar responsible for the rise in soil pH and EC caused by biochar application. A pot culture experiment conducted by Carter *et al.* (2013) revealed that adding biochar at 50 g kg^{-1} increased pH by 0.6 units from 5.5 to 6.1 and adding 150 g biochar kg^{-1} increased pH by 1.2 units from 5.5 to 6.7.

Biochar made from crop straws improved soil pH, exchangeable base cation, CEC and base saturation while reducing exchangeable acidity, exchangeable Al and reactive Al. The resulting effects were influenced by the feedstock properties as well as the pyrolysis temperature (Wan *et al.*, 2014).

The impact of biochar on soil electrical conductivity was investigated by Chintala *et al*. (2014). At 650 °C, they used biochar made from two biomass feedstocks (corn stover and switchgrass). However, application of biochar at very high rates of 150 Mg ha^{-1} resulted in a significant increase in EC.

Ghosh *et al*. (2015) found that all compost and biochar treatments had resulted in substantial increases in soil pH and EC as compared to the control. The initial pH of the soil was 5.38, but after the addition of organic amendments, it increased dramatically to a maximum of 7.10. A field experiment conducted by Chathurika *et al.* (2015) in sandy clay loam soil, after a two-season of study, found that applying sawdust biochar increased the soil's EC from 136.5 S cm^{-1} to 150 S cm^{-1}.

A pot experiment conducted by Abrishamkesh *et al.* (2015) studied that the effects of rice husk biochar on alkaline soil properties and lentil growth. The crop was grown in soil that had been amended with 0.4, 0.8, 1.6, 2.4 and 3.3 % biochar made from rice husk by weight. Biochar application increased SOC, CEC and available K while decreased soil bulk density while having no impact on available water content.

A field experiment conducted by Pandian *et al.* (2016) revealed that application of different type of biochar with different rate gives significant difference in pH and EC over the control. Among them the application of prosopis biochar at 5 t ha^{-1} resulted significant difference in pH and EC over the control, the pH increased from 5.72 to 6.33 and EC increased from 0.22 to 0.42.

Conz *et al.* (2017) found that soil EC improved significantly from 1.13 dSm^{-1} to 1.35 dSm^{-1} in clayey soil incubated with sugarcane straw biochar. Based on the pot experiment with clay soil, Trupiano *et al.* (2017) reported a substantial increase in soil EC from 0.71 to 1.5 dSm^{-1} due to application of biochar at 50 g kg^{-1} dry soil.

Effect of biochar on soil biological properties

Biochar amendments tend to promote soil fungi, which makes sense considering that biochar is a complex matrix that can only be degraded by soil fauna and soil fungi (Birk *et al*. 2009).

Deenik *et al*. (2010) evaluated that adding biochar with a high volatile matter content (22 %) stimulated microbial activity, induced net N immobilization and reduced lettuce and corn yields. The addition of biochar having a very low amount of volatile matter content (6.3 %) resulted in lower microbial action spikes and net N immobilization.

Bailey *et al.* (2011) discovered that biochar increased the activity of alkaline phosphatase, amino-peptidase and N-acetylglucosaminidase. The development of organic N- and P-mineralizing enzymes was stimulated by plant uptake of N and P, as well as the growth of fine roots and root hairs into biochar pores.

Biochar absorbed toxic and harmful compounds in the soil studied by Zavalloni *et al.* (2011), indirectly increasing soil MBC (microbial biomass carbon). These improvements in soil biochemical reaction conditions increased soil enzyme activity and created more chemicals (Lee *et al*., 2010).

Lehmann *et al*. (2011) and Steinbeiss *et al*. (2009) found that the fungal biomass has a greater capacity to decompose more complex compounds in biochar and biochar application increased fungal population rather than bacterial population.

Dempster *et al.* (2012) revealed that the biochar contains 2.5 % carbonate, which increases CO_2 after dissolution in soil solution. When biochar and organic amendments like green and pig manure are applied together, CO_2 efflux is increased compared to when they are applied separately (Luo *et al*., 2011).

A field experiment performed by Chen *et al.* (2013) found that the biochar amended soil have increased of activities of dehydrogenase, alkaline phosphatases while decreased glucosidase.

Biochar amended soils had greater rhizosphere zones than control soils (Prendergast-Miller *et al*., 2014), Furthermore, biochar particles were found in the rhizosphere, which provided additional labile biomass, nitrogen and phosphorus sources as well as habitat niches for bacterial proliferation and persistence.

Gomez *et al*. (2014) revealed that after adding biochar, the number of bacteria, fungi and actinomycetes increased significantly. Actinomycetes have a slow reproduction rate and thrive in nutrient-poor soil.

Lu *et al*. (2015) conducted an experiment and discovered that, with the exception of urease in rhizosphere soils, the activities of urease, invertase and phosphatase in both bulk and rhizosphere soils were increased by 30 - 44 %, 19 - 31 % and 25 - 36 %, respectively, compared to the control. As a result, significant increase in microbial growth and enzyme activities, combining biochar and poultry manure with pyroligneous solution may be a viable choice for alleviating salt stresses on plant and soil microbial communities and improved crop production in saline soils.

Watanabe and Sato (2015) found that when biochar and organic amendment were implemented simultaneously, net CO_2-C evolution was higher than the amount of net CO_2-C evolution from sole amendment with biochar and organic amendment.

In addition, the effects of biochar addition on CO_2 emissions are related to the rate at which it is applied. Biochar application at a low rate has a minimal impact on compost mixture properties and cannot contribute to the optimum pH and aeration conditions needed by microorganisms; these effects would be more pronounced if biochar was applied at high rates (Awasthi *et al.,* 2017).

2.2.3 EFFECT OF BIOCHAR AS A NUTRIENT SOURCE (NUTRIENT COMPOSITIONS) AND ITS NUTRIENT USE EFFICIENCY (NUTRIENT RETENTION /SLOW RELEASE)

A field experiment performed by Steiner *et al*. (2008) found that crops growing in an acidic soil amended with 11 t ha^{-1} wood biochar used nitrogen more efficiently over the course of two years. Since this was a hard-setting soil, they attributed the increased productivity to the positive effects of higher biochar rates on soil physical properties and thus root growth.

Improved P fertilizer use efficiency was discovered by Blackwell *et al.* (2010) in the biochar amended soil, where biochar attributed to better plant mycorrhizal interactions. Wheat yields was found to increase more at low fertilizer application rates than at high fertilizer application rates by applying 1 t ha^{-1} of biochar to lands.

An incubation study conducted by Widowati *et al*. (2011) reported the application of biochar affect the pattern of nitrogen release from urea fertilizer. Biochar slowed down the transformation of $N-NH_4$ to $N-NO_3$. After 28 days of incubation, the CM (Chicken manure) biochar treated soil had 62 mg kg^{-1} $N-NH_4$ and the CW (city waste compost) biochar treated soil had 52 mg kg^{-1}. These values are higher than those observed in untreated soil (12 mg kg^{-1} $N-NH_4$) or even CM-treated soil (40 mg kg^{-1} $N-NH_4$).

A field experiment conducted by Sukartono *et al.* (2011) to evaluate the effect of cattle dung biochar on sandy loam soil found that the application of cattle dung biochar with the rate of 15 Mg ha^{-1} increased N, P and K uptake from 87.31 kg ha^{-1} to 103.12 kg ha^{-1}, 12.25 kg ha^{-1} to 16.81 kg ha^{-1} and 98.65 kg ha^{-1} to 123.04 kg ha^{-1}, respectively.

A pot experiment was conducted by Nigussie *et al.* (2012) to investigate the effect of biochar on the properties of chromium-polluted soils and the absorption of lettuce grown in polluted soils. The results showed that biochar application is critical for increasing soil fertility, improving nutrient uptake, improving Cr-polluted soils and reducing the amount of carbon emitted by biomass burning.

A pot culture experiment conducted by Carter *et al.* (2013) revealed that the soil treated with biochar, helps to increase the final biomass, root biomass, plant height and number of leaves. They found that the biochar additions resulted in the greatest biomass increase (903 %) in non-fertilized soils, rather than fertilized soils (483 % with the same biochar application as in the “without fertilization” case). The effect was decreasing over the cropping cycles, with a 363 % rise in biomass in the third lettuce cycle.

A field experiment conducted by Martinsen *et al*. (2014) found that the high ash fraction of biochar one of the key nutrient factors responsible for increased biomass production. A significant positive relationship between maize biomass production and K supply rates combined with previous findings that K is the primary nutrient added by biochar.

Increased N content in soils amended with compost and biochar was experimented by Ghosh *et al*. (2015) indicated that these amendments will supply N due to their relatively high N content. The findings also indicate that increased native soil N mineralization was due to a priming effect following the application of these amendments, as a result of increased soil microorganism growth (Hamer *et al*., 2004).

A pot experiment was conducted by Ghosh *et al.* (2015) to evaluate the effect of biochar and organic compost on soil properties and on tree growth in Singapore. In response to organic amendments, *Samanea saman* showed greater stem elongation than *Suregada multiflora*. In biochar amended treatments, both tree species had substantially higher foliar N content, as well as substantial increase in P.

A greenhouse and field experiment conducted by Chathurika *et al.* (2015) found that, in the greenhouse experiment, plant dry weight increased significantly with the addition of amendments i.e., fertilizers alone site specific fertilizer (SSF), or biochar + SSF or rock powder + SSF and in field condition.

In sandy clay loam soils, Yusof *et al*. (2015) observed a substantial increase in nutrient concentrations of P (0.065 to 0.131 %), K (0.611 to 1.416 %), Ca (0.005 to 0.022 %), Mg (0.079 to 0.102 %) and Zn (4.43 to 15.34 ppm) in rice crop with the application of rice straw biochar at 5 t ha^{-1} + poultry litter biochar at 5 t ha^{-1} compared to control.

A study conducted by Gwenzi *et al.* (2018) found that biochar was an important nutrient carrier in the development of an N–P–K fertilizer. The biochar-based slow release fertilizer had lower nutrient release, higher moisture retention and a higher pH than conventional chemical fertilizer, indicating that it could be a better alternative to chemical fertilizers.

A field experiment performed by Adekiya *et al.* (2019) for over two years to investigate the effects of biochar (B) and poultry manure (PM) on different soil properties revealed that the application of biochar alone or with the combination of poultry manure, increased the N, P, K, Ca and Mg concentration in soil, leaf nutrient concentration and the yield attributes of radish and also found that the interactive effect of biochar and poultry manure was significant in the both of the year of study.

2.3 CO-APPLICATION OF BIOCHAR AND CHEMICAL FERTILIZER ON MEDICINAL HERBS

When crops are grown in cultivated soil, biochar combined with chemical fertilizer (CF) may be a viable choice for improvement of crop productivity. The combined application can improve the availability and uptake of plant nutrients while also reducing nutrient leaching by reducing the application of CF. As a result, biochars and CF must be used together or mixed in various amounts. Though, in different food crops, the value of combining biochar with CF has been reported (Sadaf *et al*., 2017).

2.3.1 Effect on Yield (economic part/essential oils)

A pot culture experiment conducted by Saha *et al.* (2019) to evaluate the effect of combined application of biochar prepared from lemongrass (*Cymbopogon flexuosus*) distillation waste and chemical fertilizers (CF) with different ratios on yield, quality of *Andrographis paniculata*. The results revealed that the application of biochar at 5 t ha^{-1} with chemical fertilizer (60:20:40 NPK kg ha^{-1}), significantly increased the fresh herbage yield (140.8 g $plant^{-1}$) over the control (72.1 g $plant^{-1}$) and also increased the total andrographolide yield content (0.57 g $plant^{-1}$) over the control (0.19 g $plant^{-1}$).

2.3.2 Effect on Quality (bio active compounds *viz., phenols, flavonoids and antioxidants etc*.)

A pot culture experiment was conducted by Saha *et al.* (2019) to evaluate the effect of combined application of biochar prepared from lemongrass (*Cymbopogon flexuosus*) distillation waste and chemical fertilizers (CF) with different ratios on yield and quality of *Andrographis paniculata*. The results showed that the application of biochar at 5 t ha^{-1} with chemical fertilizer (60:20:40 NPK kg ha^{-1}), significantly increased the total phenolic content (TPC), total flavonoid (TFC) content and antioxidant potential over the control.

3. MATERIALS AND METHODS

The research work was conducted to evaluate the effect of Integration of biochar with chemical fertilizer for improving yield and quality of Tulsi (*Ocimum sanctum* L.). The study was conducted in pot culture under natural condition of net house. The details of material used, experimental methods followed and techniques adopted during the course of investigation are given below.

3.1. EXPERIMENTAL SITE

The present experiment was carried out at net house, ICAR- DMAPR, Boriavi during *kharif* season of year 2020.

3.2. CLIMATE AND WEATHER CONDITION

The pot culture experiment was conducted at the net house of ICAR – Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand located at 22° 35′ 57″–22° 36′ 06″ N latitude and 73° 27′ 57″– 73° 27′ 16″ E longitude, at an altitude of 45.11 m above mean sea level. The climate of the study area is semi-arid and subtropical, with hot summers and cold winters with a mean annual maximum and minimum temperature of 41.5 and 9 °C, respectively. Annual average rainfall is (approximately) 860 mm, occurring mostly during the months of August to September.

The meteorological data on the average weekly maximum and minimum temperature, rainfall, bright sunshine hours and mean relative humidity recorded at the agro-meteorological observatory, during experimental period of the year 2020-21 are given in Table 3.1.

3.3. PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL

The bulk soil sample was collected from a fallow site of ICAR-DMAPR, Boriavi for pot culture experiments. The soil for experimental collected from ICAR-DMAPR, Boriavi was Fluventic Ustochrept having sandy loam texture. The physico-chemical characteristics of the soil taken at a depth of 0 - 15 cm were analyzed by standard procedures presented in Table 3.2.

Table 3.1. Mean weekly parameters recorded for the period of experiment during 2020-21

	Standard Week	Date	Temperature °C		Mean R.H. (%)	Sun shine hrs / day	Evaporation (mm)	Rainfall (mm)	Rainy days
			Max.	Min.					
September 2020	39	24-30	32.1	25.3	78.9	4.5	3.0	3.2	1.0
October 2020	40	01-07	33.9	24.9	69.4	8.8	4.2	13.2	1.0
	41	08-14	35.9	23.0	57.9	8.0	4.5	0.0	0.0
	42	15-21	35.1	26.9	72.5	7.0	4.3	10.4	1.0
	43	22-28	35.3	20.3	54.7	9.5	4.4	0.0	0.0
	44	29-04	34.0	17.6	55.5	9.1	4.0	0.0	0.0
November 2020	45	05-11	33.4	15.8	54.9	9.3	3.4	0.0	0.0
	46	12-18	32.6	19.1	59.2	8.5	3.1	0.0	0.0
	47	19-25	30.4	15.2	60.0	8.7	3.7	0.0	0.0
	48	26-02	30.4	17.6	59.1	8.4	3.6	0.0	0.0
December 2020	49	03-09	32.7	15.1	62.0	9.5	2.8	0.0	0.0
	50	10-16	27.4	17.9	78.3	4.8	2.0	16.4	2.0
	51	17-23	26.6	12.6	63.1	8.8	2.7	0.0	0.0
	52	24-31	26.8	12.3	55.1	9.2	3.1	0.0	0.0
January 2021	1	01-07	25.7	14.9	62.5	4.1	2.6	0.0	0.0
	2	08-14	27.5	16.1	74.1	5.6	2.9	0.0	0.0
	3	15-21	29.2	13.6	67.8	8.6	2.8	0.0	0.0

Table 3.2: Physico – Chemical properties of experimental soil

S.No	Particulars		Method employed	Reference
A	**Physical properties**			
	Mechanical fraction (%)		International Pipette Method	Piper (1966)
1	Sand	70.1		
2	Silt	11.3		
3	Clay	18.6		
4	Texture class	Sandy loam		
B	**Chemical properties**			
1	pH (Soil : water, 1: 2.5)	7.8	Potentiometry	Richards (1954)
2	EC (Soil : water, 1 : 2.5) (dSm^{-1})	0.28	Conductometry	Richards (1954)
3	Organic Carbon (g kg^{-1})	2.92	Wet oxidation method	Walkley and Black (1934)
4	CEC (C mol (p^+) kg^{-1})	9.6	Ammonium acetate	Jackson (1973)
5	(NH_4^+ + NO_3^-) Nitrogen (mg kg^{-1})	39.6	Kjeldahl distillation	Keeney and Nelson (1983)
6	Available P (mg kg^{-1})	16.3	0.5 M $NaHCO_3$	Olsen *et al*. (1954)
7	Available K (mg kg^{-1})	89.7	Flame photometry	Hanway and Heidel (1952)

3.4. PREPARATION OF BIOCHAR

The procedure for preparation of Biochar

- Biochar was prepared by pyrolysis of distillation waste biomass of tulsi at 350 ^{o}C in muffle furnace.
- The distillation waste biomass of tulsi was collected from hydro distillation unit of ICAR-DMAPR and chopped the biomass to reduce size to 5-6 cm.
- After that the distillation waste biomass was dried in shade.
- Crush and grind the waste biomass into powder (≤ 200 μm).
- Pyrolysis in digital temperature controller equipped Muffle furnace.
- N_2 purge (flow rate 2 mL min^{-1}) to make oxygen limited environment.
- The heating rate was 5 ^{o}C min^{-1} and residence time was 2 h at temperature 350 ^{o}C.

3.5. CHARACTERIZATION OF BIOCHAR

The biochar (BC) used in this study were finely ground and passed through the sieve for further analysis. The pH and electrical conductivity were measured by using a digital pH and conductivity meter, respectively, at a ratio of biochar: water ratio 1: 5 (w/v) (Jackson, 1973). The cation exchange capacity (CEC) was measured following the method by using 1 N sodium acetate solution (pH 8.2) exchange complex (Sumner and Miller, 1996). The Fourier transformation infrared (FTIR) spectra of BC were taken in Shimadzu IR-Prestige-21®. Potassium bromide (KBr) pellets were prepared by mixing powdered biochar and spectra were recorded in 400-4000 cm^{-1} scan range. Morphology of biochar surface was pictured in scanning electron microscope (SEM) (Philips XL-30®) at different magnification in order to confirm the complex formation. For this, powdered BC was smeared on specimen stubs and then it was gold coated under vacuum before being observed under SEM.

3.5.1 Total Nitrogen (TN)

Total nitrogen in biochar was determined by micro-Kjeldahl method (Bremner and Mulvaney, 1982). Biochar (0.5 g) was digested with concentrated

H_2SO_4 using salt-catalyst mixture. After digestion, the material was diluted, made alkaline with 40 % NaOH and steam-distilled in a micro-Kjeldahl system. The NH_3 released was absorbed in 4 % H_3BO_3 containing mixed indicator (methyl red + bromocresol green) and titrated against standard H_2SO_4.

3.5.2. Total Phosphorus

The oven dried biochar samples were ground by a Wiley mill (5 mm) and digested with di-acid mixture containing $HClO_4$: HNO_3 :: 9 : 4 on hot plate (Biswas, 2009). The digested material was cooled and diluted in distilled water. The total phosphorus content in the acid extract was determined calorimetrically after developing vanadomolybdo yellow colour complex in HNO_3 medium (Jackson, 1973).

3.5.3. Total Potassium

Total potassium was determined through in the di-acid digestion (Biswas, 2009) of biochar sample. The total potassium in the acid extract was determined in Flame photometer after suitable dilution (Page *et al*., 1982).

3.5.4.. Olsen extractable Phosphorus

Olsen extractable phosphorus in biochar sample was extracted with 0.5 M $NaHCO_3$ (pH 8.5) solution and measuring blue colour (730 nm) in spectrophotometer (Watanabe and Olsen, 1965).

3.5.5. NH_4OAc extractable Potassium

NH4OAc extractable potassium was estimated by a flame photometer after extracting the biochar with neutral normal ammonium acetate solution (Jackson, 1973).

3.5.6. Water Soluble Phosphorus

Water soluble P was extracted on 10 : 50 w/v (biochar to distilled water). The solution was agitated for 1 hour after adding 1 g activated charcoal and then filtered. P content in the extract was measured by developing blue colour (phosphovanadomolybdate complex) using ascorbic acid as reductant and subsequent estimation in spectrophotometer (Watanabe and Olsen, 1965)

3.5.7. Water Soluble Potassium

This pool of potassium was extracted using (biochar: water::1: 5) as described by Page *et al*. (1982). The K content in the water extracts was then determined by a flame photometer.

3.6. EXPERIMENTAL DETAILS:

3.6.1. Experimental design and layout

The details of the experiment conducted to evaluate the effect of Integration of biochar with chemical fertilizer for improving yield and quality of Tulsi (*Ocimum sanctum* L.) during *kharif* season of 2020is given in table 3.3.

Table 3.3 Experimental details

Location	Net house, ICAR- DMAPR, Boriavi	
Soils	Experimental farm of ICAR-DMAPR, Boriavi	
Crop and Variety	Tulsi (*Ocimum sanctum* L.) and variety: Cim-Angna	
Season and Year	*Kharif* – 2020	
Experimental design	Complete Randomized Design (CRD)	
Number of repetitions	3 (Three)	
Number of treatments	7 (Seven)	
Total number of pots	21 (Twenty one)	
Pot details	Size	Upper diameter : 45 cm, Depth : 40 cm
No. of seeds per pot	5 (Five)	

3.6.2. Treatment details:

T_1: Control

T_2: Biochar at 5 t ha^{-1}

T_3: 100% Recommended dose of fertilizer (RDF)

T_4: Biochar at 2.5 t ha^{-1} + 50 % RDF

T_5: Biochar at 2.5 t ha^{-1} + 100 % RDF

T_6: Biochar at 5 t ha^{-1} + 50 % RDF

T_7: Biochar at 5 t ha^{-1} + 100 % RDF

3.6.3. Treatment application details:

- **RDF (100-50-50 NPK kg ha^{-1})**
- Nitrogen will be applied as split dose

- 50 kg of N is applied as basal dose and remaining 50 kg (25 kg + 25 kg) of N at 21 and 35 DAS in standing crop.
- Phosphorous and potassium is applied as a whole amount (50 kg) as basal.
- **Source of Fertilizer:** Urea and Di-ammonium phosphate (DAP) and MOP
- **Seed treatment:** Seeds will be soaked for 2 hours and then shade-dried for one hour in case of seed treatment with *tricoderma*.
- **Soil application**: Chemical fertilizer and biochar at the time of sowing.

3.7. CULTURAL OPERATIONS

3.7.1. Preparation of soil

Bulk soil sample (0-15 cm) was collected from farm of ICAR-DMAPR, Boriavi. After collection, soil was ground and processed (< 5 mm size). It was placed on a clean polyethylene sheet and a calculated quantity of different nutrient sources were added to the soil and mixed thoroughly as per the treatments and 30 kg soil was filled in each earthen pot. Initial physico-chemical properties of the soil *viz.*, mechanical fraction, physical properties, pH, EC, organic carbon, CEC, (NH_4^+ + NO_3^-) N, available P and K were analyzed before sowing and the data were presented in table 3.2.

The fertilizer doses were added according to the recommended dosage of fertilizer nitrogen. As per recommended dose, 50 % of nitrogen (50 kg N ha^{-1}) supplied from urea (after subtracting N concentration from DAP) and whole of potassium (50 kg K_2O ha^{-1}) and phosphorus (50 kg P_2O_5 ha^{-1}) from muriate of potash (MOP) and Di-ammonium Phosphate (DAP) respectively, were applied as basal in each pot followed by mixing thoroughly with soil.

3.7.2. Sowing and thinning

Five seeds of tulsi (Cim-Angna variety) were sown in each pot. After germination of seeds, thinning was carried out to maintain single plant per pot.

3.7.3. Fertilizer application

Nitrogen was applied as split dose: 50 kg of N was applied as basal dose and remaining 50 kg (25 kg + 25 kg) of N at 21 and 35 DAS in standing crop. Phosphorous and potassium were applied as a whole amount (50 kg and 50 kg respectively) as basal. Biochar treatments were applied at the time of sowing.

3.7.4. Irrigation

Initially after sowing, irrigation was given at regular intervals throughout the experimental period.

3.7.5. Weeding

Hand weeding was carried out in the pots to keep the pots free from weed infestation.

3.7.6 Harvesting

Harvesting was done after 90 days after transplanting when crop was in full flowering stage.

3.8. GROWTH AND YIELD PARAMETERS

3.8.1. Plant height (cm)

The plant height was recorded from ground level to the base of last fully opened leaf at flowering stage.

3.8.2. Total number of branches (per plant)

Primary and secondary branches emerging directly from the main stem were counted in each plant at the time of harvest.

3.8.3. Fresh leaf weight (g plant^{-1})

Plants were uprooted from each pot at harvest and fresh leaves from the plants were weighed immediately with electronic top pan balance.

3.8.4. Dry leaf weight (g plant^{-1})

The fresh leaf samples from each plant were kept in an oven at 40 °C for drying and the dry weights of leaves were recorded.

3.8.5. Total fresh biomass weight (g plant^{-1})

Total fresh biomass yields were derived from the harvested leaf and branches. And the total fresh biomass yield per plant was calculated from the fresh sample.

3.8.6. Total dry biomass weight (g plant^{-1})

Total dry biomass yields were derived from dry yields of leaf and branches respectively. The total dry biomass yield per plant was calculated from weight of the dry samples.

3.8.7. L: S ratio

After uproot the plant from each pot at harvest, weighed fresh leaves and stem from the plants separately with electronic top pan balance. And made the ratio between leaf to stem wright.

3.8.8. Total chlorophyll content (mg g^{-1}) (Sadasivan and Manickam, 1991)

With the addition of 20 ml of 80 % acetone, 1 g of finely cut fresh leaves was mashed to a fine pulp in a mortar and pestle. This paste was centrifuged at 5000 rpm for 5 minutes. In a 50 mL beaker, the supernatant was transferred. The residue was then ground in 20 mL of 80 % acetone, centrifuged for 5 minutes at 5000 rpm, and the supernatant transferred to the same beaker. This process was done four times more until the leftovers were nearly colourless. The insides of the mortar and pestle were likewise rinsed with 80 % acetone, with the clear washings collected in the beaker. With 80 % acetone, the volume was increased to 100 ml. This was done with all of the leaf samples. The absorbance of the extract solutions were read at 645, 663 and 652 nm against the solvent (80 % acetone) blank.

Calculated with the formula: $[20.2(A_{645}) + 8.02(A_{663})] \times V / (1000 \times W)$

Where, A= absorbance at specific wavelengths, V= final volume of chlorophyll extract in 80% acetone which h in this case is 100 ml and W= fresh weight of tissue extracted.

3.8.9. Oil yield (mL plant^{-1})

After extraction oil, collected separately and measure in terms of mL per plant.

3.9. PLANT ANALYSIS

Plant samples were digested with strong sulphuric acid to assess total nitrogen. Using micro kjeldahl equipment, the N in the digested sample is calculated. Dry materials were ground in a stainless-steel blade Willey mill and digested in a di-acid mixture (HNO_3: $HClO_4$ – 2:1) for total P and K. The determined volume was prepared with double distilled water. Whatman filter paper No.42 was used to filter the extract. Total elemental analysis was performed on the digested extract of plant samples using standard procedures as stated in Table 3.4.

Table 3.4: Methods of plant analysis

Sr. No.	Parameters	Analytical method	References
1.	Nitrogen	Kjeldahl method	Jackson (1973)
2.	Phosphorus	Vanadomolybdate yellow colour	
3.	Potassium	Flame photometric	

3.9.1 PLANT BIOACTIVE COMPOUND ANALYSES

3.9.1.1 Essential oil extraction and content (%)

Fresh herbage (leaves and fluorescence) collected from each replicated plant was subjected to hydro-distillation for 5 hrs in Clevenger-type apparatus. The essential oil from distillate was subjected to hydrous sodium sulphate for removal water. Then extracted oils were stored in amber colour vial 4-8 ^{o}C for further analysis. The oil content was calculated based on fresh weight basis and expressed as %.

3.9.1.2. Total phenol content (mg GAE g^{-1}) (Singleton *et al*. 1999)

Well grind leaf sample (0.5 g) was taken and mortar in 10-time of 80 % of ethanol. The sample was centrifuged at 10,000 rpm for 20 minutes and save the supernatant. Centrifuge and pool the supernatant after re-extracting the residue with five times the volume of 80 % ethanol. Until the supernatant is completely dry, evaporate it. The residue was liquefied in a known volume of distilled water. Pipette out 0.1 mL aliquot into test tubes. 0.5 mL of Folin-Ciocalteau reagent followed by 2.9 mL of water was added. After 3 min, 2 mL of 20 % Na_2CO_3 solution was added to each tube. The absorbance was measured at 750 nm against a reagent blank. Standard curve was prepared by using different concentrations of catechol.

3.9.1.3 Total flavonoid content (mg Quercetin equivalent g^{-1})

Well grind leaf sample (0.5 g) was taken and mortar in 10-time of 80 % of ethanol. The sample was centrifuged at 10,000 rpm for 20 minutes and save the supernatant. Centrifuge and pool the supernatant after re-extracting the residue with five times the volume of 80 % ethanol. Until the supernatant is completely dry, evaporate it. Pipette out 0.1 mL aliquot into test tubes. 0.6 mL of Quercetin and 0.6 mL of aluminium chloride was added (Wang *et al*., 2012). The sample was kept for 1 hour at room temperature and the absorbance was measured at 420 nm against a reagent blank. Standard curve was prepared by using different concentrations of Quercetin.

3.9.1.4 Anti-oxidant potential (IC_{50} µm mL^{-1})

The antioxidant activity of extracts was evaluated by assays of radical scavenging activity using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (Liyana-Pathiranan and Shahidi, 2005). For DPPH scavenging assay, 1.0 mL methanolic solution of DPPH (mM L^{-1}) was mixed with 3.0 mL of methanolic solution of tulsi leaf extract and it was kept under dark (30 min) at room temperature followed by spectrophotometric measurement at 515 nm. Scavenging activity (%) of DPPH and ABTS radical was calculated as:

$$= (A_c - A_s) \times 100/Ac,$$

A_c and A_s corresponds to absorbance of control and sample.

3.10. SOIL ANALYSES

After harvesting of tulsi, soil samples were collected from each pot for chemical and bio chemical analysis. Immediately after collection, portion of the soil samples were kept in refrigerator (4^oC) for further bio-chemical analysis. Rest of the soil samples were air dried and processed further for chemical analysis.

3.10.1. Soil physico-chemical properties

Physico-chemical properties of the soil *viz.*, pH, EC, organic carbon, CEC, (NH_4^+ + NO_3^-) N, available P and K were analysed as per standard procedure presented in table 3.2. Soil organic C was measured by oxidation with chromic acid followed by rapid titration with ferrous ammonium sulphate as per the method outlined by Walkley and Black (1934). For CEC, soil was extracted with neutral 1N ammonium acetate (NH4OAc) solution after filtration, washing with alcohol and finally titrates with 0.1N H_2SO_4 (Jackson, 1973). (NH_4^++NO_3^-)N in soil was extracted with 2 M KCl solution (Keeney and Nelson, 1982) followed by micro-Kjeldahl distillation and titration. Available P was extracted with $NaHCO_3$ solution (Olsen *et al*., 1954) followed by estimation of blue colour developed in spectrophotometer

(Watanabe and Olsen, 1965). Available K in soil was extracted with neutral 1 N ammonium acetate (NH_4OAc) solution (Hanway and Heidel, 1952) followed by estimation of K in the extract by flame photometer.

3.10.2 Soil Biochemical Properties

3.10.2.1. Microbial Biomass Carbon

Microbial biomass carbon (MBC) in soil was determined by fumigation-extraction method as outlined by Jenkinson and Powlson (1976). Moist soil sample was fumigated with chloroform ($CHCl_3$) in a vacuum desiccator and extracted with 0.5 M K_2SO_4 solution. The extracts of non-fumigated and fumigated soil samples were subjected to wet oxidation separately with potassium persulphate and dilute H_2SO_4 by heating the contents on a digestion block for 2 hours. Evolved $CO_2$was trapped in 10 mL of 0.1 M NaOH solution. The amount of CO_2 absorbed was determined by back titration with 0.01 N HCl. The amount of the MBC in soil was calculated as follows:

Microbial biomass carbon = $(OC_F - OC_{UF}) / K_{EC}$

Where, OC_F and OC_{UF} are organic carbon extracted from fumigated and un-fumigated soil, respectively (expressed on oven dry basis), and K_{EC} is the efficiency of extraction. A value of 0.45 is considered as a general K_{EC} value for microbial extraction efficiency and used for calculation.

3.10.2.2 Dehydrogenase (Klein *et al*., 1971)

One gram of soil was incubated for 12 hours with 1 ml of 3% TTC (Triphenyltetrazolium chloride) and 0.5 ml of 1 % glucose. After incubation 10 ml of methanol was added. Then the test tube was shaken and allowed to stand in dark for 24 hours. Supernatant was withdrawn and colour intensity was measured using blue filter at 400 nm wavelength. The amount of formazan formed from standard curve prepared from TPF (Triphenylformazan) was in the range of 0.04 to 0.5 mg/10 ml. The results were expressed in the terms of TPF formed per hour per gram of soil (TPF g^{-1} soil h^{-1}).

3.10.2.3. Soil respiration (Anderson., 1982)

Take 100 g of soil sample in a conical flask, moist the soil at field capacity. Take 10 mL of 0.5 N NaOH in a tube and the tube tie in the conical flask and make the conical air tight. Keep the flask in an incubator at 30 ºC and titrate with the help of 0.5 N HCl in presence of phenolphthalein indicator after one day (24 hour), two and seven days.

3.11 STATISTICAL ANALYSIS

The data on various microbial, soil and plant parameters under laboratory as well as field conditions were subjected to statistical analysis using Completely Randomized Design (CRD) as outlined by Gomez and Gomez (1984). The Critical difference at 5 per cent and 1 per cent level was used for testing the significant differences among the treatment means.

4. RESULTS AND DISCUSSION

The present investigation was undertaken to study the **"Integration of biochar with chemical fertilizer for improving yield and quality of Tulsi (*Ocimum sanctum* L.)''** in pot culture under natural condition in net house of ICAR – Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat.

Data pertaining to the effect of different treatments on growth, herbage yield and quality of tulsi were subjected to statistical analysis in order to test the significance of results. The results recorded in the experiment are presented and discussed in this chapter under the following headings.

4.1 CHARACTERIZATION OF BIOCHAR

4.2 EFFECT OF DIFFERENT TREATMENTS ON PLANT HEIGHT AND NUMBER OF BRANCHES AT HARVEST

4.3 EFFECT OF DIFFERENT TREATMENTS ON TOTAL FRESH BIOMASS TOTAL DRY BIOMASS AND AT HARVEST

4.4 EFFECT OF DIFFERENT TREATMENTS ON FRESH LEAF YIELD AND DRY LEAF YIELD AT HARVEST

4.5 EFFECT OF DIFFERENT TREATMENTS ON L : S RATIO AND TOTAL LEAF CHLOROPHYLL CONTENT AT HARVEST

4.6 EFFECT OF DIFFERENT TREATMENTS ON ESSENTIAL OIL PERCENTAGEAT AND OIL YIELD HARVEST

4.7 EFFECT OF DIFFERENT TREATMENTS ON BIOACTIVE COMPOUNDS IN TULSI PLANT AT HARVEST

4.8 EFFECT OF DIFFERENT TREATMENTS ON PLANT NUTRIENT CONTENTAT HARVEST

4.9 EFFECT OF DIFFERENT TREATMENTS TYPE ON SOIL PROPERTIES AFTER HARVEST

4.1 CHARACTERIZATION OF BIOCHAR

4.1.1 Physico-chemical characterization of biochar

Prepared biochar was characterized for different parameters and the data is presented in the table 4.1.

Table 4.1 Physico-chemical characterization of biochar

S.No.	Parameters		Biochar
1	pH		8.2 ± 0.03
2	Electrical Conductivity (dSm^{-1})		0.68 ± 0.02
3	Cation exchange capacity [cmol(p^{+}) kg^{-1}]		27.1 ± 1.21
4	Total C (%)		43.7 ± 0.4
5	Total Kjeldahl Nitrogen (g kg^{-1})		7.7 ± 0.11
6	Phosphorus	Total P (g kg^{-1})	2.1 ± 0.06
		Olsen extractable P (g kg^{-1})	0.19 ± 0.004
		Water soluble P (g kg^{-1})	0.05 ± 0.002
7	Potassium	Total K (g kg^{-1})	7.1 ± 0.05
		NH_4OAc extractable K (g kg^{-1})	0.42 ± 0.03
		Water soluble K (g kg^{-1})	0.04 ± 0.001

4.1.1 Structural characterization of the of biochar

The physicochemical properties of biochar produce from distillation waste biomass are presented in table 4.1. The biochar contains 43.7 % total carbon and was alkaline in nature with a pH (8.2) higher than experimental soil. The FTIR spectra distillation waste biochar determines the functional group variability. The FTIR spectra of the biochar (Plate 4.1) also revealed the highly cross-linked network of carboxylic acids and hydroxyl groups in the matrix indicating its chelating potential. A comparison of surface morphology between raw distillation waste biomass and biochar are presented in Plate. 4.1. The morphology of biochar changed dramatically as compared to the raw distillation waste. The biochar showed porous morphology which can be helpful in retaining nutrients in porous structure.

4.2 EFFECT OF DIFFERENT TREATMENTS ON PLANT HEIGHT AND NUMBER OF BRANCHES AT HARVEST

Table 4.2 Plant height influenced by different treatments

Treatments		Plant height (cm)
T_1	Control	61.17
T_2	Biochar 5 t ha^{-1}	68.10
T_3	100 % RDF	80.23
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	70.87
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	78.73
T_6	Biochar 5 t ha^{-1} + 50 % RDF	80.80
T_7	Biochar 5 t ha^{-1} + 100 % RDF	86.57
S.Em ±		1.45
C.D at 0.05		4.40
C.V. %		3.34

4.2.1 Effect of different treatments on plant height at harvest

The plant height showed higher values with respect to application of biochar as sole or co-application with chemical fertilizer over control (61.17 cm). However, the treatment receiving biochar @ 5 t ha^{-1}+100 % RDF (T_7) found significantly ($P < 0.05$) higher plant height (86.57 cm). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 41.52 %and 27.12 % increase in plant height over the control and biochar @ 5 t ha^{-1} (T_7) respectively.

The increment in plant height may be due to biochar's positive interaction with chemical fertilizer. Biochar reduces nutrient loss by leaching and long and consistent supply of plant nutrient to the plant that helps in profuse plant growth. The current result is in agreement with previous studies in basil (*Ocimum basilicum* L.) (Pandey *et al.*, 2016) and kalmegh (Saha *et al.*, 2019) where, application of biochar with chemical fertilizer improved plant growth. It was also found that different organic nutrient source helps in increasing plant height of *Andrographis paniculata* (Basak *et al.*, 2020).

4.2.2 Effect of different treatments on number of branches at harvest

The effect of different treatments on number of branches is present in table 4.3. Application of biochar and chemical fertilizer in different proportion were found to increase the number of branches per plant over the control (9.00 $plant^{-1}$). However, significantly higher ($P < 0.05$) number of branches (18.67 $plant^{-1}$) was recorded in biochar @ 5 t ha^{-1} + 100 % RDF (T_7). Treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (16.33plant^{-1}) was found at par with the biochar @ 5 t ha^{-1} + 100 % RDF (T_7).

Table 4.3 Number of branches influenced by different treatments

Treatments		Number of branches (plant^{-1})
T_1	Control	9.00
T_2	Biochar 5 t ha^{-1}	11.67
T_3	100 % RDF	14.67
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	12.33
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	15.33
T_6	Biochar 5 t ha^{-1} + 50 % RDF	16.33
T_7	Biochar 5 t ha^{-1} + 100 % RDF	18.67
S.Em ±		0.92
C.D at 0.05		2.80
C.V. %		11.45

As mentioned earlier, biochar helps in reducing the possible loses and enhancing the NUE specially phosphorus which might have helped in increasing in cell division in plant. More cell division help in growth of axillary bud which directly help in increasing number of branches of tulsi plant. Similar study where, number of branches in *Andrographis paniculata* increased with the use of biochar with combination of chemical fertilizer (Saha *et al*., 2019). Similar kind of result obtained by Basak *et al*. (2020) where, application of different organic nutrient sources helps in enhancing in number of branches of *Andrographis paniculata*.

4.3 EFFECT OF DIFFERENT TREATMENTS ON TOTAL FRESH BIOMASS AND TOTAL DRY BIOMASS AT HARVEST

Table 4.4 Total fresh biomass and total dry biomass influenced by different treatments

Treatments		Total fresh biomass (g plant^{-1})	Total dry biomass (g plant^{-1})
T_1	Control	156.83	47.01
T_2	Biochar 5 t ha^{-1}	173.40	52.54
T_3	100 % RDF	253.43	74.63
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	201.17	60.59
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	271.23	82.36
T_6	Biochar 5 t ha^{-1} + 50 % RDF	246.83	74.80
T_7	Biochar 5 t ha^{-1} + 100 % RDF	320.47	95.01
S.Em ±		2.70	0.86
C.D at 0.05		8.20	2.63
C.V. %		2.02	2.16

4.3.1 Effect of different treatments on total fresh biomass at harvest

Fresh biomass of tulsi (Table 4.4) (Fig. 4.1 a) enhanced significantly with addition of different combination of biochar with chemical fertilizer or sole application of biochar @ 5 t ha^{-1} or 100 % RDF compared to control (156.83 g $plant^{-1}$). However, the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest total fresh biomass (320.47 g $plant^{-1}$). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 104.34 % and 26.45 % increase in total fresh biomass over the control and 100 % RDF, respectively.

As discussed in the 4.2.1 and 4.2.2 section, biochar with chemical fertilizer helps in increase in plant height and number of branches. Total fresh biomass is directly depending on plant height and number of branches. Similar results were reported earlier in basil (*Ocimum basilicum* L.) (Pandey *et al.*, 2016), *Andrographis paniculate* (Saha *et al*., 2019), *Withania somnifera* (Nigam *et al*., 2019) where, biomass increased with biochar application. Increase in fresh herbage yield of kalmegh (Basak *et al*., 2020) and sacred basil (Smitha *et al*., 2019) observed with the application of different organic nutrient sources.

4.3.2 Effect of different treatments on total dry biomass at harvest

Total dry biomass (Table 4.4) also followed similar trend as total fresh biomass. Application of different combination of biochar with chemical fertilizer or sole application biochar @ 5 t ha^{-1} or 100 % RDF showed significantly ($P < 0.05$) higher total dry biomass compared to control (47.01 g $plant^{-1}$). However, the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest total dry biomass (95.01 g $plant^{-1}$).

Total dry biomass is the one of the most important yield parameters contributing to the economic yield of tulsi which were significantly influenced by the application of biochar with chemical fertilizer. The findings are in consistent with earlier studies where total dry herbage yield was increased in *Andrographis paniculata* (saha *et al*., 2019). Similar kind of result was found by using different organic nutrient source (vermicompost, farm yard manure, castor cake and jivamrut) increased the total dry herbage yield of kalmegh (Basak *et al*., 2020) and sacred basil (Smitha *et al*., 2019).

4.4 EFFECT OF DIFFERENT TREATMENTS ON FRESH LEAF YIELD AND DRY LEAF YIELD AT HARVEST

Table 4.5 Fresh leaf yield and dry leaf yield influenced by different treatments

Treatments		Fresh leaf yield (g plant^{-1})	Dry leaf yield (g plant^{-1})
T_1	Control	73.50	18.67
T_2	Biochar 5 t ha^{-1}	84.43	21.54
T_3	100 % RDF	132.87	33.58
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	105.50	26.96
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	147.43	37.48
T_6	Biochar 5 t ha^{-1} + 50 % RDF	127.47	32.15
T_7	Biochar 5 t ha^{-1} + 100 % RDF	180.17	45.19
S.Em ±		2.12	0.53
C.D at 0.05		6.43	1.63
C.V. %		3.02	3.03

4.4.1 Effect of different treatments on fresh leaf yield at harvest

Fresh leaf yield of tulsi (Table 4.5) enhanced significantly with addition of different combination of biochar along with chemical fertilizer or sole biochar or recommended dose of fertilizer over control (73.50 g plant^{-1}). However, the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest fresh leaf yield (180.17 g plant^{-1}). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 145.12 % increase in fresh leaf yield over the control. It was also found that co-application of biochar with chemical fertilizer (RDF) recorded higher fresh leaf yield compared to sole application of biochar @ 5 t ha^{-1}(84.43 g plant^{-1}) and 100 % RDF (132.87 g plant^{-1}).

Fresh leaf yield is one of the most important components of total biomass production as well as economic production of tulsi and biochar treatment had a considerable impact on both. As we discussed in total fresh biomass, fresh leaf yield is integrate part of fresh biomass. Similar results were reported earlier on basil (*Ocimum basilicum* L.) (Pandey *et al.*, 2016) and *Andrographis paniculate* (Saha *et al*., 2019).

4.4.2 Effect of different treatments on dry leaf yield at harvest

Dry leaf yield (Table 4.5) also followed the similar trend as in case of fresh leaf yield. Dry leaf yield increased significantly ($P < 0.05$) with the addition of different level of biochar with chemical fertilizer over control (18.67 g plant^{-1}). However, the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest dry leaf yield (45.19 g plant^{-1}). The treatment receiving 100 % RDF (T_3) (33.58 g plant^{-1}) and biochar @ 5 t ha^{-1} (21.54 g plant^{-1}) also recorded significantly ($p<0.05$) higher dry leaf yield over the control.

Dry leaf yield is one of the most important yield parameters contributing to total herbage yield as well as economic yield of tulsi, which was significantly influenced by biochar application. Similar results have been reported in senna (Basak *et al*., 2021) where increase in dry herbage yield was observed with biochar mineral compelx application. In other studies, increase in dry leaf yield of *Andrographis paniculata* was observed due to application of biochar with chemical fertilizer (Saha *et al*., 2019).

4.5 EFFECT OF DIFFERENT TREATMENTS ON L : S RATIO AND TOTAL LEAF CHLOROPHYLL CONTENT AT HARVEST

Table 4.6 Leaf: Stem ratio and total leaf chlorophyll content influenced by different treatments

Treatments		**L:S ratio**	**Total leaf chlorophyll content (mg g^{-1})**
T_1	Control	0.88	0.94
T_2	Biochar 5 t ha^{-1}	0.95	1.12
T_3	100 % RDF	1.11	1.21
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	1.11	1.20
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	1.19	1.38
T_6	Biochar 5 t ha^{-1} + 50 % RDF	1.07	1.24
T_7	Biochar 5 t ha^{-1} + 100 % RDF	1.29	1.40
S.Em ±		0.04	0.05
C.D at 0.05		0.13	0.16
C.V. %		7.15	7.64

4.5.1. Effect of different treatments on Leaf: Stem ratio

The effect of different treatments on leaf to stem ratio (L:S ratio) is presented in the table 4.6. Integration of biochar with chemical fertilizer in different proportion showed higher L:S ratio compared to control (0.88). Significantly higher ($P < 0.05$) L:S ratio (1.29) was recorded in biochar @ 5 t ha^{-1} + 100 % RDF (T_7). However, treatment receiving biochar 2.5 @ t ha^{-1} + 100 % RDF (T_5) (1.19) was found at par with the biochar @ 5 t ha^{-1} + 100 % RDF (T_7).

Similar results were reported earlier on *Andrographis paniculata* (Saha *et al*., 2019). Also the positive effect of different organic sources (farmyard manure vermicompost, and apple pomace manure) on L:S ratio of stevia observed (Kumar *et al*., 2013). The positive effect of biochar on maize root and shoot (Ullah *et al*., 2019) and on tomato seedling (Guo *et al*., 2021) has been reported earlier.

4.5.2. Effect of different treatments on total leaf chlorophyll content

Total leaf chlorophyll content increased with the application of biochar and chemical fertilizer (Table 4.6). Treatment receiving different level of biochar + RDF or sole application of biochar or 100 % RDF recorded higher total leaf chlorophyll content over control (0.94). The treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) showed significantly (*P<0.05*) higher total leaf chlorophyll content (1.40). However, the treatment receiving biochar @ 2.5 t ha^{-1} + 100 % RDF (1.38) (T_5) and biochar @ 5 t ha^{-1} + 50 % RDF (1.24) (T_6) found at par with treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7).

Increasing total chlorophyll content may be due to consistent and steady supply of N. Similar results were reported earlier on maize (Agegnehu *et al*., 2016) and *Carya illinoinensis* (Hou *et al*.,2020) with application of biochar and compost-biochar.

4.6 EFFECT OF DIFFERENT TREATMENTS ON ESSENTIAL AND OIL PERCENTAGE OIL YIELD AT HARVEST

4.6.1 Effect of different treatments on essential oil content at harvest

Like other parameters, essential oil content also follow same trend as the treatments receiving different proportion of inputs showed higher in essential oil content (Table 4.7) (Fig. 4.1 b) as compared to control (0.043 %). The treatment receiving biochar @ 5 t ha^{-1} (T_2) and biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) recorded the highest essential oil content (0.051 %). However, biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (0.050 %) found at par with biochar @ 5 t ha^{-1} (T_2) and biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) (0.051 %). It was also found that biochar @ 5 t ha^{-1} (T_2) and biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) (0.051 %) showed 18.60 % and 10.86 % increase in essential oil content as compared to control (0.043 %), respectively.

Similar kind of results have been reported earlier by (Najafian and Zahedifar., 2018) where, biochar and potassium-nano chelate increased oil yield in sweet basil. Biochar with chemical fertilizer increased essential oil yield in *Ocimum basilicum* (Pandey *et al.*, 2016). The results are in agreement with other studies where, andrographolide content in kalmegh (Saha *et al*., 2019) improved due to application of biochar with chemical fertilizer. Similarly, application of different organic nutrient source increased essential oil content in sacred basil (*Ocimum sanctum* L.) (Smitha *et al*., 2019).

Table 4.7 Essential oil content and oil yield of fresh herbage as influenced by different treatments

Treatments		Essential oil content (%)	Oil yield (g plant^{-1})
T_1	Control	0.043	0.315
T_2	Biochar 5 t ha^{-1}	0.051	0.434
T_3	100 % RDF	0.046	0.608
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	0.051	0.533
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	0.045	0.666
T_6	Biochar 5 t ha^{-1} + 50 % RDF	0.050	0.632
T_7	Biochar 5 t ha^{-1} + 100 % RDF	0.045	0.804
S.Em ±		0.001	0.006
C.D at 0.05		0.003	0.019
C.V. %		3.81	1.88

4.6.2 Effect of different treatments on oil yield at harvest

Treatment receiving biochar with recommended dose of fertilizer in different proportion or sole application of biochar or RDF found higher in oil yield (g plant^{-1}) (table 4.7) (Fig. 4.1 c) in tulsi as compared to control (0.315 g plant^{-1}). However, treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest in oil yield (0.804 g plant^{-1}). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) showed 155 %, 85.25 % and 32.23 % increase in oil yield over control (T_1), biochar @ 5 t ha^{-1} (T_2) and 100 % RDF (T_3), respectively.

Oil yield is one of the important quality parameter of tulsi crop and also the economically important. The results (table 4.7) revealed that the application of biochar sole or with chemical fertilizer significantly increased the oil content in leaves. The improved quality parameters may be due to improved growth characteristics, which may have resulted from improved nutrient uptake and photosynthetic activities (Shani *et al*., 2016). Similar kind of results have been reported earlier by Najafian and Zahedifar, 2018 where, biochar and potassium-nano chelate increased oil yield in sweet basil and in basil (*Ocimum basilicum*) with addition of biochar with chemical fertilizer (Pandey *et al*., 2016). It was also found that different organic nutrient source increased oil yield in sacred basil (*Ocimum sanctum* L.) (Smitha *et al*., 2019).

4.7 EFFECT OF DIFFERENT TREATMENTS ON BIOACTIVE COMPOUNDS IN TULSI PLANT AT HARVEST

Table 4.8 Total phenol content in herbage as influenced by different treatments

Treatments		Total phenol content (mg GAE g^{-1} dry leaf extract)
T_1	Control	24.70
T_2	Biochar 5 t ha^{-1}	30.77
T_3	100% RDF	24.30
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	27.23
T_5	Biochar 2.5 t ha^{-1} +100 % RDF	25.77
T_6	Biochar 5 t ha^{-1} + 50 % RDF	30.13
T_7	Biochar 5 t ha^{-1} + 100 % RDF	29.03
S.Em ±		1.35
C.D at 0.05		4.11
C.V. %		8.57

4.7.1 Effect of different treatments on total phenol content

Effect of different level of input on total phenol content (mg GAE g^{-1} dry leaf extract) is present in table 4.8 (Fig. 4.2 a). Addition of biochar with or without different combination of chemical fertilizer showed higher total phenol content as compared to control (24.70 mg GAE g^{-1} dry leaf extract). Application of biochar @ 5 t ha^{-1} (T_2) showed the highest total phenol content (30.77 mg GAE g^{-1} dry leaf extract). However, treatment receiving biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) (27.23mg GAE g^{-1} dry leaf extract), biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (30.13 mg GAE g^{-1} dry leaf extract) and biochar 5 t ha^{-1} + 100 % RDF (T_7) (29.03 mg GAE g^{-1} dry leaf extract) were found at par with biochar @ 5 t ha^{-1} (T_2).

The increment in total phenol content may be due to application of biochar which attributed to increase soil water holding capacity, plant metabolic activities, and increased secondary metabolite production. Biochar also improves nutrient availability and plant uptake, which might have impact on secondary metabolite production. Similar kind of results has been reported earlier (Saha *et al*., 2019) where, biochar and chemical fertilizer helped in improving total phenol content in kalmegh and in lettuce (Quartacci *et al*., 2017).

Table 4.9 Total flavonoid content in herbage as influenced by different treatments

Treatments		Total flavonoid content (mg QE g^{-1} dry leaf extract)
T_1	Control	52.93
T_2	Biochar 5 t ha^{-1}	61.40
T_3	100% RDF	58.50
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	74.83
T_5	Biochar 2.5 t ha^{-1} +100 % RDF	63.47
T_6	Biochar 5 t ha^{-1} + 50 % RDF	76.63
T_7	Biochar 5 t ha^{-1} + 100 % RDF	65.40
S.Em ±		1.00
C.D at 0.05		3.06
C.V. %		2.70

4.7.2 Effect of different treatments on total flavonoid content

Effect of different level of input on total flavonoid content (mg QE g^{-1} dry leaf extract) is present in table 4.9 (Fig. 4.2 b). Use of different combination of biochar with chemical fertilizer as well as sole application of biochar and recommended dose of fertilizer resulted higher total flavonoid content over control (52.93 mg QE g^{-1} dry leaf extract). Application of biochar @ 5 t ha^{-1} + 50 % RDF (T_6) recorded the highest total flavonoid content (76.63 mg QE g^{-1} dry leaf extract). However, treatment receiving biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) (74.83 mg QE g^{-1} dry leaf extract) was found at par with biochar @ 5 t ha^{-1} + 50 % RDF (T_6). The treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF (T_6) showed 44.7 % and 30.9 % increase in total flavonoid content (mg QE g^{-1} dry leaf extract) over control, respectively.

As discussed earlier in 4.7.1, biochar might have helped in improving secondary metabolites concentration. Similar kind of results has been reported earlier (Saha *et al.*, 2019) where, combined application of biochar and chemical fertilizer improved total flavanoid content in kalmegh, lettuce (Quartacci *et al.*, 2017) and tomato fruit (Petruccelli *et al.*, 2015)

4.7.3 Effect of different treatments on anti-oxidant potential

Anti-oxidant potential of tulsi (Table 4.10) (Fig. 4.2 c) improved significantly with addition of different combination of biochar with chemical fertilizer as well as sole application of biochar and recommended dose of fertilizer over control (56.50 IC_{50} value µm ml^{-1} % inhibitions). However, treatment receiving biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4) recorded the highest anti-oxidant potential (64.13 IC_{50} value µm ml^{-1} % inhibition). Treatment receiving biochar @ 5 t ha^{-1} (T_2), 100 % RDF (T_3), biochar @ 2.5 t ha^{-1} +100 % RDF(T_5), biochar @ 2.5 t ha^{-1} +100 % RDF (T_6) and biochar @ 5 t ha^{-1} + 100 % RDF (T_7) were found at par with biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4).

Table 4.10 Anti-oxidant potential in herbage as influenced by different treatments

Treatments		Anti-oxidant potential (IC_{50} value µm ml^{-1}) % inhibition
T_1	Control	56.50
T_2	Biochar 5 t ha^{-1}	62.17
T_3	100 % RDF	60.30
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	64.13
T_5	Biochar 2.5 t ha^{-1} +100 % RDF	61.13
T_6	Biochar 5 t ha^{-1} + 50 % RDF	63.20
T_7	Biochar 5 t ha^{-1} + 100 % RDF	61.43
S.Em ±		1.411
C.D at 0.05		4.281
C.V. %		3.99

Enhancement of phenolic and flavonoid biosynthesis, as evidenced by TPC and TFC levels, could be one factor contributing to improved antioxidant activity. Similar kind of results have been reported earlier by (Saha *et al*., 2019) where, biochar in combination with chemical fertilizer improved anti-oxidant potential in kalmegh, lettuce (Quartacci *et al*., 2017) and tomato fruit (Petruccelli *et al*., 2015).

4.8 EFFECT OF DIFFERENT TREATMENTS ON PLANT NUTRIENT CONTENTAT HARVEST

4.8.1 Effect of different treatments on plant nutrient (N %) content at harvest

Effect of different treatments on plant nutrient (N %) content is presented in table 4.11. Integration of biochar with chemical fertilizer significantly increased the plant N content compared to control (1.60 %). However, treatment receiving biochar @5 t ha^{-1}+ 100 % RDF (T_7) showed the highest plant N content (1.94 %).

Similar kind of results was - obtained due to application of biochar and compost in *Samanea saman* and *Suregada multiflora* (Ghosh *et al*., 2015). Applications of organic nutrient source (vermicompost) were found to increases shoot N content in savory (*Satureja hortensis* L.) (Esmaielpour *et al*., 2018).

Table 4.11 Plant nutrient content as influenced by different treatments

Treatments		Plant nutrient content (%)		
		N	**P**	**K**
T_1	Control	1.60	0.41	2.82
T_2	Biochar 5 t ha^{-1}	1.63	0.43	3.07
T_3	100 % RDF	1.73	0.46	3.31
T_4	Biochar 2.5 t ha^{-1}+ 50 % RDF	1.80	0.46	3.17
T_5	Biochar 2.5 t ha^{-1}+100 % RDF	1.86	0.48	3.42
T_6	Biochar 5 t ha^{-1}+ 50 % RDF	1.80	0.49	3.35
T_7	Biochar 5 t ha^{-1}+ 100 % RDF	1.94	0.51	3.52
S.Em ±		0.02	0.004	0.03
C.D at 0.05		0.05	0.01	0.11
C.V. %		1.78	1.33	1.99

4.8.2 Effect of different treatments on plant nutrient (P %) content at harvest

Plant P content also followed similar trend as plant N. Co-application of biochar and chemical fertilizer significantly increased the plant P content as compared to control (0.41 %) (Table 4.11). Application of biochar @ 5 t ha^{-1}+ 100 % RDF (T_7) recorded the highest plant P content (0.51 %). However, treatment receiving biochar @ 5 t ha^{-1}+ 50 % RDF (T_6) (0.49 %) was found at par with the application of biochar @ 5 t ha^{-1}+ 100 % RDF (T_7).

Similar kind of results was found in previous study, where application of organic nutrient sources increased the plant shoot P content in savory (*Satureja hortensis* L.) (Esmaielpour *et al*., 2018).

4.8.3 Effect of different treatments on plant nutrient (K %) content at harvest

Treatments receiving different level of biochar with chemical fertilizer significantly increased the plant K content as compared to control (2.82 %). Application of biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest plant K content (3.52 %) (Table 4.11). However, treatment receiving biochar @ 2.5 t ha^{-1} + 100 % RDF (T_5) (3.42 %) was found at par with application of biochar @ 5 t ha^{-1} + 100 % RDF (T_7)

Similar kind of results was found in previous study where, application of cattle dung biochar on sandy loam soil increases the content and uptake of K (Sukartono *et al.,* 2011)

4.9 EFFECT OF DIFFERENT TREATMENTS ON SOIL PROPERTIES AFTER HARVEST

Table 4.12 Soil pH and EC influenced by different treatments after harvest

Treatments		Soil $pH_{1.25}$	$EC_{1.25}$ (dSm^{-1})
T_1	Control	7.63	0.30
T_2	Biochar 5 t ha^{-1}	7.57	0.37
T_3	100 % RDF	7.75	0.32
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	7.67	0.34
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	7.81	0.36
T_6	Biochar 5 t ha^{-1} + 50 % RDF	7.71	0.39
T_7	Biochar 5 t ha^{-1} + 100 % RDF	7.53	0.41
S.Em ±		0.014	0.008
C.D at 0.05		0.042	0.025
C.V. %		0.31	3.97

4.9.1 Effect of different treatments on soil pH after harvest

The results of soil pH (1:2.5) as influenced by different treatments are presented in table 4.12. Application of biochar with chemical fertilizers significantly ($P < 0.05$) increased soil pH over the control (7.63). Sole application of biochar @ 5 t ha^{-1} (7.57) did not show any significant increment in soil pH over control (7.63). However, combined application of biochar and chemical fertilizer in different proportion found resulted higher soil pH. The treatment receiving biochar @ 2.5 t ha^{-1} + 100 % RDF (T_4) was recorded the highest soil pH (7.81).

Increasing in soil pH may be due to the alkaline nature of applied biochar. Similar trend was observed in earlier studies due to application of different organic inputs also. Increase in soil pH by addition of biochar with green manure in maize (Partey *et al*., 2014; Shah and Shah, 2018), and biochar with inorganic fertilizer in kalmegh (Faloye *et al*., 2017; saha *et al*., 2019) were also reported.

4.9.2 Effect of different treatments on soil EC after harvest

Application of biochar with chemical fertilizer as well as sole application of biochar @ 5 t ha^{-1} and 100 % recommended dose of fertilizer showed higher soil EC as compared to control (0.30 dSm^{-1}). However, treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded highest soil EC (0.41 dSm^{-1}). The EC value in the treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (0.39 dSm^{-1}) found at par with biochar application @ 5 t ha^{-1} + 100 % RDF (T_7).

The pyrolysis of the organic residue produced ash, which is mainly made of calcium, magnesium, and potassium oxides (Jien and Wang, 2013) which might have contributed to higher soil EC upon biochar application. The findings of the present study are consistent with those of other investigations where, the application of biochar increased soil EC (Saha *et al.*, 2019; Pandian *et al.*, 2016). Significant improvement in soil EC was observed due to application of biochar prepared from feedstock like corn stover and switchgrass (Chintala *et al.*,2014), sawdust (Chathurika *et al.*, 2015) and prosopis (Pandian *et al.*, 2016).

Table 4.13 Soil organic carbon and CEC influenced by different treatments after harvest

Treatments		Soil organic carbon (g kg^{-1})	CEC (cmol(p+kg^{-1})
T_1	Control	2.69	19.3
T_2	Biochar 5 t ha^{-1}	3.32	21.6
T_3	100 % RDF	2.68	20.2
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	2.91	20.3
T_5	Biochar 2.5 t ha^{-1} + 100 % RDF	3.13	20.5
T_6	Biochar 5 t ha^{-1} + 50 % RDF	3.29	20.9
T_7	Biochar 5 t ha^{-1} + 100 % RDF	3.36	21.3
S.Em ±		0.024	0.43
C.D at 0.05		0.074	1.41
C.V. %		1.38	4.29

4.9.3 Effect of different treatments on soil organic carbon after harvest

Application of biochar alone or in combination with chemical fertilizer significantly ($P < 0.05$) increased the soil organic carbon (g kg^{-1}) over the control (2.69 g kg^{-1}) as shown in table 4.13. The treatment receiving biochar @ 5 t ha^{-1}+ 100 % RDF (T_7) recorded the highest SOC content (3.36 g kg^{-1}). However, treatment receiving biochar @ 5 t ha^{-1} (T_2) (3.32 g kg^{-1}) and biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (3.29 g kg^{-1}) were found at par with treatment containing biochar @ 5 t ha^{-1}+ 100 % RDF (T_7). Treatment receiving biochar @ 5 t ha^{-1}+ 100 % RDF (T_7) recorded 23.42 %

and 25.37 % higher soil organic carbon over the control and 100 % RDF, respectively.

The results corroborate the findings of other studies where, application of biochar improved soil organic carbon content. Significant improvement of SOC was observed due to biochar application under maize-wheat cropping system (Arif *et al*., 2017). Application of biochar in wheat (Chaudhry *et al*., 2016), also improved the carbon content in soil.

4.9.4 Effect of different treatments soil CEC after harvest

Cation exchange capacity of soil (table 4.13) increased significantly with the addition of biochar alone or integration with chemical fertilizer as compared to control (19.3 cmol(p+)kg^{-1}). The treatment receiving biochar @ 5 t ha^{-1} (T_2) was recorded highest soil CEC (21.6 cmol(p+)kg^{-1}). However, treatment receiving 100 % RDF (T_3), biochar @ 2.5 t ha^{-1} + 50 % RDF (T_4), biochar @ 2.5 t ha^{-1} + 100 % RDF (T_5), biochar @ 5 t ha^{-1}+ 50 % RDF (T_6) and biochar @ 5 t ha^{-1} + 100 % RDF (T_7) were found at par with biochar @ 5 t ha^{-1} (T_2). Treatment receiving biochar @ 5 t ha^{-1} (T_2) recorded 11.9 % increase in soil CEC over the control.

Cation exchange capacity of soil is one of the important soil chemical properties. Soil organic matter content has positive relation with CEC of soil. As we discussed earlier in 4.9.3, application of biochar enhanced the soil organic carbon content which might have attributed higher soil CEC. Similar kind of results has been reported earlier where, significant increase in CEC of soil was recorded when corn stover and switch grass biochar applied to acidic soil (Chintala *et al*., 2013).

Table 4.14 (NH_4^+ + NO_3^-) N, available P and available K influenced by different treatments after harvest

Treatments		(NH_4^+ + NO_3^-) N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
T_1	Control	31.37	16.47	76.23
T_2	Biochar 5 t ha^{-1}	39.03	18.80	95.27
T_3	100 % RDF	35.33	19.87	83.77
T_4	Biochar 2.5 t ha^{-1}+ 50 % RDF	37.03	20.90	91.50
T_5	Biochar 2.5 t ha^{-1}+100 % RDF	41.23	21.53	93.50
T_6	Biochar 5 t ha^{-1}+ 50 % RDF	43.03	23.70	106.00
T_7	Biochar 5 t ha^{-1}+ 100 % RDF	46.67	24.77	116.80
S.Em ±		1.15	0.70	1.74
C.D at 0.05		3.38	2.14	5.29
C.V. %		4.94	5.85	3.19

4.9.5 Effect of different treatments on (NH_4^+ + NO_3^-) N after harvest

Significant ($P < 0.05$) increase in (NH_4^+ + NO_3^-) N was observed with the application of biochar and chemical fertilizer over the control (31.37 mg kg^{-1}) as given in table 4.14. However, the highest soil (NH_4^+ + NO_3^-) N content was observed under treatment receiving biochar @ 5 t ha^{-1}+ 100 % RDF (T_7) (46.67 mg kg^{-1}). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 48.77 %, 19.57 % and 32.09 % increase in (NH_4^+ + NO_3^-) N over the control, biochar @ 5 t ha^{-1} and 100 % RDF, respectively.

Biochar, in addition to its role as a soil conditioner, aids in the improvement of soil physico-chemical properties, resulting in increased nutrient retention and supply. Simultaneously, it decreases nutrient losses due to leaching and volatilization (Major *et al.,* 2010). These properties might have played important role in help in increasing available N content in soil. The outcomes of previous studies corroborate the findings of the current investigation. Significant increase in soil N recorded due to application of biochar prepared by using *Cymbopogon flexuosus* (Saha *et al*., 2019) and *V. faba* and *T. diversifolia* residue (Partey *et al*., 2014) also support the finding of present study.

4.9.6 Effect of different treatments on available P after harvest of tulsi plant

Effect of different level of biochar and chemical fertilizer on available P (mg kg^{-1}) is present in table 4.14. Use of different combination of biochar with chemical fertilizer as well as sole application of biochar and recommended dose of fertilizer showed significantly ($P < 0.05$) higher available P over control (16.47 mg kg^{-1}). The treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest available P (24.77 mg kg^{-1}) in soil. However, treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (23.70 mg kg^{-1}) found at par with biochar @ 5 t ha^{-1} + 100 % RDF (T_7). Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 50.39 %, 31.75 % and 24.66 % increase in available P over control, biochar @ 5 t ha^{-1} and 100 % RDF, respectively.

Biochar also improves the physico-chemical characteristics of the soil, resulting in increased nutrient retention and supply. The pH of the soil plays an important role in nutrient availability by influencing the soil pH (Van *et al.,* 2010) which could be another reason for boosting soil nutrient availability. Similar kind of results has been reported earlier by (Saha *et al*., 2019) where, biochar and chemical fertilizer improved available P. Biochar along with *Azospirillum* and green manures application on maize (Saranya*et al*., 2011; Partey *et al.,* 2014) also improved the soil available P.

4.9.7 Effect of different treatments on available K after harvest

Application of biochar with chemical fertilizer as well as sole application of biochar @ 5 t ha^{-1} or 100 % recommended dose of fertilizer showed significantly ($P < 0.05$) higher available K (mg kg^{-1}) over the control (76.23 mg kg^{-1}) as shown in table 4.14. However, treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest available K (116.80 mg kg^{-1}) in soil. Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 53.22 %, 22.63 % and 39.42 % increase in available K content over the control, biochar @ 5 t ha^{-1} and 100 % RDF, respectively.

The findings of this study supported by previous research where, increasing in available K was observed with the application of biochar in rice-based cropping systems (Haefele *et al*., 2011), maize (Saranya *et al*., 2011) and tomato (Vaccari *et al*., 2015).

Table 4.15 Microbial biomass carbon, dehydrogenase activity and soil respiration as influenced by different treatments

Treatments		Microbial biomass carbon (mg kg^{-1})	Dehydrogenase activity (µTPF g^{-1} h^{-1})	Soil respiration (mg CO_2-C kg $soil^{-1}$ day^{-1})
T_1	Control	143.90	20.57	2.22
T_2	Biochar 5 t ha^{-1}	172.07	37.49	2.66
T_3	100 % RDF	148.73	25.63	2.47
T_4	Biochar 2.5 t ha^{-1} + 50 % RDF	183.43	35.06	2.59
T_5	Biochar 2.5 t ha^{-1} +100 % RDF	184.17	32.91	2.52
T_6	Biochar 5 t ha^{-1} + 50 % RDF	193.80	41.75	3.77
T_7	Biochar 5 t ha^{-1} + 100 % RDF	215.13	44.55	3.91
S.Em ±		1.97	1.71	0.03
CD (p=0.05)		6.00	5.19	0.08
C.V. %		1.93	8.73	1.73

4.9.8 Effect of different treatments on microbial biomass carbon after harvest

The soil microbial biomass carbon (MBC) content was significantly ($P<0.05$) influenced by the application of different level of biochar with chemical fertilizer over the control (143.90 mg kg^{-1}) as given in table 4.15. Sole application of biochar @ 5 t ha^{-1} (T_2) recorded 19.57 % and 15.69 % increase in soil microbial biomass carbon over control and 100 % RDF, respectively. However, the highest soil microbial biomass carbon (215.13mg kg^{-1}) was observed in the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7).

The presence of an intrinsic carbon source in biochar may be responsible for the increased MBC in soil. Biochar (BC) is generally poor in natural mineral nutrients but high in carbon content which might have supplied the carbon for energy as well as a habitat for microorganisms. In combined with CF, biochar works as a source of mineral nutrients for microorganism growth and proliferation. Because of the rapid development and proliferation of microorganisms, the combined application of BC and CF resulted in greater soil MBC. The result of the present study is also in agreement with previous investigation where, the application of biochar enhanced MBC (Zavalloni *et al*., 2011; Saha *et al*., 2019).

4.9.9 Effect of different treatments on dehydrogenase activity after harvest

Effect of different treatments had variable response on soil dehydrogenase activity presented in the Table 4.15. It was observed that biochar application significantly ($P < 0.05$) enhanced dehydrogenase activity as compared to the control (20.57 µTPF g^{-1} h^{-1}). The treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest dehydrogenase activity (44.55 µTPF g^{-1} h^{-1}). However, application of biochar @ 5 t ha^{-1} + 50 % RDF (T_6) (41.75µTPF g^{-1} h^{-1}) found at par with biochar @ 5 t ha^{-1} + 100 % RDF (T_7). Sole application of biochar @ 5 t ha^{-1} (T_2) recorded 82.25 % and 46.27 % increase in dehydrogenase activity over control and 100 % RDF, respectively. While biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded 116.5 % and 73.81 % increase in dehydrogenase activity over control and 100 % RDF, respectively.

The amount of dehydrogenase activity in the soil is directly influenced by the amount of organic matter in the soil. As a result, adding biochar increased the availability of substrate needed for microbes responsible for dehydrogenase activity. Biochar, in addition to providing substrate, creates a reducing environment in the soil, which speeds up the electronic reduction process and enhances dehydrogenase activity (Jain *et al*., 2016). The current investigation indicates an increase in soil dehydrogenase activity in biochar amended soil, which is closely observed in previous study (Chen *et al*., 2013). Increase in soil dehydrogenase activity was also observed when biochar applied with chemical fertilizer (Saha *et al*., 2019).

4.9.10 Effect of different treatments on soil respiration after harvest

The soil respiration was significantly (P<0.05) increased by the application of different level of biochar with chemical fertilizer over the control (2.22 mg CO_2-C kg soil^{-1} day^{-1}) as given in table 4.15. However, treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF (T_7) recorded the highest in soil respiration (3.91mg CO_2-C kg soil^{-1} day^{-1}). Biochar @ 5 t ha^{-1} + 100 % RDF (T_7) received treatment was recorded 78.82 % and 58.29 % increment in soil respiration over control and 100 % RDF, respectively.

Increasing in soil respiration may be due to increase activity of microorganisms induced by biochar application. Biochar can boost the microbial activity by providing substrate as an energy source. The results of present study corroborate the finding of other work where, soil respiration increased with the application of biochar (Watanabe and Sato, 2015; Smith *et al*., 2010) and effect of biochar addition on CO_2 emissions are also influenced by the rate at which it is applied (Awasthi *et al.,* 2017).

5. SUMMARY AND CONCLUSION

A pot culture experiment on "Integration of biochar with chemical fertilizer for improving yield and quality of Tulsi (*Ocimum sanctum* L.)" was carried out in the net house, ICAR- DMAPR, Boriavi during *kharif* season of year 2020.

The experiment was laid out on completely randomized design. The pot culture experiment was conducted by integrating the biochar prepared from tulsi (*Ocimum sanctum* L.) distillation waste and chemical fertilizers (CF) in different proportions. The biochar was prepared from distillation waste biomass of tulsi (*Ocimum sanctum* L.) after extraction of essential oils. The treatment comprised of seven Treatments *viz.*, T_1: Control, T_2: Biochar 5 t ha^{-1}, T_3: 100 % RDF, T_4: Biochar 2.5 t ha^{-1} + 50 % RDF, T_5: Biochar 2.5 t ha^{-1} + 100 % RDF, T_6: Biochar 5 t ha^{-1} + 50 % RDF and T_7: Biochar 5 t ha^{-1} + 100 % RDF.

The results presented and discussed in the preceding chapter are briefly summarized here.

EFFECT OF DIFFERENT TREATMENTS ON GROWTH AND YIELD PARAMETERS

Plant growth parameters such as plant height and number of branches at harvest showed significant ($P = 0.05$) higher values in treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF. However, in case of number of branches, treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF was found at par with biochar application @ 5 t ha^{-1} + 100 % RDF.

In case of total fresh biomass, fresh leaf yield, total dry biomass and dry leaf yield at harvest showed significant ($P = 0.05$) higher values in treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF.

L: S ratio and total chlorophyll content at harvest showed significantly ($P = 0.05$) higher in treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF. However, in case of both L : S ratio and total chlorophyll content treatment receiving biochar @ 2.5 t ha^{-1} + 100 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF. While, in case of total chlorophyll content treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF.

Oil yield at harvest showed significantly (*P = 0.05*) higher in the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF.

EFFECT OF DIFFERENT TREATMENTS ON BIOACTIVE COMPOUND TULSI

Essential oil content (%) in leaves was recorded significantly (*P = 0.*05) higher in treatment receiving biochar @ 2.5 t ha^{-1} and biochar @ 2.5 t ha^{-1} + 50 % RDF. However, treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF was found at par with biochar @ 2.5 t ha^{-1} and biochar @ 2.5 t ha^{-1} + 50 % RDF.

Total phenol content was recorded significantly (*P = 0.*05) higher in treatment receiving biochar @ 5 t ha^{-1}. However, treatment receiving biochar @ 2.5 t ha^{-1} + 50 % RDF, biochar @ 5 t ha^{-1} + 50 % RDF and biochar 5 t ha^{-1} + 100 % RDF were found at par with biochar application @ 5 t ha^{-1} (T_2).

Treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF recorded significantly (*P = 0.*05) higher value of total flavonoid content. However, treatment receiving biochar @ 2.5 t ha^{-1} + 50 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 50 % RDF.

In case of anti-oxidant potential, application of biochar @ 2.5 t ha^{-1} + 50 % RDF recorded significantly (*P=0.05*) higher value. The treatment receiving biochar @ 5 t ha^{-1}, 100 % RDF, biochar @ 2.5 t ha^{-1} +100 % RDF, biochar @5 t ha^{-1} + 50 % RDF and biochar @ 5 t ha^{-1} + 100 % RDF were found at par with the application of biochar @ 2.5 t ha^{-1} + 50 % RDF.

Plant nutrient N, P and K content were recorded significantly (*P = 0.*05) higher in treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF. However, in case of plant P content treatment receiving biochar @ 2.5 t ha^{-1} +100 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF. While in case of plant K content, treatment receiving biochar @ 2.5 t ha^{-1} +100 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF.

EFFECT OF DIFFERENT TREATMENTS ON SOIL PROPERTIES AFTER HARVEST

After harvesting of tulsi plant, soil samples were analyzed to study the influence of biochar and chemical fertilizer on soil properties. The study found that pH, EC, organic carbon (g kg^{-1}) and CEC significantly (*P = 0.05*) influenced by the biochar application.

Treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF significantly (*P = 0.05*) increased EC and soil organic carbon (g kg^{-1}). Application of biochar @ 2.5 t ha^{-1} +100 % RDF was found at par with biochar @ 5 t ha^{-1} + 100 % RDF in case of EC. While in case of SOC, treatment receiving biochar @ 5 t ha^{-1} found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF. The highest soil pH was recorded in the treatment receiving biochar @ 2.5 t ha^{-1} + 100 % RDF. Similarly, the highest soil CEC was also recorded in treatment receiving biochar @ 5 t ha^{-1}. However, treatment receiving 100 % RDF, biochar @ 2.5 t ha^{-1} +50 % RDF, biochar @ 2.5 t ha^{-1} +100 % RDF, biochar @ 5 t ha^{-1} + 50 % RDF and biochar @ 5 t ha^{-1} + 100 % RDF were found at par with the application of biochar @ 5 t ha^{-1}.

The soil is analyzed for mineral (NH_4^+ + NO_3^-) N (mg kg^{-1}), available P (mg kg^{-1}) and available K (mg kg^{-1}) after harvest of tulsi plant. All three major nutrients (N. P and K) in soil showed significantly (*P = 0.05*) higher value in the treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF. In case of available K (mg kg^{-1}), treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF.

Soil biological properties such as microbial biomass carbon (mg kg^{-1}), dehydrogenase activity (µTPF g^{-1} h^{-1}) and soil respiration (mg CO_2-C kg $soil^{-1}$ day^{-1}) were analyzed after harvest of tulsi plant. The result indicated significantly higher (*P=0.05*) MBC, DHA and soil respiration under treatment receiving biochar @ 5 t ha^{-1} + 100 % RDF. In case of dehydrogenase activity (µTPF g^{-1} h^{-1}), treatment receiving biochar @ 5 t ha^{-1} + 50 % RDF was found at par with the application of biochar @ 5 t ha^{-1} + 100 % RDF.

CONCLUSION

The present investigation demonstrated that biochar with chemical fertilizer had a synergistic effect on plant growth and nutrition. The combined application of biochar (5 t ha^{-1}) and recommended chemical fertilizer (100:50:50 NPK kg ha^{-1}) improve the plant growth as well as the bioactive compounds (total phenol content, total flavonoid content and anti-oxidant potential). The application of chemical fertilizer along with biochar improved soil pH, soil organic carbon, available nutrients and soil biological activity. This study shows that biochar can successfully reduce 50% of chemical fertilizer application while enhancing yield and quality of medicinal herbs as well as soil properties.

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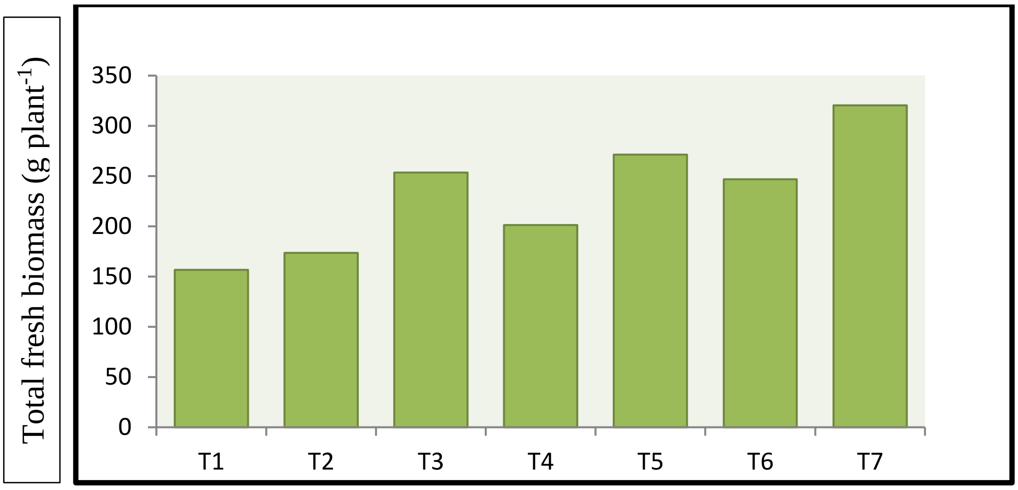
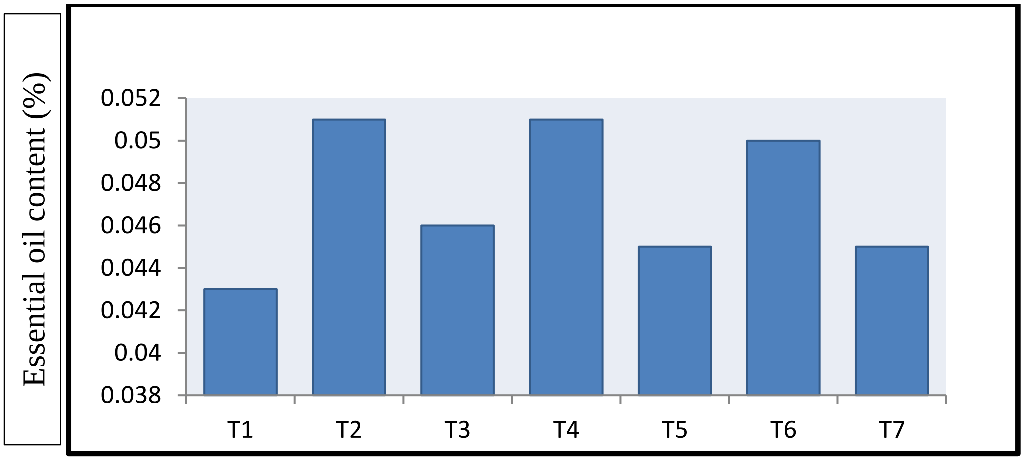


(a)



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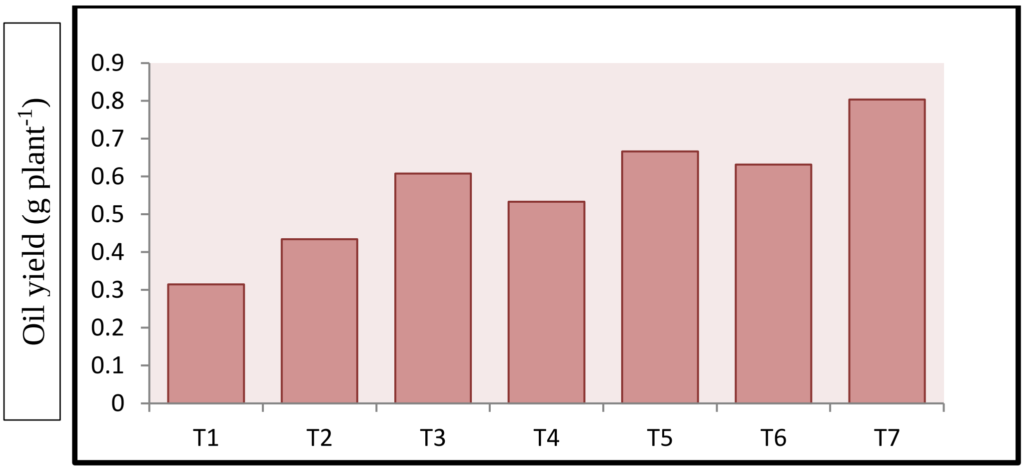
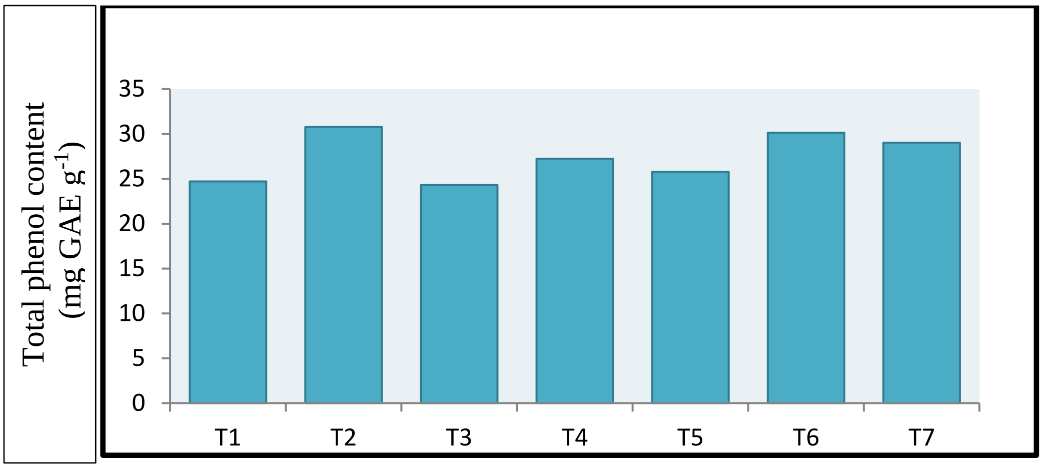
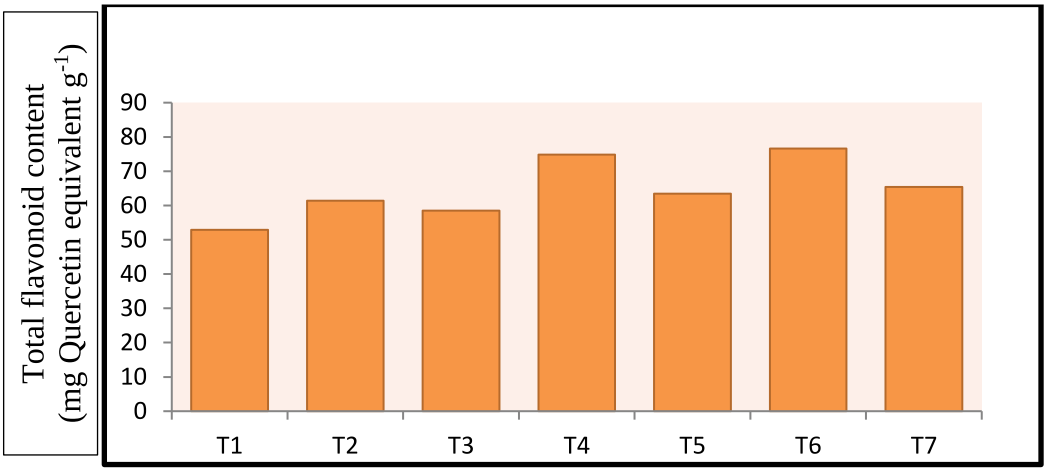


(c)

Fig 4.1 Effect of different on (a) Total fresh biomass (g plant^{-1}) (b) Essential oil content (%) and (c) Oil yield (g plant^{-1}).



(a)



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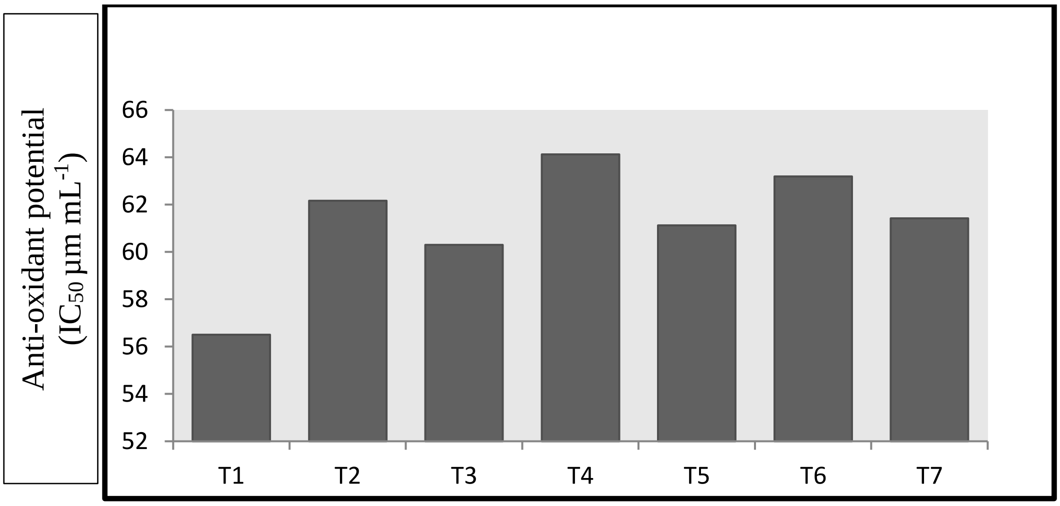
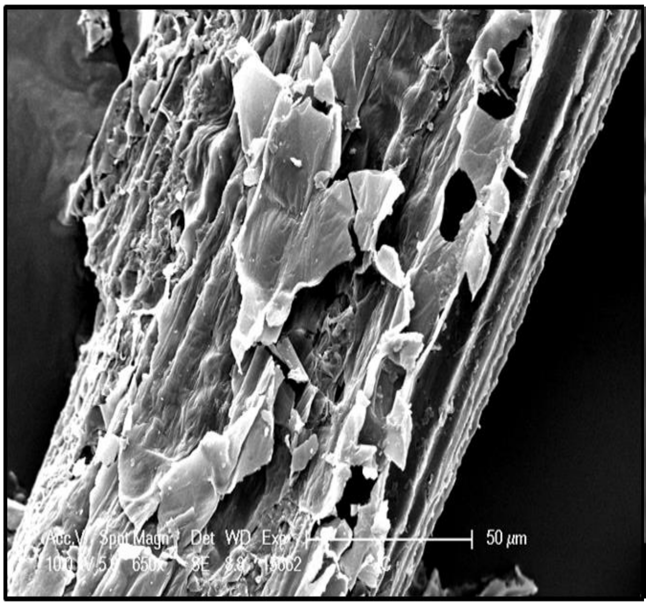
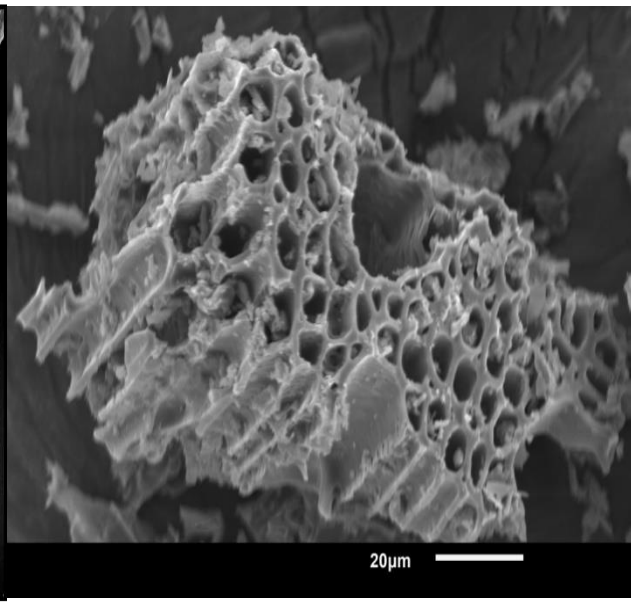


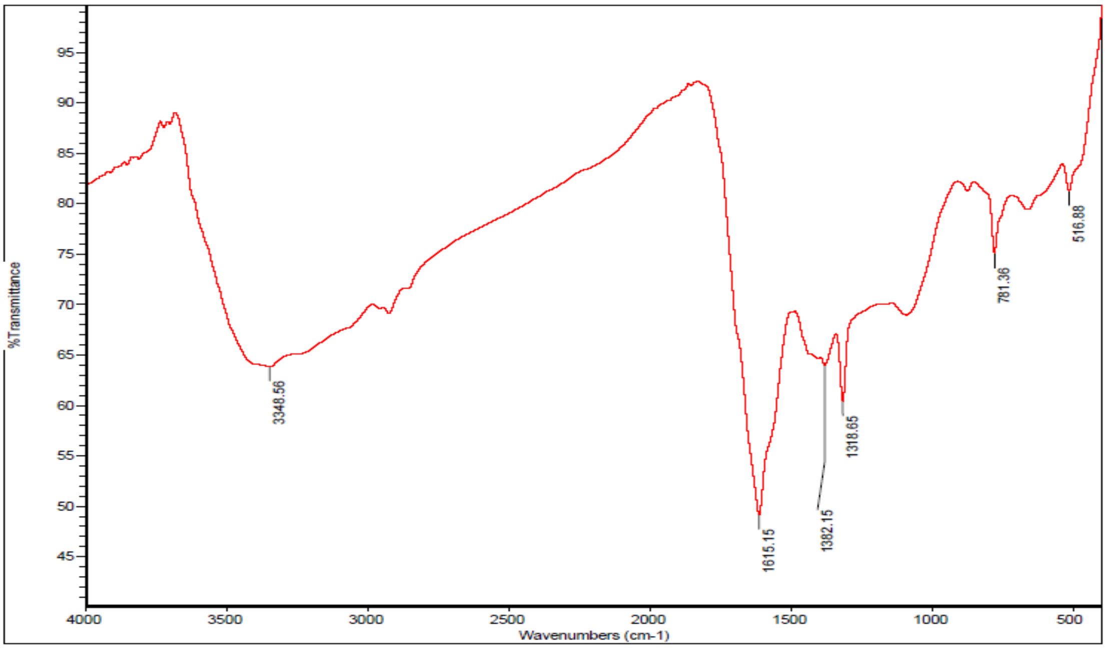
(c)

Fig. 4.2 Effect of different treatments on (a) Total phenol content (mg GAE g^{-1}) (b) Total flavonoid content (mg Quercetin equivalent g^{-1}) and (c) Anti-oxidant potential (IC_{50} µm mL^{-1})





(a) **(b)**



(c)

Plate 1. Scanning electron micrographs of (a) distillation waste (b) biochar of distillation waste and (c) FTIR spectra of distillation biochar

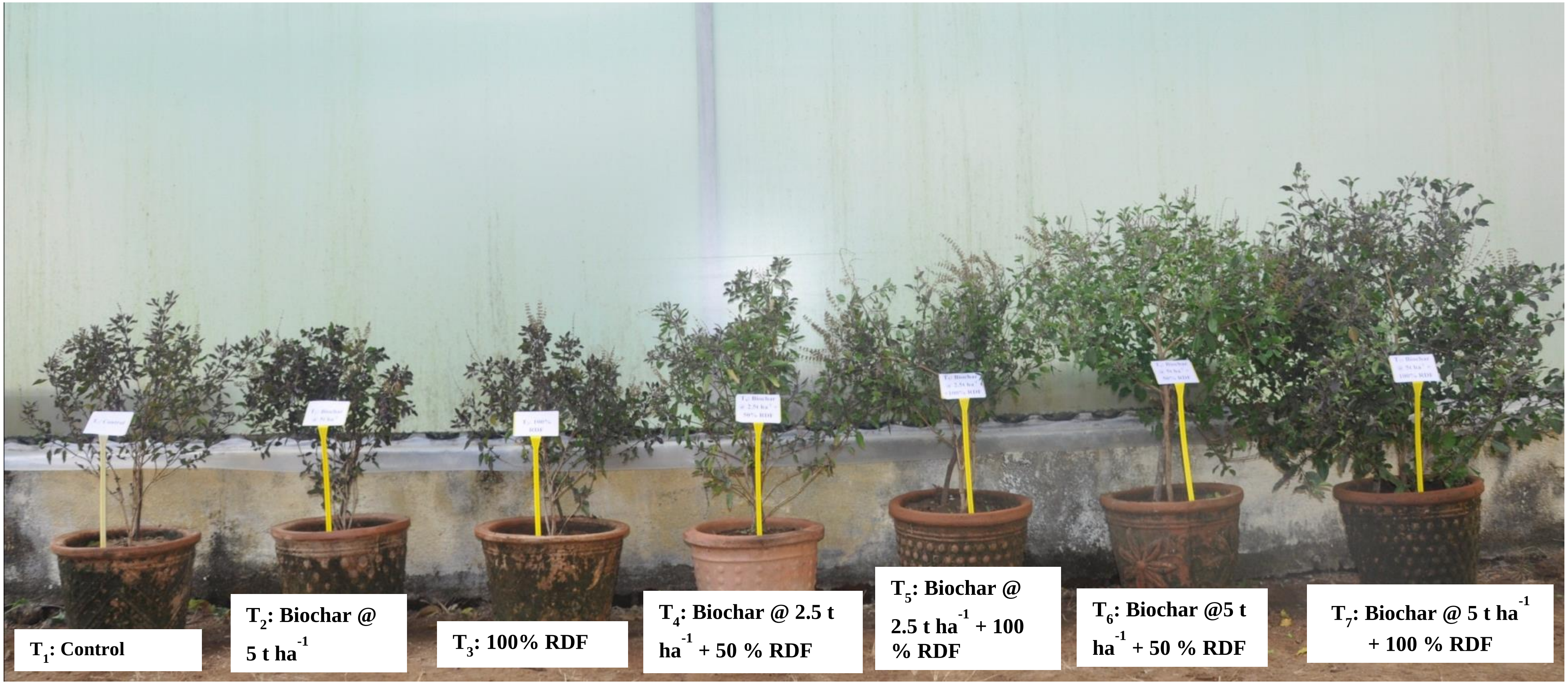


Plate 2: Plant growth affected by different treatments after 90 days after transplanting.