T.R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF SOIL SCIENCE AND PLANT NUTRITION

THE EFFECTS OF DIFFERENT PARTICLE SIZES OF BIOCHAR ON SOIL BIOLOGICAL PROPERTIES AND YIELD OF WHEAT CROP

Master's Thesis

Nabeela MAQBOOL

supervisor
Prof. Dr. Ridvan KIZILKAYA

II. Supervisor

Prof. Dr. Andon Vasilev ANDONOV

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2022

ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"THE EFFECTS OF DIFFERENT PARTICLE SIZES OF BIOCHAR ON SOIL BIOLOGICAL PROPERTIES AND YIELD OF WHEAT CROP"** prepared by **Nabeela Maqbool**, and supervised by **Prof. Dr. Rıdvan KIZILKAYA** and **Prof. Dr. Andon Vasilev ANDONOV**, was found successful and majority of votes accepted by committee members as Master thesis, following the examination on the date 6.7.2022

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Chairman	Prof. Dr. Rıdvan KIZILKAYA Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☐Accept ☐ Reject
Member	Prof. Dr. Andon Vasilev Andonov Agricultural University Plovdiv Department of palnt physiology, biochemistry &Gentaics		☐Accept ☐ Reject
Member	Prof. Dr. Coşkun GÜLSER Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☐Accept ☐ Reject
Member	Prof. Dr. Orhan DENGIZ Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☐Accept ☐ Reject
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This thesis has been approved by the committee members that already stated above and determined by the Institute Executive Board.

APPROVAL
… / … /2022
Prof. Dr. Ali BOLAT
Head of Institute of Graduate Studies

DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC

I hereby declare and undertake that I complied with scientific ethics and academic rules in all stages of my Master's Thesis, that I have referred to each quotation that I use directly or indirectly in the study, and that the works I have used consist of those shown in the sources, that it was written in accordance with the institute writing guide and that the situations stated in the article 3, section 9 of the Regulation for TÜBİTAK Research and Publication Ethics Board were not violated.

Is Ethics Committee Necessary?

Yes ☐ (If it is necessary, please add appendices.)

No ☒

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17/06/2022

Nabeela MAQBOOL

DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT

Thesis Title: THE EFFECTS OF DIFFERENT PARTICLE SIZES OF BIOCHAR ON SOIL BIOLOGICAL PROPERTIES AND YIELD OF WHEAT CROP

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Prof. Dr. Rıdvan KIZILKAYA

ÖZET

FARKLI PARÇACIK BÜYÜKLÜĞÜNE SAHİP BİYOKÖMÜR’IN TOPRAKLARIN BİYOLOJİK ÖZELLİKLERİ İLE BUĞDAY BİTKİSİNİN VERİMİ ÜZERİNE ETKİLERİ

Nabeela MAQBOOL
Ondokuz Mayıs Üniversitesi
Lisansüstü Eğitim Enstitüsü
Toprak Bilimi Ve Bitki Besleme Bölümü
Yüksek Lisans, Şubat/2022
Danışman I: Prof. Dr. Rıdvan KIZILKAYA
Danışman II: Prof. Dr. Andon Vasilev ANDONOV

Toprak ıslahında biochar'a artan ilgiye rağmen, biyokömür’ün killi toprak üzerindeki etkileri ve toprak mikrobiyotası üzerindeki etkisi hakkında bilgi boşluğu devam etmektedir. Farklı parçacık büyüklüğüne sahip biyokömürün farklı uygulama dozlarının toprak mikrobiyotasının iyileştirilmesinde rol oynayabileceğini ve bunun sonucunda ürün verimini artıracağı hipotezi üzerine bu çalışma planlanmıştır. Dört farklı parçacık boyutuna sahip Ağaç dallarının pirolizi sonunda elde edilen biyokömürün Gazlaştırma işlemiyle üretilen sert ağaç biyokömürün (ince (< 0,5 mm), ince-orta (0,5-1 mm), orta-kaba (1-2 mm) ve kaba (2-4 mm)) artan seviyelerdeki 3 dozu (%05, %1 ve %2 w/w) topraklara ilave edilmiştir. Farklı parçacık büyüklüğüne ve uygulama dozlarına sahip biyokömürün, buğday bitkisinin verimi üzerine etkisinin belirlenmesi amacıyla dört aylık bir sera denemesi yapılmıştır. Bitkilerin haşatını takiben buğday bitkisinin ürün verimi ile verim unsurları belirlenmiş, her bir saksıdan alınan toprak örneklerinde bazı kimyasal ve biyolojik analizler yapılmıştır. Deneme sonunda, Orta-kaba (1-2mm) ve kaba (2-4mm) biokömür fraksiyonların bitki biyokütlesi ve başak yüksekliğinin daha yüksek olduğu, diğer tüm verim parametrelerinin ise kontrolden genel olarak daha yüksek olduğu saptanmıştır. Toprakların biyolojik analizine yönelik bulgular ise, topraklara biyokömür uygulamasının toprak mikrobiyal aktivitesini artırdığını ortaya koymuştur. Elde edilen sonuçlara göre, biyokömürün %1 ve %2 uygulama dozunda orta-iri (1-2 mm) ve kaba (2-4 mm) fraksiyonlarının diğer fraksiyonlara göre daha yüksek etki gösterdiğini ortaya koymaktadır. Buna karşın, biyokömürün %1 ve %2 uygulama dozundaki ince (<0.5 mm) ve ince- orta (0,5-1 mm) fraksiyonların kontrole göre toprakların biyolojik özelliklerini daha az etkilediğini ortaya konulmuştur.

Anahtar Kelime: Biochar, partikül boyutu, toprak mikrobiyotası, bitki verimi

ABSTRACT

THE EFFECTS OF DIFFERENT PARTICLE SIZES OF BIOCHAR ON SOIL BIOLOGICAL PROPERTIES AND YIELD OF WHEAT CROP

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Master’s Thesis, June/2022
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Despite the increasing interest in biochar as a soil amendment, a knowledge gap remains on its impacts on clay soil and its effect on soil microbiota. We hypothesized that biochar particle size and application rate can play a role in the betterment of soil microbiota in turn have improved crop yield. Hardwood biochar produced by the process of gasification was incorporated in soil at four particle size classes: fine (< 0.5 mm), fine-medium (0.5-1 mm), medium-coarse (1-2mm), and coarse (2-4mm), and at three dose level: 05%, 1% and 2% w/w. A four-month green-house experiment was conducted using wheat as an indicator plant to check the effects of biochar fraction and concentration on the yield of the crop. Thereafter, plants were harvested and plant yield parameters were recorded while soil samples were collected for further lab soil chemical and biological analysis. It was noted that plant biomass & spike height was higher for biochar fraction class of medium-coarse(1-2mm) and coarse(2-4mm) whereas all other yield parameter were overall higher than control. Findings for soil biological analysis implies that treatment of biochar application improved soil microbial growth activity. Results suggest that for biochar particle size class: medium- coarse(1-2mm) and coarse (2-4mm) at 1% and 2% concentration had higher results than other biochar fraction. It was also note that biochar treatment with fine(<0.5mm) and fine- medium (0.5-1mm) at 1% and 2% concentration in some cases show even decreased results than control.

Keywords: biochar, Particle size, Soil microbiota, Plant yield

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17th June 2022
Samsun, Turkey
Nabeela MAQBOOL

CONTENTS

SYMBOLS AND ABBREVATION

DHA	: Dehydrogenase activity
OM	: Organic matter
MBC	: Microbial Biomass Carbon
OC	: Organic Carbon
g	: Grams
ODS	: Oven dry soil
CEC	: Cation Exchange Capacity
Kg	: Kilogram
L	: Liter
Mm	: milli meters
Mg	: milligram
EC	: Electrical Conductivity
ExC	: Exchangeable cations
MC	: moisture content
BM	: Plant Biomass
E	: Cumulative Evapotranspiration
N	: Nitrogen
E	: Cumulative evapotranspiration

FIGURES LEGENDS

TABLES LEGENDS

1. INTRODUCTION

Climate change is a critical issue that must be addressed immediately. The primary driver of anthropogenic climate change is the gradual rise in the amount of carbon in the atmosphere. This increased concentration is the result of GHG (greenhouse gas) emissions from economic activities such as energy, industry, transportation, and land use, all of which rely heavily on fossil fuels (Banuri & Opschoor, 2007). Renewable energy is one of the possible and viable alternatives to fossil fuels in the context of climate mitigation strategies(Goldemberg, 2004). The discovery of Terra Preta (black soil) in the Amazon rainforest and the practice of utilizing charcoal to enhance soil fertility is suggested as a significant method for producing bioenergy, biofuel, and biogas, while also allowing for CO_2 storage in the soil; a strategy named “carbon-negative”.

Terra Preta is frequently referred to as fertile/dark/black/super soil from the Amazon basin or Indian black earth Thousands of hectares of planted plots comprised of extraordinarily productive black soil occur in the Amazon Basin. It is thought to have resulted through the pedological change of a previous soil as a result of indigenous cultures' activities. These soils appeared to be perpetually fruitful, yielding twice as much as neighboring soils and containing almost three times as much phosphate and nitrogen, while containing approximately 9% carbon, compared to around 1% in surrounding soils (*A Perspective on Terra Preta and Biochar - ALL Power Labs*, n.d.). In contrast to typical soils of the Amazon rainforest which are red and have a lot of kaolinite, aluminum, and a very acidic pH, soils called Terra Preta do Indio’s are very fertile and have a black color, an alkaline pH, and unique microorganisms that live there.

The soils found naturally in the Amazon region contain trace levels of the majority of nutrients. In comparison, terra preta is characterized by high P, N, Ca, and basic nutrient concentrations. According to Glaser & Birk, 2012 Terra preta has many times the nutrient reserves of adjacent soil. In comparison to adjacent soils with 20-30 g C/kg soil, Amazonian Dark Earths contain high carbon content of up to 150 g C/kg soil (Glaser et al., 2000; Woods et al., 2009) that were voluntarily introduced into the soil by local people (slash and char strategy) over thousands of years. According to Glaser et al., 2014 the carbonaceous fraction's chemical and microbiological stability can be attributed to its complex aromatic polycyclic chemical structure, which can survive in the environment for centuries. As the aromatic structure gradually oxidizes at the surface, forming carboxylic groups, the carbonaceous particles' ability to retain nutrients is enhanced.

Figure 1.1. (a)Typical profile of terra Preta, (b) Profile of Oxisol normally found in the Amazon basin (Glaser et al., 2014)

It's likely that the pre-Columbian civilization, which lived in the Amazon between 2500 and 500 B.C., was credited for these “dark earths” which can be found throughout the Amazon basin, other parts of South America and probably beyond.(Lehmann et al., 2003) Slash-and-char is ancient, deceptively easy farming technique said to have been used by pre-Columbian Amerindians to generate the well-known 'Amazonian dark earths' that is still used in modern civilization (or its analog) (Niu et al., 2015)

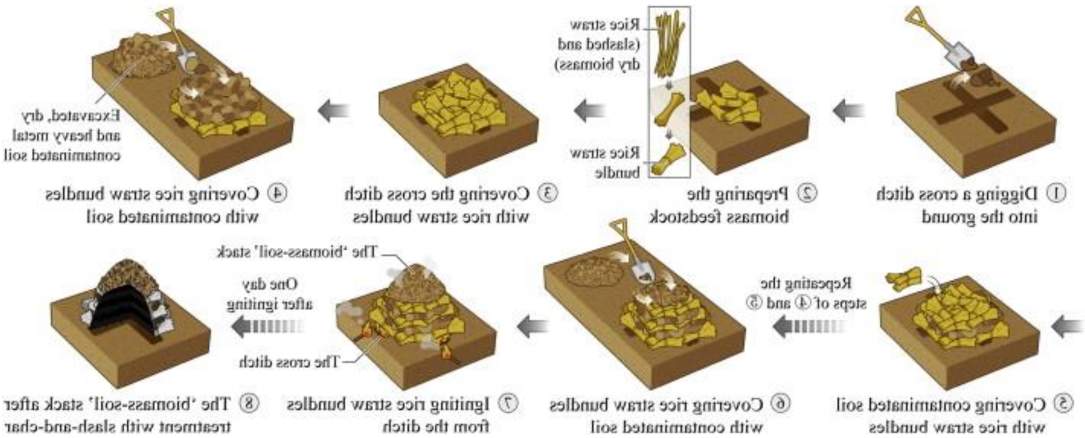

Figure 1.2. Schematic diagram showing the major steps of the slash-and-char technique (Niu et al., 2015)

Carbonaia o pojat is another ancient technique frequently employed in Italy to turn wood into charcoal, primarily beech, but also spruce, larch, chestnut, Holm, oak, and pine by suppling limited amount of oxygen. The carbonization process could take up to 5 or 6 days. Typically, 30 to 40 tons of wood were heaped up in the Carbonaia, yielding 6 to 8 tons of coal.

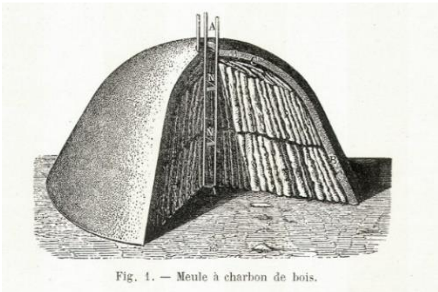

Figure 1.3. Carbonaia o Pojat from inner side (https://it.wikipedia.org/wiki/Carbonaia)

There has been a noticeable surge in the study, inspired by Terra Preta's research, have included the examination of biochar (black carbon or charcoal formed from biomass) as a soil supplement to improve nutrient availability and retention as means of sustainable agriculture because of its wide range of applications, availability, and manufacturability.

Biochar is an intriguing carbon-based compound created by repurposing waste materials generated in our ecosystem by thermal decomposition of organic material at relatively low temperatures (<700) and with absence or a limited supply of oxygen (O2) (*Biochar for Environmental Management*, n.d.). Biochar is solid fraction of pyrolysis along with which other products that are produced are syngas and bio-oil (a fuel that can be converted into biodiesel and other biofuels)(Brewer & Brown, 2012)

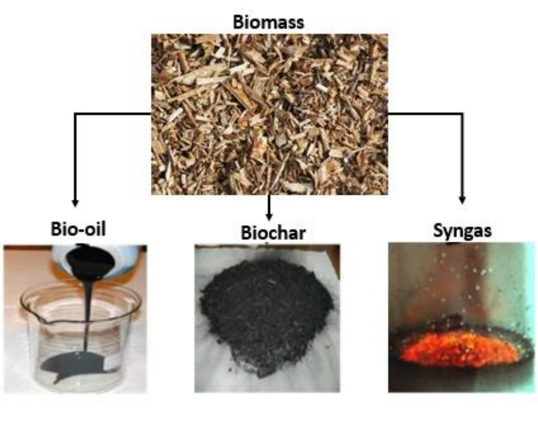

Figure 1.4. Products obtained as result of pyrolysis of woody biomass

Biochar is a type of charcoal that is black, porous, light, fine-grained, and has a large surface area. Carbon accounts for more than 70% of its mass. The remainder is made up of nitrogen, hydrogen, and oxygen.

Biochar soil amendment is being studied around the world as a way to improve soil fertility and mitigate climate change. The method and success of biochar application depend predominately upon the purpose of applying biochar to soil (*Biochar for Environmental Management: Science and Technology - Google Books*, n.d.). Due to the unique features of biochar, its addition may have a substantial effect on the chemical and physical properties of soil, as well as on biochemical processes and microbiological functions.

Originally, the term "biochar" was used to refer to a particular method of manufacturing known as "slow pyrolysis" where biomass is heated at the temperature in the range of 300–600 °C with a heating rate of 5–7°C min^{-1}, slow pyrolysis yields biochar as a major product (35–45%)(Gabhane et al., 2020), however, the word "biochar" has been expanded to encompass products of rapid pyrolysis at elevated temperatures, termed "fast pyrolysis," as well as other new techniques such as Gasification, Torrefaction, Flash pyrolysis, Vacuum pyrolysis, etc.

Biochar is characterized as porous structure with abundant functional groups; it is rich in surface free radicals and surface charges, a high surface area, and also contains minerals and trace metals(Wang & Wang, 2019). Their ability to adsorb organic debris, nutrients, and gas makes it a perfect environment for bacteria, actinomycetes, and arbuscular mycorrhizal fungus to colonize, thrive, and reproduce. Natural predators would be shielded from microbes.

In view of what discussed above, the aim of this research is to:

1) Assess the possibility of using hardwood biochar produced by gasification, as soil amendment.

2) Analyze Interaction of soil microbiota in response of different particle size and their application rate.

3) To observe the morphological properties of indicator plant (wheat) in response to application of various dosses and particle size of hardwood biochar.

4) To study the possibilty of using gassification Biocahr for improving cicrular economy.

Measurable targets:

1) Yield of wheat crop
2) Health of the plant
3) Soil chemical properties
4) Soil biological characteristics
5) Interaction of soil chemical properties with soil biome

2. LITERATURE REVIEW

2.1. Biochar Production technology

A biochar production occurs under anaerobic (limited supply of oxygen) condition. Various method of production is listed in literature but no proper classification is available. Several traditional biochar production methods have been replaced by modern approaches as humans and science continue to evolve. The methods for producing biochar are primarily classed as traditional or modern based on their developments and modernization.

Thermochemical conversion is a common technique for biochar production (Yaashikaa et al., 2020). Production methods include slow or fast pyrolysis, hydrothermal carbonisation, torrefaction, and flash carbonisation, as well as the regulation of the pyrolysis process and subsequent modification(Li et al., 2020). Gasification is an effective thermochemical conversion process for biomass into energy fuel while producing biochar as a byproduct(Gabhane et al., 2020). Pyrolysis and gasification are said to be the two most common methods for producing biochar (Kambo & Dutta, 2015; Qian et al., 2015). Compared to other conventional processes, gasification yields a greater amount of syngas and lower levelized emissions. Hydrogen is the primary byproduct of gasification. Nonetheless, a substantial amount of biochar can be produced during the gasification process (Gabhane et al., 2020; Guan et al., 2016). In biochar production, It's important to pay attention to changes in the elemental makeup of C, H, O, and N, as well as the interactions between them. The molar relationship between H to C and O to C, in particular, is utilized to determine the degree of aromaticity(Lehmann & Joseph, 2012)

During the gasification process, heat transfer within a particle raises the temperature of a small area of biomass. This causes water to leave the particle, which is followed by the gradual release of pyrolytic volatiles. Biochar is a main result of biomass degradation, which occurs between 400 and 500 °C (Mohan et al., 2006). Brief comparison between different type of biochar production technique is given the table 1.1

Table 1.1. Various biochar production methods and their application

Technique	Temperature (°C)	% Biochar production	Applications	References
Traditional approach				
Pyrolysis	250-900			(Cantrell et al., 2012; Lai et al., 2013)
Slow pyrolysis	300-700	35	Soil amendment, Bio-oil, Syngas as an energy fuel	
Fast pyrolysis	500-1000	12	Adsorbent, Soil amendment, Bio-Oil, Syngas	
Modern approaches				
Gasification	750-900	10	Dye Removal, Adsorbent, Carbon Sequestration Soil Amendments, Syngas	(Gabhane et al., 2020)
Hydrothermal carbonization	220-240	50-80	Retention of nutrients High calorific value, Better grindability, Improved hydrophobicity	(Yaashikaa et al., 2020)
Torrefaction	290-300	80	Regarded as a pre-treatment step to improve the physical, chemical, and biochemical characteristics of raw biomass	(Gabhane et al., 2020; Yaashikaa et al., 2020)

Acording to Tripathi et al., 2016 biochar yield, characteristics (amorphous or porous), and quality (shape, size, and chemical composition) are highly affected by manufacturing parameters (temperature, residence time, heating rate, and pressure). During pyrolysis, cellulose is first transformed into amorphous intermediates, then irregular carbohydrates, and finally aromatic carbon. Intramolecular and intermolecular processes convert biomass to biochar. Temperature increases turn hemicellulose into porous, smooth solids, reducing functional groups (e.g. hydroxyl and methoxy groups) (Li et al., 2020). Whereas, in gassification process thermochemical decopostion carbonaceous material into gaseous products are carried out i.e. the syngas comprising CO, CO_2, CH_4, H_2 and traces of hydrocarbons in presence of gasification agents such as oxygen, air, steam, etc and high temperature(Yaashikaa et al., 2020).

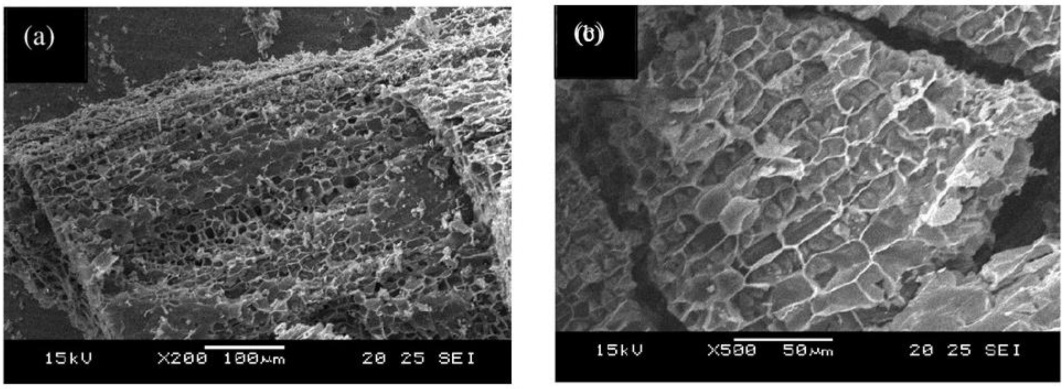

Figure 2.1. Scanning electron microscopy (SEM) images of biochar from pure wood chips gasification (Ng et al., 2017)

The composition and availability of feedstock are two of the most important factors in the efficient and cost-effective production of biochar The physical and chemical properties of biochar, such as porosity, surface area, and surface function, are influenced by the type of biomass feedstock(Li et al., 2020). Agricultural residues, urban waste, paper waste, woody biomass, aquatic biomass, animal and human excreta, industrial waste, food and kitchen waste, dairy and paper mill waste, poultry waste, and other waste are all used to make biochar.

2.2.Physical and chemical attributes of biochar

Biochar is made up of a complex blend of organic carbon-based compounds and mineral phases. Within the carbon matrix, metals and nonmetals can coexist (Joseph et al., 2013) The mineral matter content of biochars derived from wood is typically very low (5% by weight), whereas the mineral matter content of biochars derived from crop residues and manures is high (>10%). Keiluweit et al., 2010 anticipated that most biochars produced under 550°C have an uneven amorphous carbon lattice with high and low condensation while, as the pyrolysis temperature rises from 200 to 700°C, a complex transformation of organic structures occurs. Joseph et al., 2013 explains Low-temperature biochars produced under 450°C have a high concentration of water-soluble and organic volatile compounds, high-temperature biochars, on the other hand, have much lower concentrations of organic molecules and are mostly made up of low-molecular-weight acids (especially carboxylic) and neutrals. Amorphous and crystalline minerals can occur as micro and nano phases (oxides, sulphates/sulphides, carbonates, chlorides, and phosphates) in organic lattices. Some minerals are conductors, semiconductors, or insulators. Complex structures make up the edges between the mineral phase and the organic phase.(Joseph et al., 2013) In addition, biochar can be a source of micronutrients such as boron, molybdenum, potassium, phosphorus, and calcium, all of which are essential components for the nodulation process of Rhizobia(Rondon et al., 2007).Biochar is a reservoir of electron acceptors and donors with the ability to buffer pH and exchange cations (Leng et al., 2020).

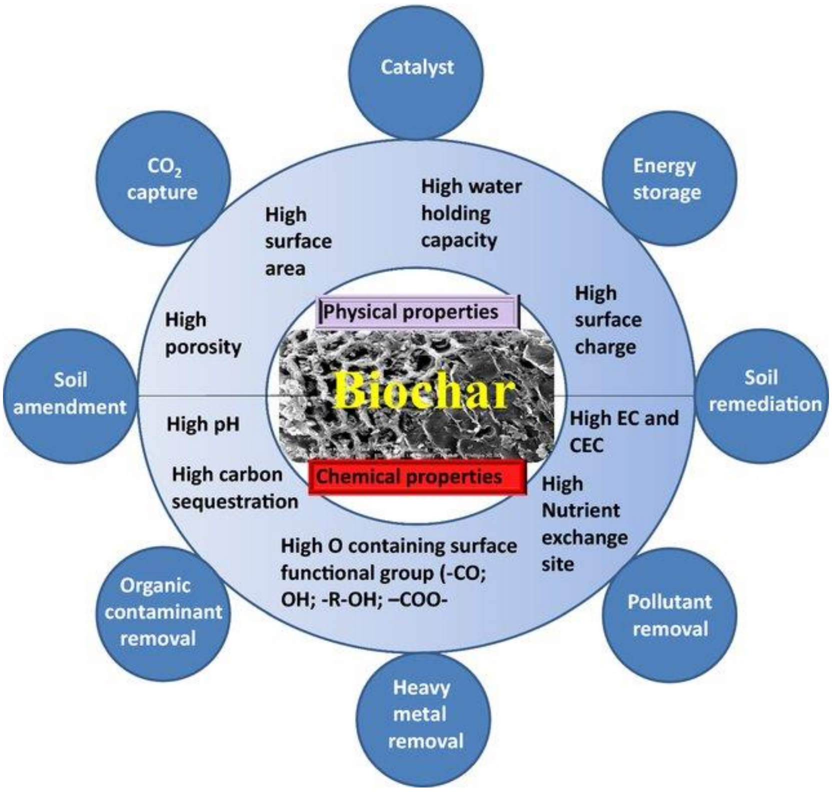

Figure 2.2. schematic diagram showing potential use of biochar because of its physiochemical properties (Qian et al., 2015)

2.3.Application of bichar and its potentinal as a soil amendment

Biochar additions have been observed to enhance microbial biomass in most investigations, with significant changes in microbial community composition and enzyme activity, which could explain biogeochemical effects of biochar on element cycles, plant diseases, and crop growth.(Lehmann et al., 2011) Potential Use of Biochar as an amendment adding biochar to soil or container substrate has several potential benefits, such as modifying soil physical and chemical properties by:

a) Increasing cation exchange capacity (CEC).

b) Increasing surface area.

c) Increasing pH.

d) Increasing plant nutrient availability.

e) Enhancing water-holding capacity

(also see figure 6)

Biochar's vast surface area and pores increase soil water holding capacity, bind heavy metals and pollutants, and encourage soil microbial growth.(You et al., 2017) Nitrogen found in soil is amongst the significant elements for plants, but it can only up taken by plants in inorganic Nitrogen form. However, various microbial activity degrades it to un-available organic Nitrogen form. Biochar acts as a soil an additive, reducing nitrogen loss and improving soil fertility (Borchard et al., 2019). Biochar can lower disease severity of numerous pathogen types and even stimulate system-wide defense responses in host plants, in addition to carbon sequestration, nutritional enrichment, improved soil quality, and stimulatory effects on microbial diversity and extracellular enzymes(Gabhane et al., 2020; Gluszek et al., 2017; Huang et al., 2017)

3. MATERIALS AND METHODS

3.1. Description of the soil sampling area

The soil for the experiment was collected from the agriculture field at Ondokuz mayis University in Samsun, Turkey. The site location of which is 41.2797°, 36.3361°. The mean annual maximum and minimum temperature are 5°c to 27.7°c and relative humidity is 73%. The average annual precipitation is 36.9 inches per year. The soil was observed to be dark brown to black and is classified as clay soil. The soil has a pH of 6.80 and an electrical conductivity of 0.60 dS/m, indicating that it is slightly acidic.

3.2. Pre-treated soil physical and chemical Analysis

Physiochemical analysis of the soil used in the experiment was carried out in order to compare the results with treated soils. About 100g of soil was collected as a sample to conduct a pre-treatment analysis. For each test three replicates were made to reduce variability in experimental results. The results of the analysis are listed below in table 2.

Table 3.1. Physio-chemical analysis for pre-treated soil

Soil Properties	Results
pH	6.58
EC	451.5 µs/cm
Moisture content (MC)	97.3%
Soil texture (By using Bouyoucos Hydrometer)	Clay Soil
% Silt	
% Sand	21.95%
% Clay	58.50%
	17.28%
Soil Organic Matter (Wet oxidation method)	4.17%
Lime ($CaCO_3$) content (calcimeter method)	1.39% (low)
Exchangeable cations	
Ca	26.96 meq/100g
Mg	21.68 meq/100g
P (Olsen method)	14.64 ppm

3.3. Biochar production technology and its properties

Biochar used in the experiment was obtained by "Kastamonu Entegreted" the company that uses hardwood to produce wooden flooring and furniture based in Samsun, the black sea region of Turkey. Biochar is produced as a by-product in the processing of wooden products as a result of the gasification of hardwood at 700°c. The chemical properties of biochar are shown in the table 3.2.

Table 3.2. Physiochemical properties of hardwood biochar

Parameters	Results
pH	9.42
E.C (Ds/m)	0.31
CEC (meq/100g)	47.82
OC (%)	4.88
Zn (g/kg)	0.01
P(g/kg)	0.21
Ca (g/kg)	7.50
Mg(g/kg)	1.56
K(g/kg)	0.92

Biochar was observed to be black with coarse particles, and extremely light in weight. Biochar was manually crushed by hammer followed by sieving with a different mesh size sieve to separate it into various fractions.

Figure 3.1. (a) Manual crushing of biochar (b) sieving biochar through different mesh sizes for separation

3.4. Description of the indicator plant used

Wheat variety ‘Altındane’ was used as an indicator plant. It is developed by BlackSea Agricultural Research Institute and can be recommended to spring sowing conditions. This cultivar has awn, white spike, white kernel, and medium early maturation. Mature Spikes lean forward. In ideal conditions, it can reach a height of 80-100cm.

3.5. Green-House experiment

The experiment was conducted in controlled environmental conditions where the temperature was maintained. Rain water was used to irrigate the plants. Soil moisture content was maintained close to field capacity by weighing the pots every two days to determine the moisture depletion and replenishing the moisture to field capacity.

3.5.1 Description of experiment

A total of 39 pots were utilized in the experiment, with three replicates of each dose (including three pots used as controls) being set up for each treatment. About 500kg of soil was collected and placed in the shade for 15-20 days to allow it to dry naturally. It was then crushed with a wooden hammer and manually sieved through a 4mm sieve, for preparing homogenized soil conditions for each treatment. Each pot was filled with 3kg of soil.

Figure 3.2. (a) Experimental soil kept for air drying, (b) Application of biochar with air-dried and sieved soil.

Biochar treatment was applied in three separate doses of 0.5 %, 1%, and 2% (percentage weight by weight w/w) for each particle size fraction <0.5, 0.5–1.0, 1.0–2.0, and 2.0–4.0 mm. In order to apply treatment, each fraction was weight according to the dose level to be applied and mixed with 3kg soil as shown in Fig 8 (b).

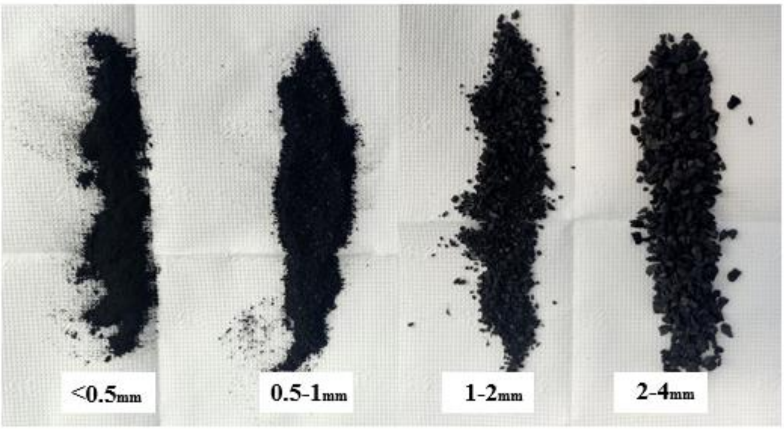

Figure 3.3. Different biochar fractions used in the experiment

3.5.2 Experimental design and Data collection

A completely randomized plot design was set, where similar experimental units are grouped into plots. A total of 13 treatments were decided including control, in which each biochar fraction was decided to be applied in three different levels of doses.

Table 3.3. Experimental Design of Experiment

TREATMENT NO	FRACTION SIZE (mm)	DOSE (%W/W)	ABBREVIATIONS
1	Control	Null	C
2	<0.5	0.5	0.5%(<0.5)
3	<0.5	1	1%(<0.5)
4	<0.5	2	2%(<0.5)
5	0.5-1	0.5	0.5%(0.5-1)
6	0.5-1	1	1%(0.5-1)
7	0.5-1	2	2%(0.5-1)
8	1-2	0.5	0.5%(1-2)
9	1-2	1	1%(1-2)
10	1-2	2	2%(1-2)
11	2-4	0.5	0.5%(2-4)
12	2-4	1	1%(2-4)
13	2-4	2	2%(2-4)

Data collection for the greenhouse phase was divided into two periods:

3.5.2.1 Plant growth stage:

During the plant growth stage data of each plant was collected after two days which included:

1. Weight of the pot (g)

İn the initial stages of the experiment field capacity of the soil was calculated and the final weight of the pot was determined that was to be used as a reference to irrigate the plants every two days, so that pot was not over or under irrigated.

2. Calculation of evapotranspiration (ml)

During the experimental period, the loss of water in the pots as a result of evaporation by plants was determined by comparing the weight of the pot right before irrigation with its final weight.

3.5.2.2 Maturation stage

After the end of 4 months and 9 days, each plant was harvested by clipping the shoot at soil level on 26-04-202. Before harvesting the mature plants, agronomical factors of plants were collected that included:

1. Number of plants per pot,
2. Height of each plant,
3. Number of spikes per plant,
4. Average spike height per pot,
5. Measuring plant biomass by taking the difference between the weight of pot with plants and after harvesting

Lab- Analysis

After harvesting the plants, soil samples were collected in two badges from each pot, First badge of soil from each pot was collected and sealed in a plastic bag immediately and kept in a fridge. These soil samples were used for the soil biological analysis later on. Whereas the other badge of soil sample was collected and air-dried for 10 days and was used for soil physiochemical analysis.

3.6.1 Post-treatment soil biological analysis

Following tests were carried out to obtain data.

1. Microbial biomass Carbon analysis using substrate-induced respiration method.

2. Soil respiration: Carbon dioxide (CO_2) production using the Isermeyer method.

3. Dehydrogenase's enzyme activity using the Pepper method

4. Soil Ureases activity using the Hoffman method

3.6.2 Post-treatment physiochemical analysis

Following are the list of analysis that was carried out for each pot to generate data for observation:

1. pH and EC were measured in 1:1 (w/v) soil: water suspension with pH and EC meter (Bayraklı, 1987)

2. Soil Organic Matter by using 'Walkley-Black' chromic acid wet oxidation method and the organic matter amount was calculated from this as stated by Nelson & Sommers (1982)

3. Lime content analysis by using calcimeter

4. Phosphorous (P) content analysis using the Olsen method

5. Exchangeable cation ExC, content analysis was evaluated in the soil suspension filtrate produced with the 1.0 N soil samples neutral ammonium acetate solution. The exchangeable Ca and Mg quantities were evaluated by titration with EDTA, and the amount of K and Na in the filtrate was determined by Atomic Absorption Spectrophotometer (AAS) (Kacar, 1994).

Figure 3.4. Lab analysis in process

The outcomes of this experiment were statistically evaluated using the SPSS 17 program in a completely randomized plot design. The means of results were compared using the Duncan test at 0.01 and 0.05 levels and F values of significant applications were given with ** at 0.01 and * at 0.05 levels.

4. RESULTS AND DISCUSSION

4.1 The effect of various biochar fractions and dose treatment on Plant yield and properties

4.1.1 Plant Biomass (BM)

The treatment of biochar with various fractions and doses increased the plant biomass (BM) significantly ($p<0.05$). The effect of biochar application under different treatments is given in the Fig 4.1.

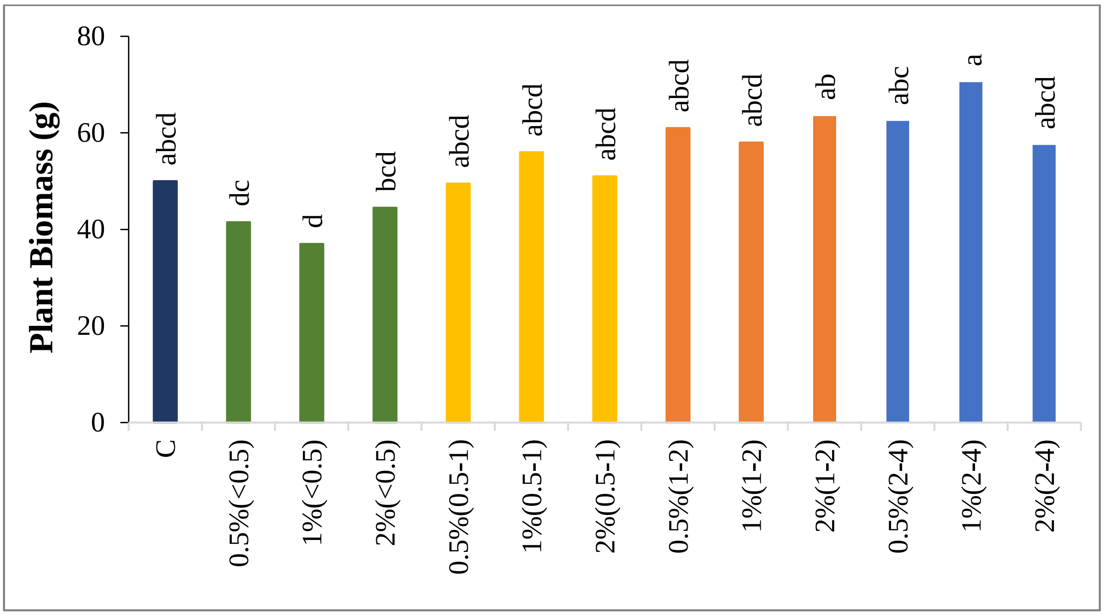

Figure 4.1. The effects of biochar amendments on plant biomass (BM), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign. (F=3.988*)

In general, the results show that using biochar with coarse fractions produces more biomass than using biochar with fine particle sizes, although using percentage dosages produces varied results for each fraction group. The maximal plant BM values were determined to be 70.5g for coarse fractions of 2-4mm with a 1% dose level, and the lowest value was 37.0g for fine fractions of <0.5 with a 1% dose level. The BM for the control treatment was 50g, which is statistically near to the value of each treatment.

Verheijen et al., 2010 explains that when the biochar interacts with the soil's physicochemical qualities, the physical properties of the soil such as texture, structure, pore size distribution, and density, which can affect soil aeration, water holding capacity and soil workability changes, which in turn have effect on plant development. (Verheijen et al., 2010) also reported that Biochar's inherent porosity and other features can influence the soil pore network in a variety of ways. As the soil used in experiment is clay, the smaller biochar particle size (,0.5mm and 0.5-1mm) may have decreased soil porosity, which explains the increased BM in medium-coarse(1-2mm) and coarse(2-4mm) particle size in comparison to fine (<0.5mm) and medium-fine (0.5-1mm) biochar fraction where decrease of BM is observed.

4.1.2 Plant length (PL)

The treatment of biochar with various fractions and doses increased the plant length (PL) statically ($p<0.05$). Figure 12 depicts the effect of biochar application in various treatments on plant length.

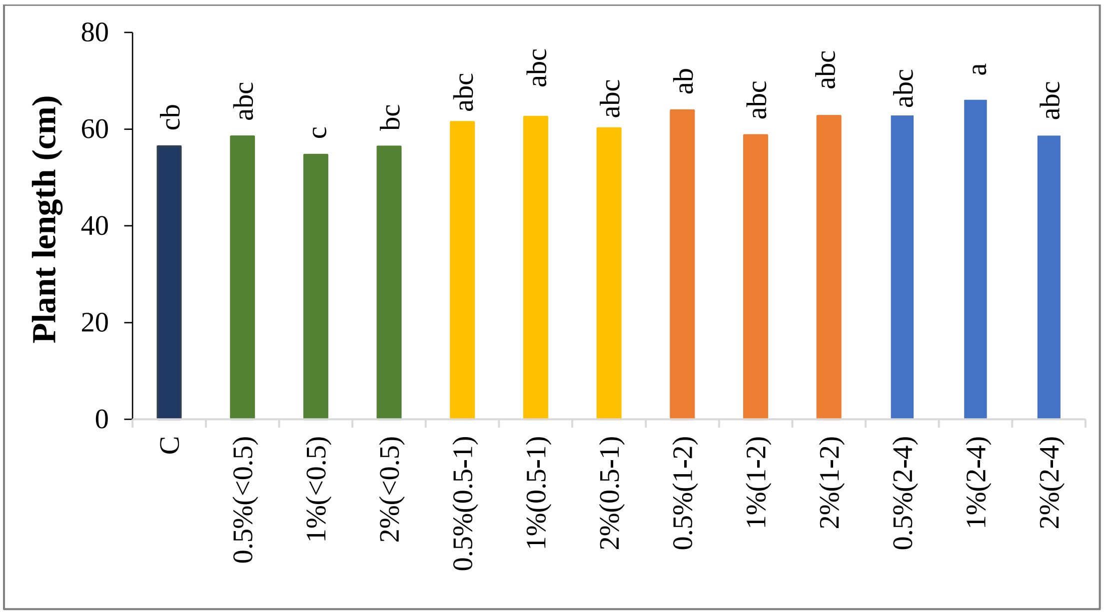

Figure 4.2. The effects of biochar amendments on plant length (PL), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F=3.457*)

Overall, plant length (PL) was statically observed to be longer for coarse biochar application in comparison to fine biochar application and control combined. Highest PL was 66.1cm for coarse biochar application 1-2mm with 1% dose. The shortest PL was for treatment of biochar particle size of <0.5 with 1% dose, while control treatment is 56cm which is grouped in one of middle to shorter length category statically.

4.1.3 Average spike height

Average spike height of the treated plants with hardwood biochar of various fractions at different doses level observed to be increased statically ($p<0.01$). Figure 4.3 depicts the effect of biochar application in various treatments on average SH of treated plants.

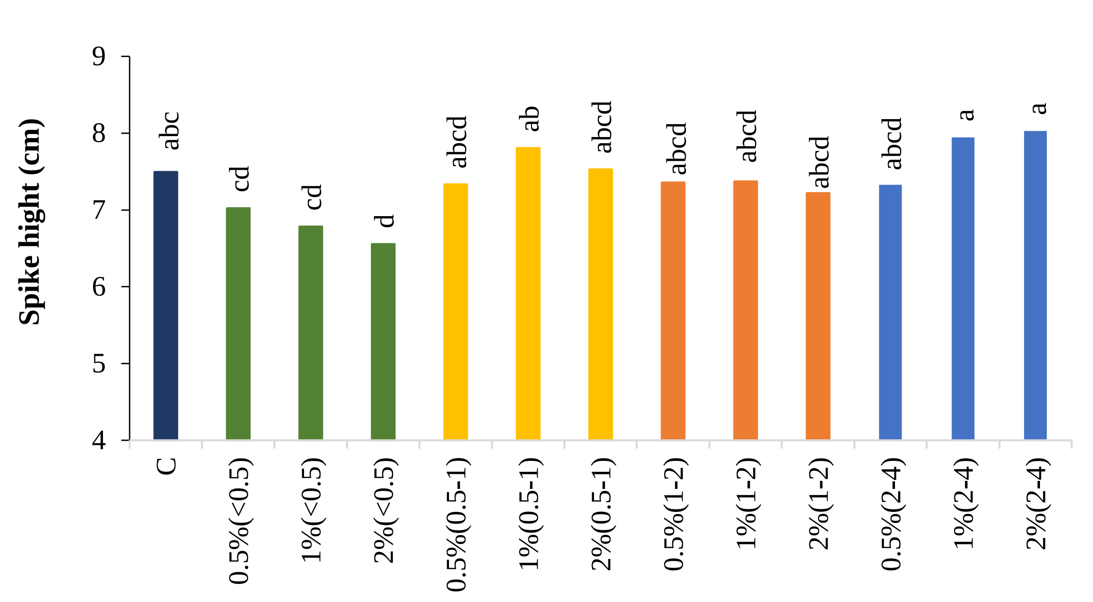

Figure 4.3. The effects of biochar amendments on spike height (SH), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F=2.870*)

The maximum SH was recorded for coarse biochar treatment with dose levels of 1% and 2%, respectively, with SH of 7.94cm and 8.03cm, respectively, while the smallest value of 6.56cm was recorded for biochar treatment with fine particles <0.5 at 2% dose level. The spike height was observed to rise when the biochar particle size transitioned from fine to coarse. Whereas C treatment was grouped under higher to middle value statically.

4.1.4 Cumulative evapotranspiration (E)

According to statical analysis there is no significant difference in the cumulative evapotranspiration of treated soil ($p>0.05$). The results are indicated in the figure 4.4

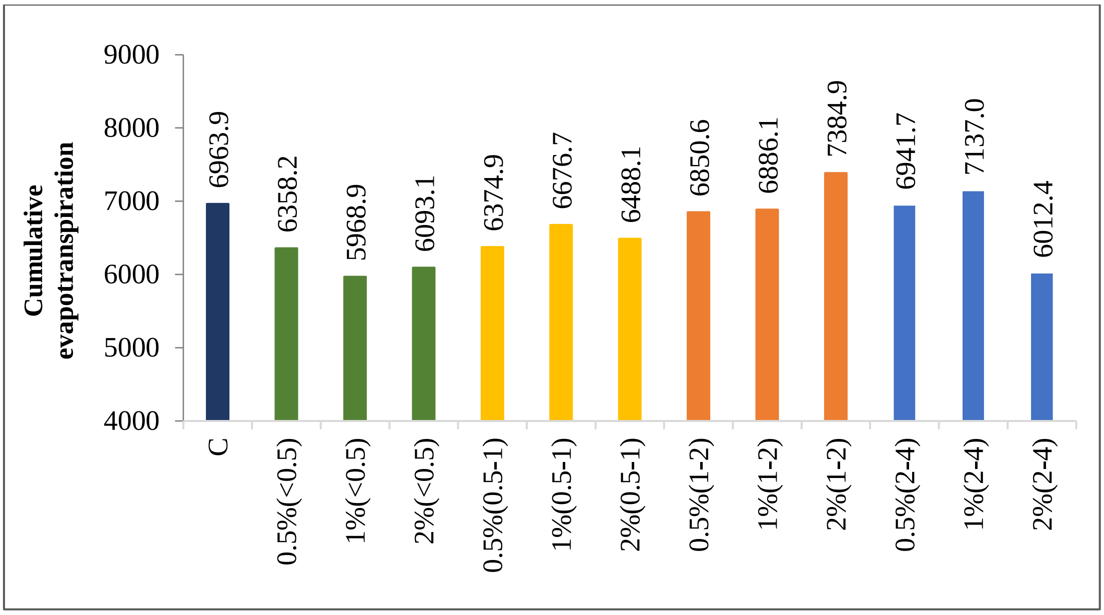

Figure 4.4. The effects of biochar amendments on Cumulative evapotranspiration (E), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis.

Cumulative evaporation is almost same for each treatment though the highest value is observed for 2% (1-2) biochar treatment. We observed that even though there were no statical differences control show much higher E as compared to many other treatments.

4.2 The impact of different biochar fractions and dose treatments on soil chemical characteristics

4.2.1 Soil Reactions (pH)

Biochar application of different particle size at various dose level statically increases the pH of soil ($p<0.01$). The effects of treatment application are show in Fig. 4.5

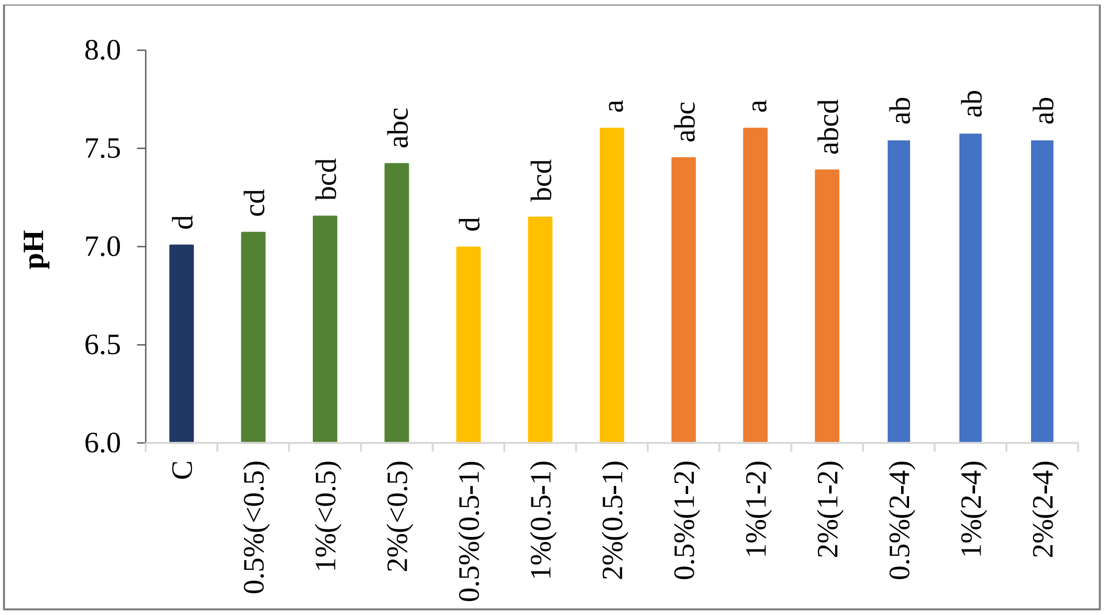

Figure 4.5. The effect of treatment on soil reaction(pH) C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F= 6.202**)

The addition of biochar improved the pH value of the soil in general. Fine fractions of biochar (0.5mm and 0.5-1mm) show a growing trend in pH with increasing dose level, whereas coarse biochar particle treatments (1-2mm and 2-4mm) show higher pH values but no exponential rise with dosage level. The maximum pH values were 7.60 for treatments 2%(0.5-1) and 1%(1-2), while the lowest values were 7.01 for control and treatment of 0.5% dose fraction being 0.5-1mm.

4.2.2 Electrical conductivity (EC)

Biochar application of different particle size at various dose level statically increases the EC of soil ($P<0.05$). Effects of treatment application are show in fig.4.6

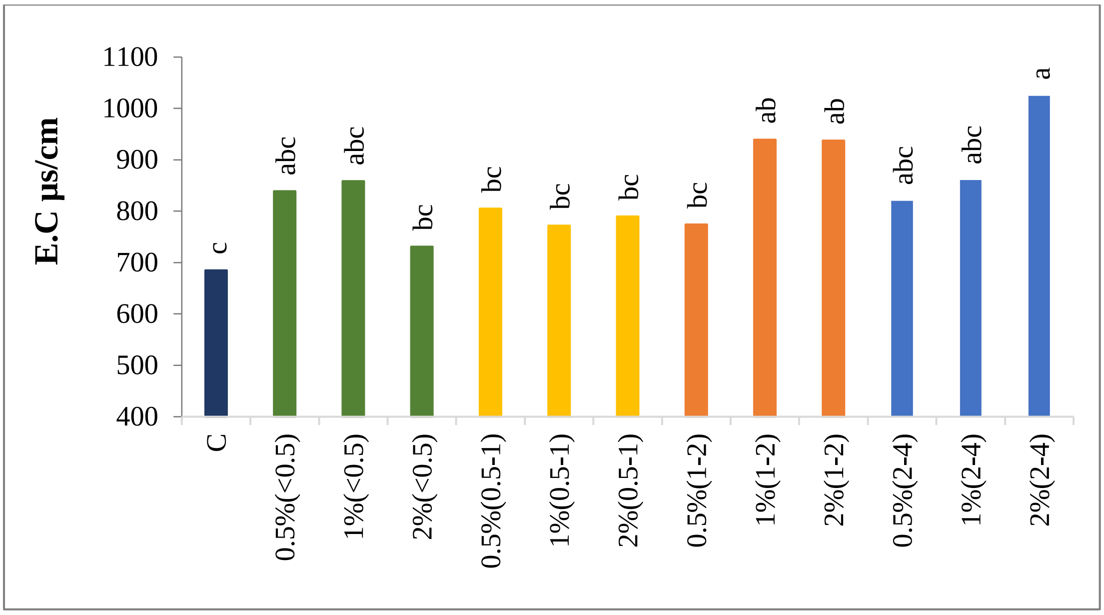

Figure 4.6. The effect of treatment on soil Electrical conductivity (EC). C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F= 2.063*)

In general, the value of EC increases with application of the biochar treatments. The heights value 1024.73 µs/cm of EC was noted for coarse biochar 2-4mm treatment at 2% dose level, while the lowest value 684.9µs/cm noted for control (C).

4.2.3 Organic Matter (OM)

Biochar application of varying fraction at numerous doe level decreases the % organic matter in soil significantly ($p < 0.05$) except biochar fraction of 1-2mm at 1%dose level. The effect of different biochar application is given in Fig 4.7.

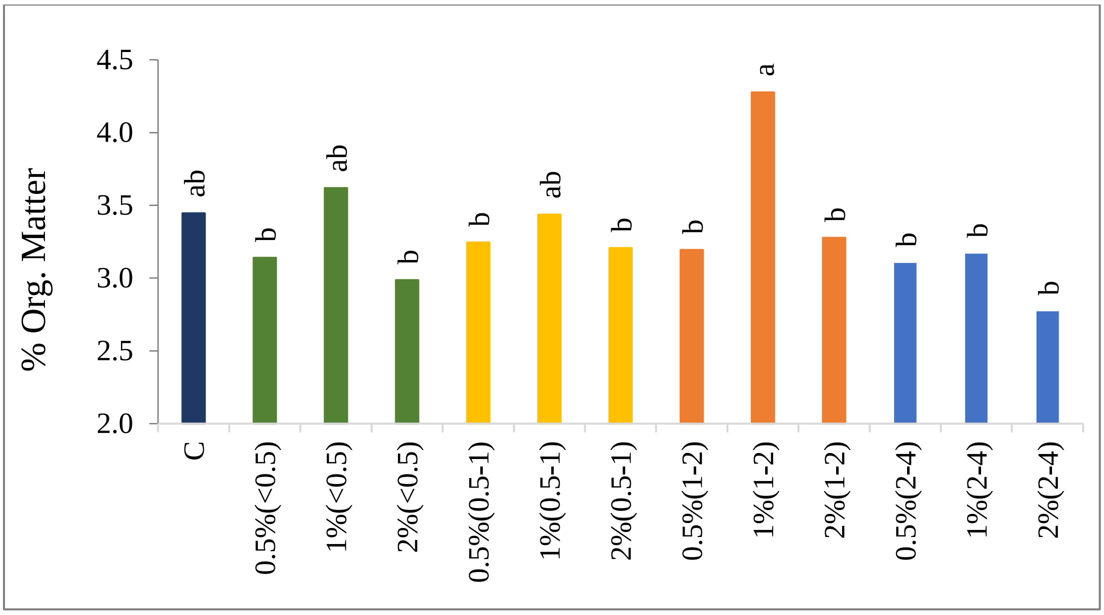

Figure 4.7. The effect of treatment on soil %Organic matter (OM). C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F=3.248*)

The OM value generally decreased with application of biochar treatment in comparison to control, except for biochar fraction of 1-2mm at 1%dose level in which high amount of OM was noted. The highest value of OM was 4.28% for 1%(1-2) treatment of biochar. While the lowest value 2.77% was for biochar treatment with 2%(2-4). It is observed that within each biochar fraction the values of OM were greater for 1% dose level as compared with other dose of 0.5% and 2%.

4.2.4 Available Phosphorus (P) Content

Biochar application with biochar fractions <0.5mm, 0.5-1mm and 2-4mm dose level mostly reduced the available P content in the soil, while treatment with biochar fraction 1-2mm increased significantly ($p<0.01$). The effect of the treatment of application on P content of soil in shown in fig17.

Figure 4.8. The effect of treatment on soil Available phosphorus (P), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F=3.758**)

The highest available phosphorus (P) content value statically is 22.7 ppm for coarse biochar size 1-2mm at 2% dose. The lowest statical values were observed 14.80ppm, 14.6ppm and 14.39ppm for treatments 0.5%(<0.5), 2%(0.5-1) and 2%(2-4) respectively. It is observed that P content in soil is low-medium when biochar treatment fraction <0.5, 0.5-1 and 2-4mm were applied whereas medium-high P was detected for control and biochar fraction 1-2mm.

4.2.5 Exchangeable Calcium (Ca) Content

The values of exc. Ca increased significantly with treatment of biochar at various doses and fraction level (p<0.01) the effect of biochar treatment on exc. Ca content in shown in figure below.

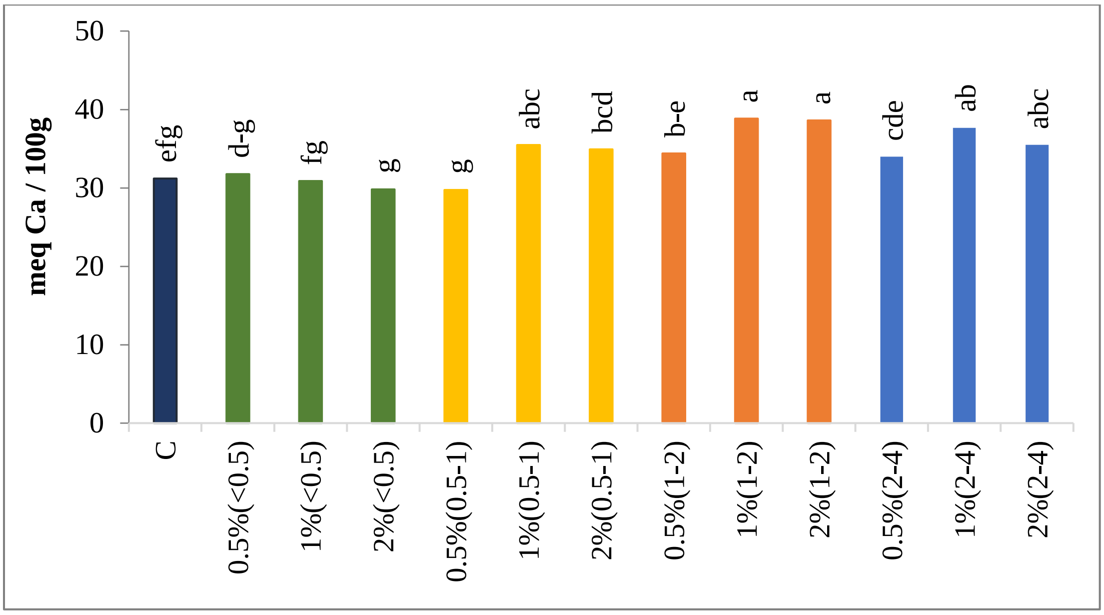

Figure 4.9. The effect of treatment on soil Exchangeable Calcium (Ca), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on x-axis. (F=15.243**)

In general, there is increase of value of exc. Ca with the shift of biochar particle size from fine to coarse and with higher level of dose percentages. The value of control was found to be in lower ranges group. Statically the highest values were 38.8 and 38.6 meqCa/100gsoil for coarse biochar 1-2mm with dose level of 1% and 2% respectively. Whereas the lowest values were for treatment with fine biochar particles of 0.5-1mm with 1% dose to be 29.7 meq Ca/100g and <0.5mm biochar particle with 2% dose to be 29.8 meq Ca/100g.

4.2.6 Exchangeable Magnesium (Mg) Content

The values of exc. Mg statically decreased in most of biochar application ($p<0.01$). The effect of treatments on exc. Mg in soil is depicted in the figure below.

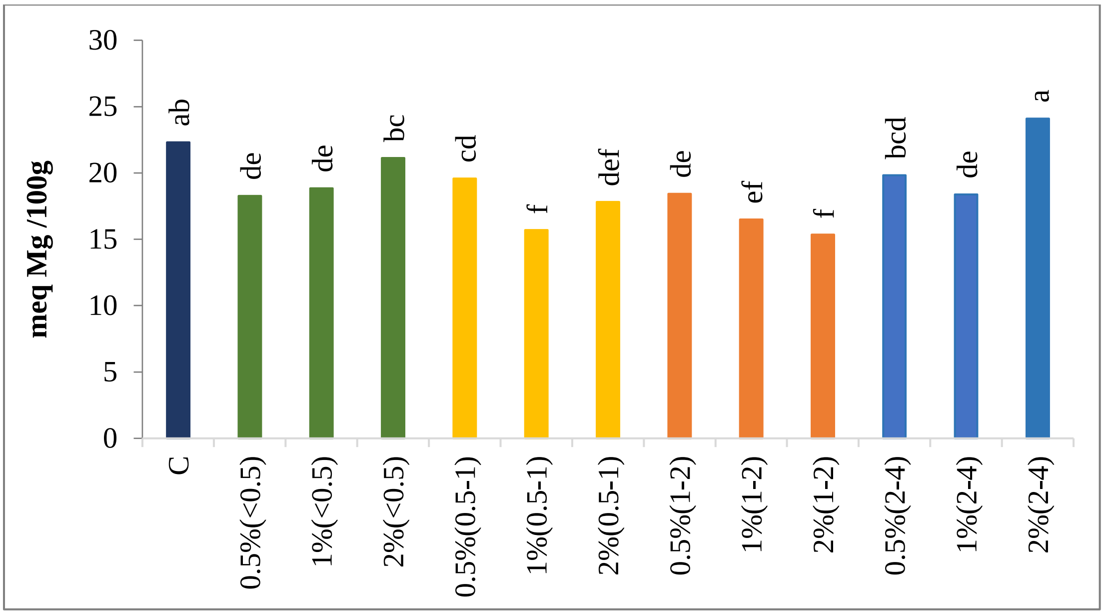

Figure 4.10. The effect of treatment on soil Exchangeable Magnesium (Mg), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis. (F=16.969**)

Generally, the values for the exc Mg are statically reduced by the application of biochar treatments in comparison to control treatment. Biochar fractions <0.5, 0.5-1, and 1-2mm showed a decrease in value, whereas biochar fractions 2-4mm showed a gain. The highest statical value 24.09meq Mg/100g soil was for 2%(2-4) treatment while, the lowest value 15.35 meq Mg/100g was for 2%(1-2) biochar treatment.

4.2.7 Exchangeable Potassium (K) Content

The values of exc K increase significantly with various biochar treatments ($p<0.01$). The effect of different treatments on exc K is given in the figure below.

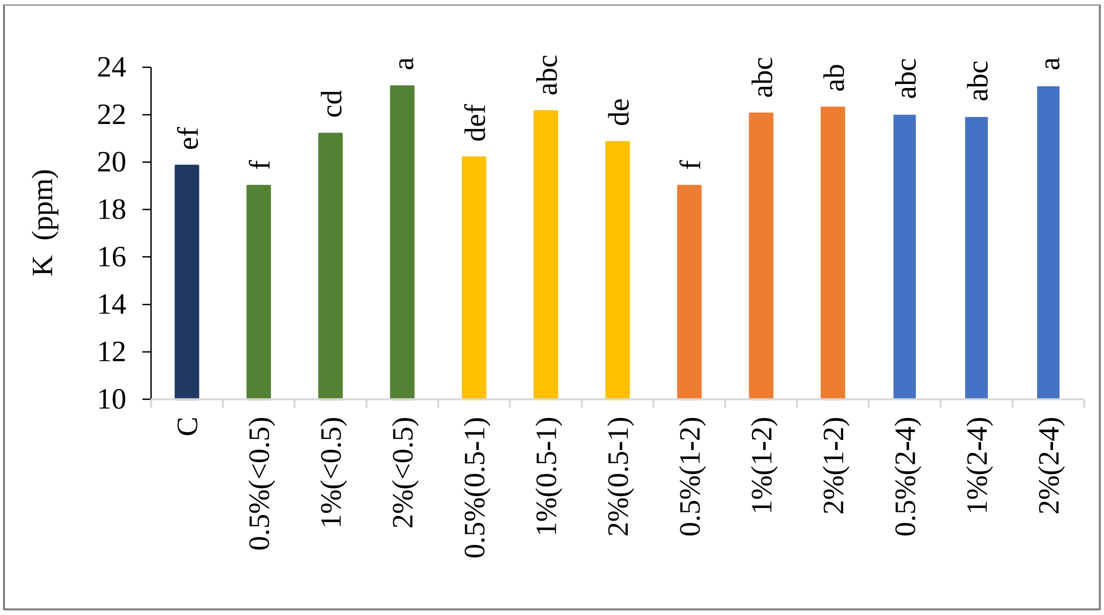

Figure 4.11. The effect of treatment on soil Exchangeable Potassium (K), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis. (F=21.661**)

The levels of exc K rise in general when biochar is treated with varied fractions. The highest result of 23.20 ppm was found to be similar for the two treatments: 2% (<0.5) and 2% (2-4). Whereas, the smallest value of 19ppm is also noted for two treatments: 0.5%(<0.5) and 0.55%(1-2). The figure shows that the exc K increases with increasing dose from 0.5 % to 2 % for each biochar fraction, with the smallest results for the lowest percent dose. C is shown to belong to the lower value group statistically.

4.3 The impact of different biochar fractions and dose treatments on soil biological characteristics.

4.3.1 Basal Soil Respiration (BSR)

The values of basal soil respiration increase significantly with various biochar treatments($p<0.01$). The effect of various treatments on BSR is given in the figure below.

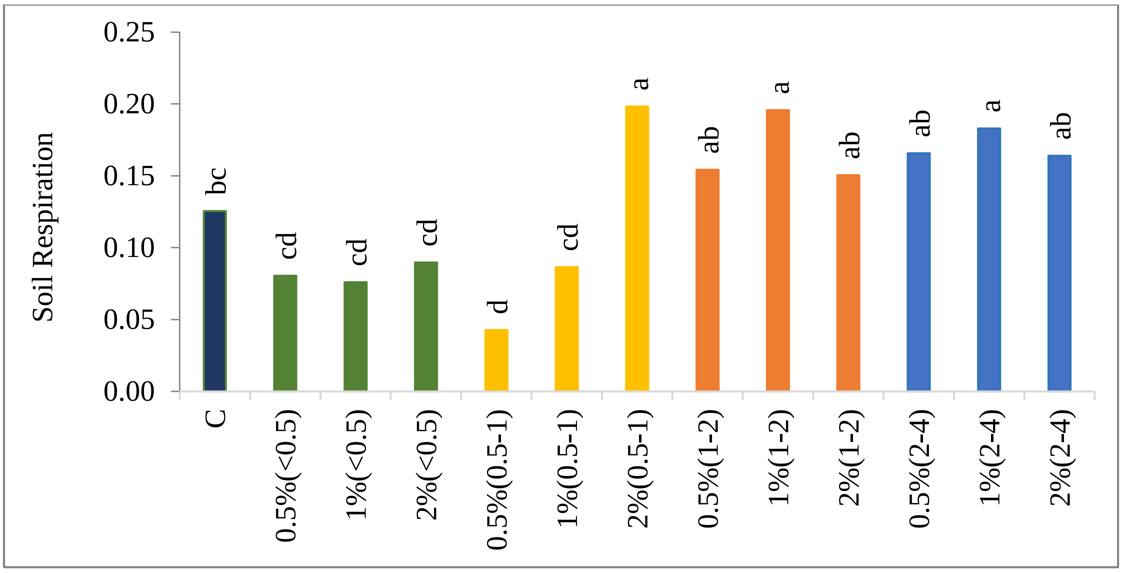

Figure 4.12. The effect of treatment on Basal Soil Respiration (BSR), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis. (F=17.552**)

In general, the BSR increase when varied biochar application is applied to soil in comparison to control. The highest BSR value 0.1982mg CO2/Day was observed for biochar fraction 0.5- 1mm at a 2% dose level. The lowest BSR value on the other hand was 0.04275mg CO2/day for 0.5%(0.5-1) biochar treatment. Within each biochar fraction 2% dose level indicates the highest values of BSR except for 2-4mm coarse biochar treatment where 1% dose indicates the maximum result

4.3.2 Microbial Biomass Carbon (MBC)

According to statical analysis there is no significant difference in the cumulative evapotranspiration of treated soil ($p>0.05$). The results are indicated in the figure 4.13

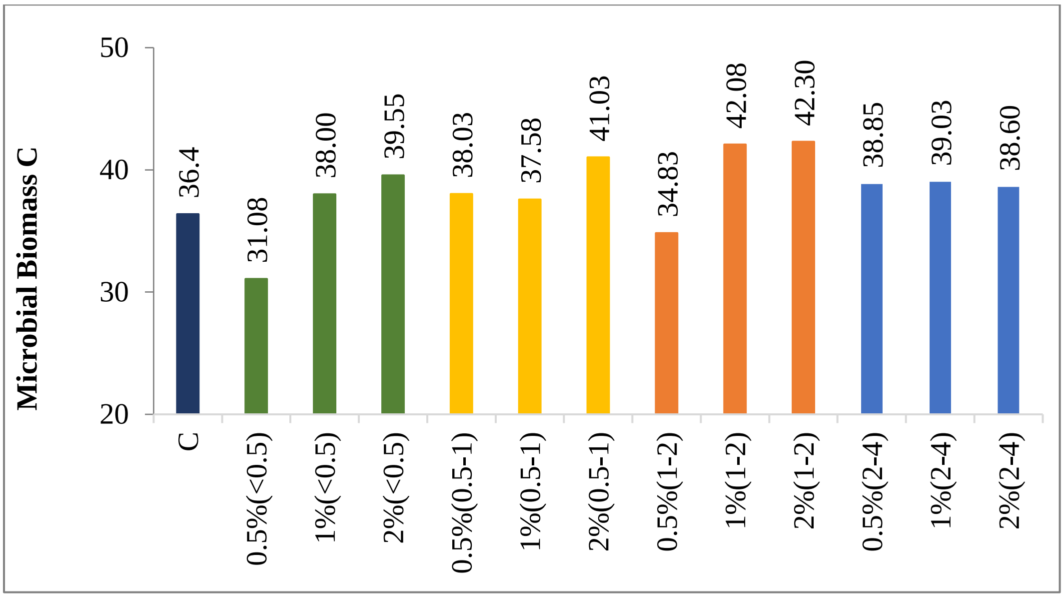

Figure 4.13. The effect of treatment on Microbial Biomass carbon (MBC), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis.

4.3.3 Ureases Enzyme Activity (UA)

The values of urease enzyme activity increase significantly with various biochar treatments ($p<0.05$). The effect of various treatments on UA is given in the figure below.

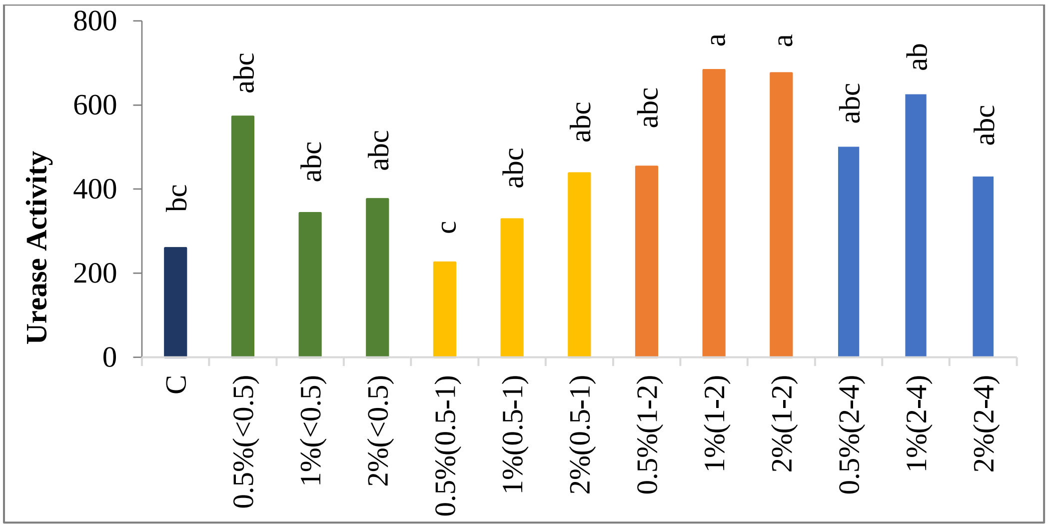

Figure 4.14. The effect of treatment on soil Urease Enzyme Activity (UA), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis. (F=3.263*)

In general, the amount of UA increased with biochar treatment of any fraction as compared to control treatment. The highest value of UA 683.15µgN/ODS/day observed for biochar fraction 1-2mm with 1% dose level, whereas, the lowest UA value is 225.5µgN/ODS/day.

4.3.4 Dehydrogenase Enzyme Activity (DHA)

The values of dehydrogenase enzyme activity (DHA) mostly decreased significantly with various biochar treatments($p<0.01$). The effect of various treatments on DHA is given in the figure below

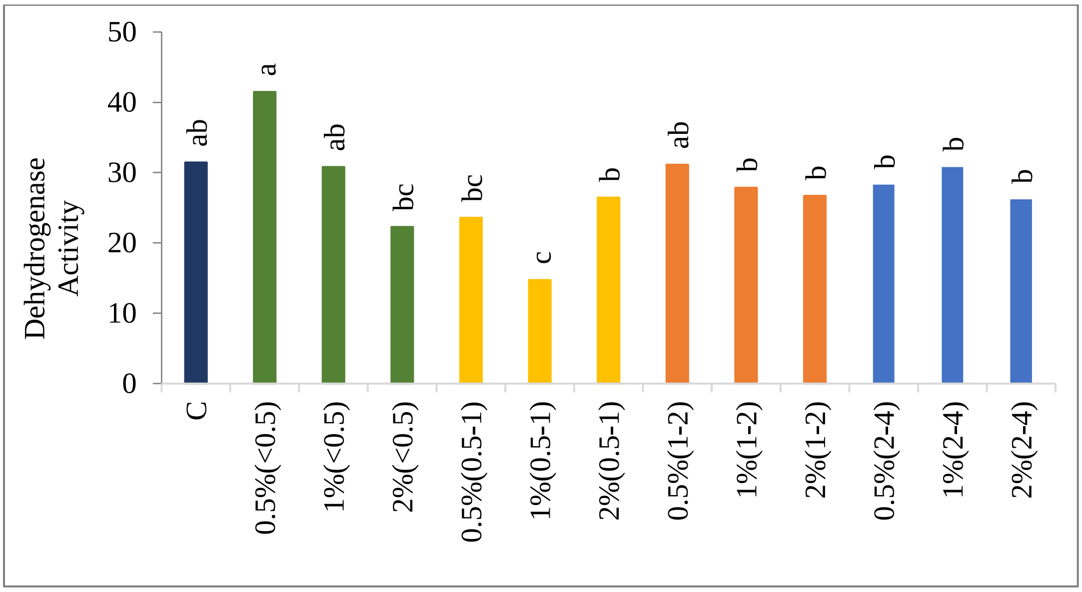

Figure 4.15. The effect of treatment on soil Dehydrogenase Enzyme Activity (DHA), C= control. Each color indicates a similar biochar fraction whereas, the amount of each dose applied is mentioned outside the bracket with the % sign on the x-axis. (F=5.446**)

The results show that with biochar treatments, levels are either decreased or recorded close to control values; nonetheless, DHA values are decreasing in general. The highest DHA value 41.48µgTPF/Day is recorded of fine biochar fraction <0.5 at 0.5% dose. whereas, the lowest value is 14.7348µgTPF/Day is noted for biochar fraction 0.5-1mm at 1% dose.

5. CONCLUSION

In this research, we used biochar of various fractions and dose levels in clay soil to assess their potential in boosting soil microbiota and its effects on wheat crop yield. All the plant growth parameters revealed as significant difference with control. It was noted biochar application with medium-coarse (1-2mm) and coarse (2-4mm) fraction led to better results as compared to biochar with medium-fine (0.5-1mm) and fine (<0.5) fraction. Observation also revealed that different percent dosages had no discernible effect on plant growth. The cumulative evapotranspiration didn't show any significance differences by the biochar amendment.

Biochar treatments had a considerable impact on the chemical and biological properties of the soil. Despite the fact that organic matter decreased in treated pots, soil pH and EC exhibited a substantial increase trend, implying that cations and anions are becoming more available to plants for better growth and development. In biochar application, the exchangeable cations Ca, Mg, and K had varying effects. Observation revealed that, with the exception of Mg, the other two significantly increased as a result of biochar amendments. Availability of Phosphorus in treated soil show particularly higher results of 1% concentration for <0.5mm, 0.5-1mm and 2-4mm biochar fraction.

Biochar treatment was found to be quite effective in promoting microbial growth and activity, according to the findings of soil microbiological properties. However, soil basal respiration ramped up with coarse and medium-coarse biochar fractions, while it decreased dramatically with fine (0.5) and medium-fine biochar particle sizes (0.5-1). The amount of carbon in microbial biomass grew dramatically, indicating that the soil has become more favorable to microbial population proliferation. The biochar treatments increased soil urease activity, but dehydrogenase activity decreased.

Biochar has been reported time by time to upsurge the soil properties: both chemical and biological but it is apparent from experiment conducted that the effect is: biochar particle size, its concentration of application and soil type dependent. It is also evident that mostly the larger the biochar particle sizes the more positive its effect on the soil physiochemical and biological properties. Smaller particle size biochar, on the other hand, not only has lower outcomes, but it can also have detrimental impacts on general soil health, which affects plant growth. Therefor before suggesting biochar application as sustainable agriculture practice further research related to what biochar fraction, its dose level and type of soil to be applied on should be considered. At the same time, further research is necessary to find out their field level efficacy to improve the crop growth and sustainable soil health management.

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