SYNERGISTIC EFFECTS OF BIOCHAR AND HALOTOLERANT ON WHEAT GROWTH AND THEIR POTENTIAL TO RECLAIM SALINE-SODIC SOIL

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ABSTRACT

SYNERGISTIC EFFECTS OF BIOCHAR AND HALOTOLERANT ON WHEAT GROWTH AND THEIR POTENTIAL TO RECLAIM SALINE-SODIC SOIL

The problem of soil salinization is one of the major threats to agricultural productivity worldwide. To address this issue, a wide range of adaptations and mitigation strategies are needed. Biochar, an activated carbon soil conditioner can help in alleviating the deleterious effects of salinity.

The current research aimed at evaluating the effectiveness of biochar in the redemption of saline-sodic soil, as well as improving the growth and yield of wheat when used in conjunction with halotolerant plant growth promoting bacteria (PGPBs). For this purpose two experiments were established as incubation and greenhouse. Firstly, high salt tolerant bacterial strains to be used in the study were isolated and screened. Then an incubation study was established for 3.5 months to monitor the biochar and halotolerant PGPBs effect on chemical properties of saline sodic soil. Cotton stalk and Olive pulp biochar were used for this purpose together with their feedstocks as positive controls. In accordance with the results each biochar type, whether applied alone or together with halotolerant was effective in improving the overall quality of degraded soil. Soil organic matter, (OM), cation exchange capacity (CEC), total nitrogen (TN) and exchangeable cations (calcium (Ca^{+2}), magnesium (Mg^{+2}) and potassium (K^{+}) were significantly increased while soil sodium (Na^{+}) content, sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) were greatly reduced. Higher the application rate better was the result.

Results of greenhouse experiment indicated that growth and yield of wheat was favorably increased with biochar application and effect was more prominent for the co-application of biochar with halotolerant. The post harvest soil analysis revealed a significant reduction in soil pH and EC in comparison to the control.

In light of the obtained results, co-application of biochar and halotolerant could be a sensible approach to reclaim the saline-sodic soils, thereby making the conditions suitable for plant growth under salinity stress.

ÖZET

BİYOKÖMÜR VE HALOTOLERANT BAKTERİLERİN, BUĞDAY BİTKİSİNİN BÜYÜMESİNDE SİNERJİSTİK ETKİLERİ VE TUZLU-SODİK TOPRAĞIN ISLAHINDAKİ ROLÜ

Toprak tuzluluğu dünya çapında tarımsal verimliliği etkileyen en büyük problemlerdendir. Bu tür problemlerle savaşmada türlü adaptasyonlara ve azaltma stratejilerine ihtiyaç vardır. Aktif karbonlu biyokömür tuzluluğun negatif etkilerini azaltmada kullanılan bit yöntemdir.

Araştırmanın amacı, biyokömür ve halotolerant bakterilerinin birlikte kullanılmasının, tuzlu-sodik toprağın geri kazanılmasında ve aynı zamanda buğdayın büyümesinde ve verim artışındaki etkinliğinin değerlendirilmesidir. Bu sebeple inkübasyon ve sera olarak iki farklı çalışma üzenlenmiştir.

İlk olarak, araştırmada kullanılacak yüksek tuz toleranslı bakteriler izole edildi. Sonra, biokömürün tuzlu sodik toprağın bazı kimyasal özelliklerine etkisini belirlemede 3.5 ayı geçen inkübasyon deneyleri gerçekleştirildi. Bu amaçla, Pamuk ve Zeytinin biokömürleri ve onların hammadeleri kontrol amaçlı kullanıldı. Elde edilen sonuçlara göre, her iki biyokömür tipinin (tek başına kullanılan biyokömür ve PGPR ile birlikte kullanılan biyokömür) degrade olan toprağın kalitesini iyleştirdiği tespit edilmiştir. Toprağın organik madde, total nitrojen, ve değiştirilebilir katyonları (Ca^{+2}, Mg^{+2}, K^{+}) önemli ölçüde artmış, toprak Na^{+} içeriği sodyum absorbe oranı ve değişebilir sodyum yüzdesi ise büyük ölçüde azaltılmıştır. Uygulama oranları arttıkça daha iyi sonuçlar gözlemlenmektedir.

Yapılan sera deneyleri sonucunda, tekbaşına biokömür uygulaması buğday büyümesini ve verimini önemli ölçüde artmıştır. Ancak biokömürün halotolerant bakteriler ile birlikte uygulanması ile daha etkili sonuçlar elde edilmiştir. Hasat sonrası toprak analizleri, toprağın pH ve elektriksel iletkenliği değerlerinde, kontrole kıyasla önemli bir azalma olduğunu ortaya koymuştur.

Elde edilen sonuçlar ışığında, biokömür ve halotolerant bakterilerin birlikte uygulanması, tuzlu-sodic toprakların ıslahı için makul bir yaklaşım olabilir, böylece tuzluluk stresi altında bitki büyümesi için uygun koşullar elde edilmiş olunur.

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LIST OF SYMBOLS/ABBREVIATIONS

%	Percent
Al	Aluminium
B	Boron
C	Carbon
˚C	Degree celsius
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CAS	Chrome azurole S
C_2H_5OH	Ethanol
CH_3COOH	Acetic acid
$CH_3COONa.3H_2O$	Sodium acetate trihydrate
CH_4	Methane
Cl^-	Chloride
cm	Centimeter
cm^2	Centimeter square
Cmol (+) kg^{-1}	Centimol per kilogram
CO_2	Carbondioxide
CO_3	Carbonates
Cu	Copper
$CuSO_4$	Copper sulphate
dSm^{-1}	Desiceimen per meter
Fe	Iron
$FeCl_3$	Iron chloride
$FeSO_4.7H_2O$	Ferrous sulphate
g	Gram
g cm^{-3}	Gram per cubic meter
g kg^{-1}	Gram per kilogram

$g\ L^{-1}$	Gram per liters
H	Hydrogen
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3PO_4	Orthophosphoric acid
HCO_3	Bicorbonate
HCL	Hydrocloric acid
$HClO_4$	Perchloric acid
HCN	Hydrogen cyanide
HNO_3	Nitric acid
K^+	Potassium
$K_2Cr_2O_7$	Potassium dichromate
K_2SO_4	Potassium sulphate
Kg	Kilogram
Ks	Hydraulic conductivity
L	Liter
M	Molar
mg	Milligram
Mg^{+2}	Magnesium
$mg\ kg^{-1}$	Milligram per kilogram
$mg\ L^{-1}$	Milligram per liter
Mg^{2+}	Magnesium
mL	Milliliter
mM	Milli mole
mm	millimeter
Mn	Manganese
N	Nitrogen
N_2O	Nitrous oxide
Na^+	Sodium
Na_2CO_3	Sodium bicorbonate

Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4OH	Ammonium hydroxide
$NH_4H_2PO_4$	Ammonium phosphate monophosphate
NH_4^+	Ammonium
NH_4OAc	Ammonium acetate
Ni	Nickel
NO_3^-	Nitrate
NO_3^--N	Nitrate nitrogen
P	Phosphorus
pH	Potential of hydrogen
t ha^{-1}	Tons per hectare
rpm	Revolutions per minute
S	Sulfur
µm	Micrometer
Zn	Zinc

ACC	Aminocyclopropane-1-carboxylate
ANOVA	Analysis of variance
BPC	Biochar poultry manure compost
CB	Cotton biochar
CEC	Cation exchange capacity
CS	Cotton stalk
Cfu	Colony forming unit
CMC	Carboxymethyl cellulose
Conc	Concentrated
CRD	Complete randomized design
D	Days
DAP	Diammonium phosphate

DF	Dilution factor
DOC	Dissolved organic carbon
EC	Electrical conductivity
EPS	Exopolysaccharides
ESP	Exchangeable sodium percentage
FAME	Fatty acid methyl ester
FUE	Fertilizer use efficiency
GHGs	Greenhouse gases
hrs	Hours
IAA	Indole acetic acid
ICP-MS	Inductively coupled plasma mass spectrometry
LB	Luria-bertani medium
LSD	Least significant difference
M9	Minimal salts
NA	Nutrient Agar
NPK	Nitrogen, phosphorus, potassium
OB	Olive biochar
OP	Olive pulp
OD	Optical density
OM	Organic matter
PBS	Phosphate buffer solution
PGPB	Plant growth promoting bacteria
PM	Poultry manure
PS	Pyroligneous solution
SAR	Sodium adsorption ratio
SMB	Soil microbial biomass
SOC	Soil organic carbon
SOM	Soil organic matter
sp	Species
T	Treatments
TN	Total nitrogen

TSA	Tryptic soy agar
TSB	Tryptic soy broth
Var.	Variable
WHC	Water holding capacity
yr^{-1}	Per year

1. INTRODUCTION - STUDY BACKGROUND

Sustainable agriculture is a feasible way to meet the future food requirements of a growing world population. However, as an anthropogenic pressure, it strikes a balance between agricultural productivity, economic stability, use of resources and land degradation. Managing soil resources are one element of sustainable agriculture to overcome productivity constraints while preserving or improving the quality of the environment.

The current research aimed at evaluating the effectiveness of two different biochar (Cotton stalk and Olive pulp) in the redemption of saline-sodic soil. Moreover, their aftermath impacts on the nutrient status of the soil, crop growth, yield and physiological processes were also assayed when used in conjunction with halotolerant PGPBs.

1.1. SOIL SALINIZATION AND AGRICULTURAL PRODUCTIVITY

The major environmental stresses like extreme temperature, soil salinity/sodicity, drought and flooding have affected the development of agricultural crops. The soil salinization issue is a scourge for global agricultural productivity. Salinization ascribes to the process that ends up in the soil with too much water-soluble salts to such an extent that soil fertility is significantly impacted.

Worldwide, around 1/5th of the irrigated agricultural lands are severely salt-affected negatively influencing plant growth both in the plant and cellular levels [1]. According to different estimates, up to 7 percent of the total land surface, amounting to 1000 million hectares on the earth is saline [2]. Countries with serious salinity problems are Turkey, Australia, Pakistan, United States, China, India and Indonesia [3-6].

Figure 1.1. An overview of salt affected soils [4]

Salt-affected soils stem either from primary or secondary soil salinization processes or both. Worldwide, approximately 95 million hectares of soils suffer from primary salinization whereas 77 million hectares are under secondary [3]. Primary soil salinization involves salt buildup through naturally present parent rock rich in salt contents or seawater intrusions [7]. The secondary salinization process is human-induced and is generated by continued irrigation with brackish water deprived of satisfactory leaching, thereby compiling salts in the root zone [8]. Moreover, low graded irrigation waters, shallow groundwater tables with inadequate drainage and rapid evaporation over precipitation intensify salt piling in the topsoil layer [9].

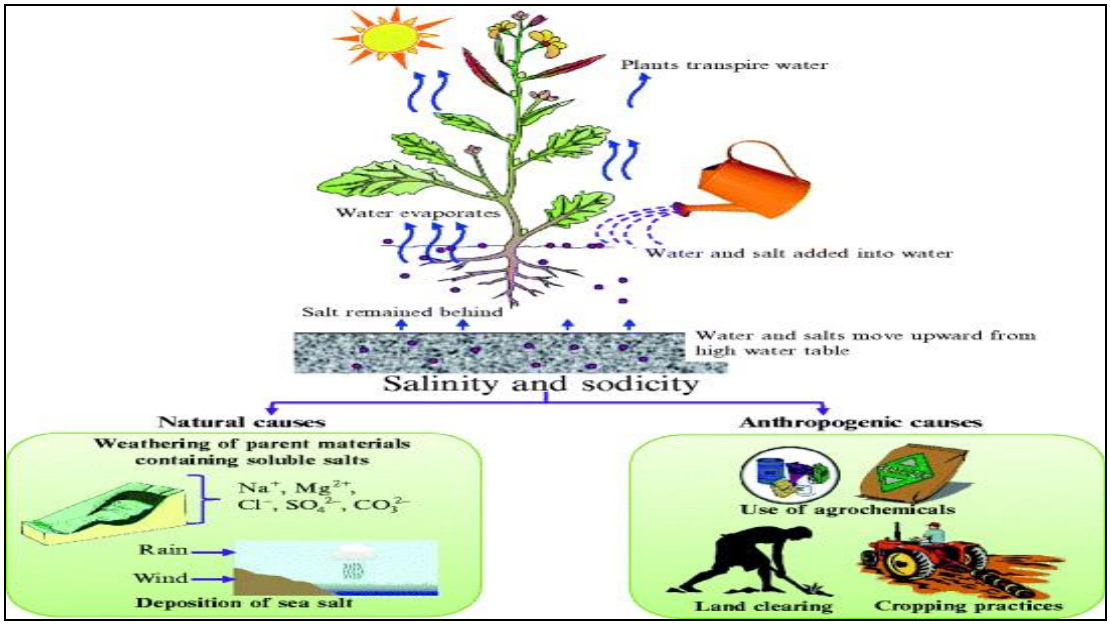

Figure. 1.2. The process of soil salinization [7, 8]

A great number of agronomic crops are rather salt susceptible and is unable to withstand even for a small amount of salinity. Hence, the magnitude of salinity problem is the gap between the production and demand for food all over the world. Salts amassment in the soil not only hamper plant growth, but also deteriorate soil status by disturbing soil physical, chemical and biological environment [10, 11].

In many cases, increased soil salinity can also result because of modifications in vegetation, this change the water balance of the ecosystem. In Australia, for instance, by changing deep-rooted crops to shallow ones, expansive areas are experiencing dryland salinization. Which in turn leads to the lower rainfall evapotranspiration and water logging of saline groundwater areas.

A vegetation change from grassland to the forest, on the other hand, results in increased evapotranspiration over groundwater recharge, thereby leading to soil salinization [12, 13].

The general category of salted soils is based on ESP (exchangeable sodium percentage), SAR (sodium adsorption ratio) and EC (electrical conductivity of saturated soil extract) [14]. Based on said properties, soils are graded as saline; sodic or saline-sodic (Table 1.1). Saline-sodic soils are thought to be highly degraded due to the cumulative impact of sodium and salinity on crop physiology and quality of soil [15].

Table 1.1. Properties of salt affected soils [14]

Classification			
Class	**EC (dSm^{-1})**	**ESP (%)**	**pH**
Normal	<4	<15	<8.5
Saline	≥4	<15	< 8.5
Sodic	<4	≥15	8.5-10
Saline-Sodic	≥4	≥15	Varies

1.1.1. Soil Physical Properties as Affected by Salinity and Sodicity

Clay swelling and dispersion are influenced positively by the elevated soil solution and irrigation water salt concentration. The salinity of the soil solution can influence the physical characteristics of the soil by holding together clay particles in aggregates in a method called flocculation. This process results in relatively larger voids between the soil aggregates contrasted with non-flocculated soil, thereby making the soil more porous and less prone to be waterlogged after watering. Root growth, root penetration, and soil aeration become better because of enhanced aggregation [16, 17]. Despite the fact that soil, water salinity has a promising effect on improving and stabilizing soil aggregates, however, salinity at its high level can impart adverse and substantially harmful effects on plants [18]. Tejada and Gonzalez (2005) demonstrated that increased electrical conductivity has an adverse effect on soil structural stability, bulk density and permeability. Many scientists have revealed that high salt exposure is known to hamper crop yield e.g. barley [19, 20] cotton [21], sugar cane [22], maize [23], wheat [24], sugar beet [25] and rice [23]. The reduction in growth is because of the negative effects of salts on protein synthesis, photosynthesis, gas exchange, high osmotic potential and disruption in water retention of plants [26]. Therefore, one cannot solely increase the salinity level to modify the deteriorated soil structure without taking into account the potential after effects of elevated salinity on plant health [27].

Sodium affects soil with the opposite effect of salinity. The most important physical processes affected by elevated sodium concentration are aggregate swelling, clay platelet and soil dispersion [17, 28]. Soil dispersion stems when excessive Na^+ ions in between the clay minerals start disrupting the attractive force which binds clay particles together. As a result, the soil gets dispersed because of the dominance of repulsive forces [27] and soil pores get plugged. Dispersed soil particles upon continuous wetting and drying are reformed and solidified into a compact soil with no or poor structure, depending on the clay type and Na^+ percentage [17]. This leads to an environment generally poor in soil-water and soil-air relations [29]. Sodium-induced dispersion exerts highly negative impacts on infiltration rate, hydraulic conductivity and surface crusting. Thereby, making it hard for the roots to permeate the soil, for the plantation to be established and absorb sufficient

water and nutrients. On the whole, these conditions negatively hit plant survival and overall crop yield [27].

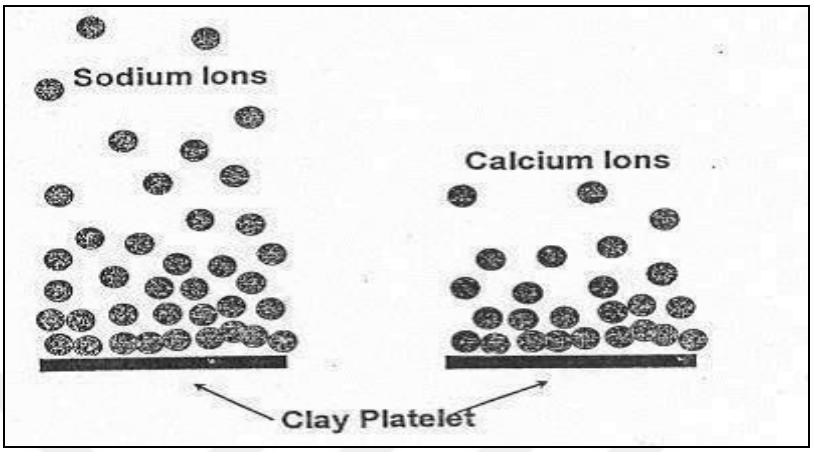

Figure 1.3**.** Sodium and calcium behavior associated with clay particles [17]

Sodicity can be seen in many different soil types and at any soil depth. Surface soils are more vulnerable to sodium and electrolyte concentration. Low EC and high ESP of these soils result in the aggregate breakdown of mechanical slaking and dispersion [30]. Thereupon, rearrangement of individual soil particles upon drying results in the formation of a thin layer of high shear strength known as a 'surface crust' [31]. This crust formation leads to soil surface sealing and a considerable reduction in the water infiltration rate, thereby causing excessive surface soil erosion and waterlogged conditions [32, 33].

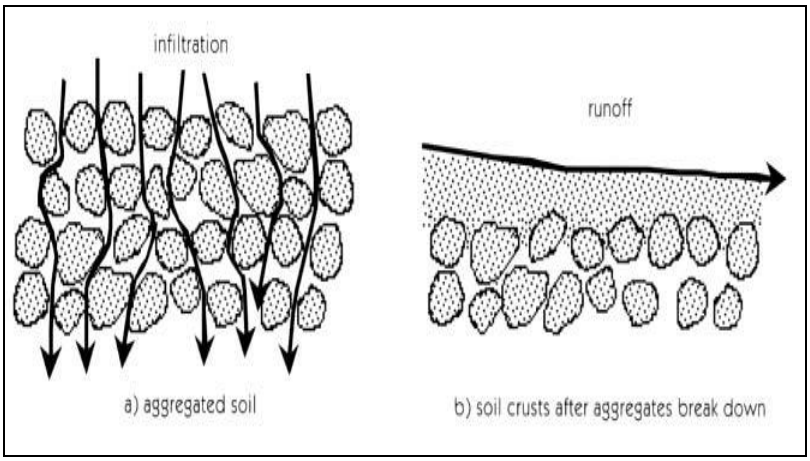

Figure 1.4. Difference between soil aggregation and soil dispersion [32]

In contrast to surface crusts, elevated sodium accumulation at relatively lower soil depths encourages the appearance of ‘hard setting’. Which, as a consequence enhances soil bulk density, thus leading to poor soil structure [34]. Development of bulk, high strength, no aerated and poorly drained soil with high sodium concentration decrease the overall potency of that soil to favor plant growth [35].

1.1.2. Chemical Parameters of Soil as Influenced by Salinity and Sodicity

Not only does organic matter play an important part in the formation of soil structures, but also serves as a nutrient and a water reservoir in the soil. Due to its limited supply and high losses, soils rich in salinity and sodicity are poor in organic matter [36]. Surface crusts and sealing results in substantial erosive losses of organic matter in such soils [35]. The type of salts found predominantly in the soil contributes to the determination of soil organic matter (SOM) solubility. Moreover, polyvalent cations like Ca^{2+} in the soil solution may bind negative loaded clay particles and organic compounds [37]. Thus, the existence of multivalent cations can boost soil particles sorption of organic matter, [38, 39] thus reducing the availability of organic matter for decomposition [40].

Affected by salt toxicity, carbon (C) inputs are usually less in salt-affected soils due to a decline in vegetation growth, differences in osmotic potential and degraded soil structure [41]. A considerable reduction in organic soil carbon and total nitrogen (N) content was observed by Chander *et al*. because of irrigating soils with sodic water [42]. Furthermore, McClung and Franken Berger, reported a considerable reduction in enzyme activities and C and N mineralization in high salinity levels [43]. According to their study increasing salinity up to 20 dSm^{-1} resulted in reduced rates of nitrification and enhanced losses of ammonia by volatilization.

The rate of nitrification is also strongly affected by the source of salinity (e.g., NaCl, Na_2SO_4), with NaCl salts greatly inhibiting nitrification [44]. Similarly, reduced N mineralization and increased gaseous NH_3 losses with increasing salinity are found by Gandhi and Paliwal [45]. Pathak and Rao also reported decreased mineralization of C and N with increased salinity and alkalinity in arid soils amended with organic residues [46]. However, Nelson *et al*. reported a decrease in C mineralization with enhanced salinity, but sodicity, on the other side, improved C decomposition owing to organic matter

solubilization [47]. Increasing salinity leads to decreased soil enzymatic activities that play a major part in C, S, P and N cycles [48].

Saline-sodic soils, mostly have high EC, ESP, and SAR. Further, these soils also own high levels of carbonates (CO_3) and bicarbonate (HCO_3) salts resulting in high pH. High soil pH gives rise to high osmotic pressure makes it hard for crops absorb soil water. Moreover, salt-affected soils with high pH have more deleterious effects on microelement accessibility, for example Cu, Fe, Mn, and Zn. These soils also have a high macronutrient (N, P, and K) deficiency.

1.1.3. Soil Microbial Properties as Affected by Salinity and Sodicity

The soil is a dynamic natural body, housing ample biological diversity; with a novel genetic profile, where the huge number and a variety of microbiota can be found serving as a nutrient reservoir. Changes in soil chemical conditions have an adverse effect on the ecology and biochemical processes of soil organisms [49]. A considerable reduction in microbial growth and activity can be seen due to osmotic stress, dehydration, and lysis of cells induced by increasing salinity [50].

Not only the Na^+ toxicity, but the concentrations of other ions (CO_3, HCO_3 and Cl^-) up to toxic levels, limited availability of nutrients like Ca^{2+} and potential loss of soil organic matter, all collectively discourage the microbial population and their activities in saline-sodic soils [35, 51].

Soil salinity effects on microbial growth and dynamics are reported by many researchers [52-54]. Rietz and Haynes proposed that salinity and sodicity caused by irrigation ended up in a lesser, more stressed and metabolically less efficient microbial community [55]. Also, in the same study, a significant linear reduction in extracellular enzyme activities was found with increasing EC, ESP and SAR.

A study conducted by Wichern *et al*. found fungal communities more susceptible to salt stress than bacterial ones [56]. The negative impact of increasing salinity and sodium on soil microbiota is also well documented in some other studies [57, 58]. Microbes play a significantly active part in the transformations of organic matter. A significant reduction in

saline soil microbial activity can cause piling up of non-decomposed organic matter, which in turn will contrastingly affect the sub sequential nutrient release for seedling growth [56].

Various scientists revealed that in soils affected by salinity there were either no or very limited organic matter retention, reduced microbial activity and mineralization of nitrogen [55, 58]. Yuan *et al*. found lowest organic carbon content with the highest salinity [58].

An enhanced metabolic quotient (respiration per unit biomass) with enhanced salinity and sodicity has also been reported, indicating a more stressed microbial community [52]. Soil biological activities and intensity of biochemical reactions are crucially important in maintaining the soil ecological functions, besides soil aggregates formation and stability to modify the soil structure [50, 59].

1.2. SALT AFFECTED SOILS RECLAMATION

1.2.1. Organic Amendments in Redemption of Salt Affected Soils and Improving Soil Health

For the reclamation of saline-sodic soils, irrigation water, abundant in divalent cations and chemical amendments containing Ca^{2+} are used for the replacement of Na^{+} ions on the cation exchange complex.

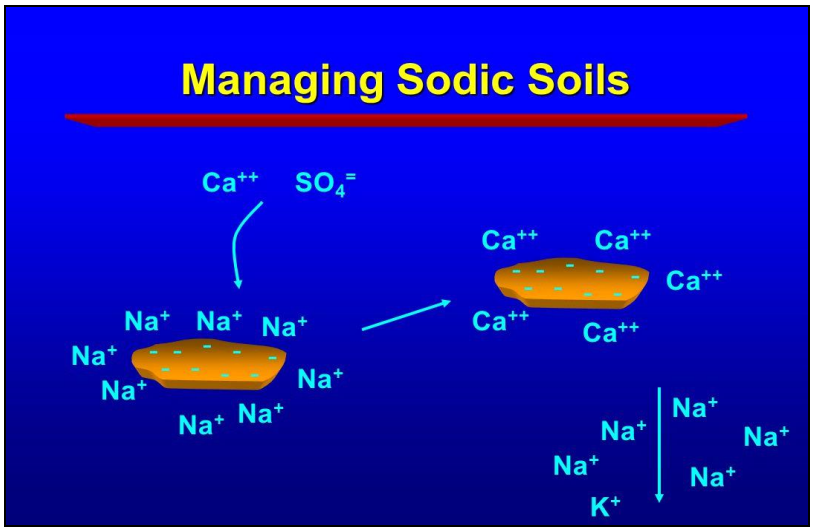

Figure 1.5. Management of sodic soils [60]

In case of dry soils with pH on the higher side, application of soil amendments in combination with organic residues is a good way to manage saline-sodic soils [35]. The efficacy of different organic amendments like compost, manures and mulch for reclamation of soils is a focus of researchers now for their soil remediation efficiency. Various kinds of organic amendments act differently within the soil structure, but the most common effect is to enhance soil aggregation [35].

Salty soils are mostly depleted in organic matter due to their low production capacity and associated low organic matter inputs. The little organic matter that they hold is highly susceptible to losses either by erosion or microbial decomposition [35]. Promoting clay mineral flocculation can be an important erosion control strategy in said soils. A combined application of organic inputs, in this regard, can lead to considerable flocculation thereby forming a plenty of soil aggregates [60]. As a result of enhanced soil structure, soil physical (porosity and water holding capacity) and hydraulic properties (infiltration rate and hydraulic conductivity) are improved, thereby lowering the impact of drought [61].

Use of organic matter in salt soils is highly beneficial as it reduces electrical conductivity and exchangeable sodium percentage while increases water holding capacity, aggregate stability and salt leaching [18]. Moreover, an increased organic matter in the topsoil layer can be helpful in absorbing raindrop energy and improving the water infiltration rate, thereby reducing the overland flow and erosion [62]. An experiment on tomato crop by Lax *et al*. [63] in soil irrigated with saline water concluded betterment in physical soil properties, with the municipal solid waste application. With an improved cation exchange capacity (CEC), the addition of organic matter in salt-affected soil encourages the Ca^{2+} exchange over Na^{+}, as a result, sodium is leached down.

A direct link between inputs of organic matter and a decrease in bulk density of soil results in improved soil porosity and, in turn leading to enhanced saline water leaching. Wang *et al*. in this context, found that a blend of organic byproducts considerably reduced soil EC, bulk density and ESP by 87 percent, 11 percent and 71 percent, respectively, while organic carbon and total porosity increased by 96 percent and 25 percent respectively [64]. Above all, these findings strongly encourage the potency of combining various organic soil amendments for the redemption of salt-stressed soil.

Sodic soils have motivated scientists around the globe to explore feasible alternative organic ameliorations that could boost soil organic matter and stimulate microbial activity by providing energy substrates, so the structural properties of the soil are improved to the extent that could lead to desodification [65]. As a consequence, some organic matter ameliorates has been studied more successfully, ranging from cottage cheese whey to green manure or pig bedding litter. However, there is very little work done to explore the ameliorative significance of biochar in salt-affected soils.

1.3. BIOCHAR AND ITS HISTORY

The term ‘biochar’ is rather new even to soil scientists, however, its concept roots back to an ancient tradition of native Amazonian. Their production method involved igniting the biomass in deep earth pits, under limited oxygen environment to make charcoal. The addition of this carbon-rich charcoal to the soils over a period of many years created “Terra Preta”, or black earth, which has served the Amazonians needs for centuries and still stable after hundreds of years [66]. Terra Preta soils are rich in most of the soil fertility measures such as cation exchange capacity, macro (C, N, P, K^{+}, Ca^{2+} and $Mg^{2+)}$ and micronutrients (Mn, Zn and Cu), as well as stable organic matter [67, 68].

Figure 1.6. Soils of Terra Preta [67]

The pH of these soils is neutral compared to the neighboring acidic soils, which low in nutrients and organic matter are considered incapable to support agriculture [67, 69]. Almost similar soils can be found around the globe, and their high fertility is consistently associated with an abundance of black carbon [70].

The charcoal is being used to improve soil status and in turn promote agriculture productivity for centuries [71]. In the recent years, the tradition of using charcoal has been modernized by means of pyrolysis or gasification systems to heat liquid or solid biomass in temperatures below zero or under low oxygen conditions [72]. The resulting solid product is termed as 'biochar' and has been greatly used in a wide range of different areas [73].

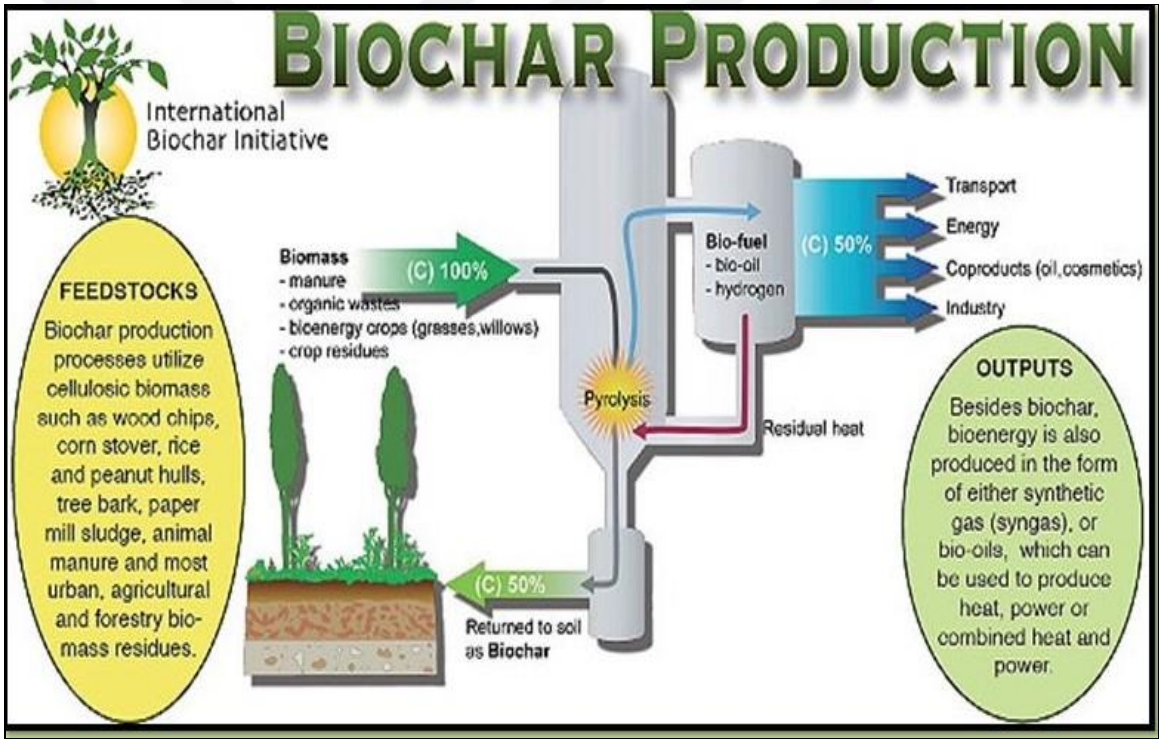

Figure 1.7. Production of biochar [69]

1.3.1. Biochar in Carbon Sequestration: A Strategy for Reducing Greenhouse Gas Emissions and Global Warming

Soil organic matter (SOM) occupies a vital position in the global carbon cycle for its importance in climate regulation and ecosystem functioning. Agro-ecosystems emit

significant amounts of GHGs to the atmosphere either directly or indirectly by 1) combustion of fossil fuel during the manufacture of synthetic fertilizer, Agrochemicals and on-farm machinery operations, 2) changes in land use and 3) microbial mediated processes such as decomposition of soil organic matter (SOM), nitrification and denitrification [73].

At present, CO_2 in the atmosphere represents the highest concentration during the last 650,000 to 800,000 years [74]. Similarly, N_2O concentration in the atmosphere has been raised by 20 per cent since last century and is further rising at a rate of 0.2–0.3 percent yr^{-1} [75]. On an average, agricultural soils are reported to contribute about 20 percent to the total emission of CO_2, 60 percent of the anthropogenic N_2O and 12 percent of CH_4 [76].

However, the soils, on the other hand, could serve as a sink for atmospheric CO_2 at low-to-no cost ratio [77]. Therefore, efforts aiming at reducing CO_2 concentration in the atmosphere are the burning issues of the day [76], and scientists and policymakers are looking for ways to reduce or reverse the trend i.e. from the atmosphere to the soil. An important option to cope with climate change [77] and reducing CO_2 emissions from soils is the sequestration of atmospheric CO_2 into soil C pools [78].

Biochar has appeared in latest years as a potential organic amendment to sustain soil productivity and an effective approach to blunt global warming [79]. Usage of biochar as a strategy to minimize climate changes depends on its capacity to release CO_2 slowly from an organic carbon source to the atmosphere and its resistibility to microbial decay [68, 80].

Researchers have estimated that mean biochar residence time in temperate soils is between 1000-2000 years, whereas fresh organic matter may be degraded in less than a decade [66]. Amending soils with biochar is an effective technique to increase soil carbon storage [81]. Pyrolyzing waste products sequester approximately 50 percent of carbon compared to traditional slash-and-burn techniques, which sequester only 3 percent, and natural decomposition, which holds 10– 15 percent [66].

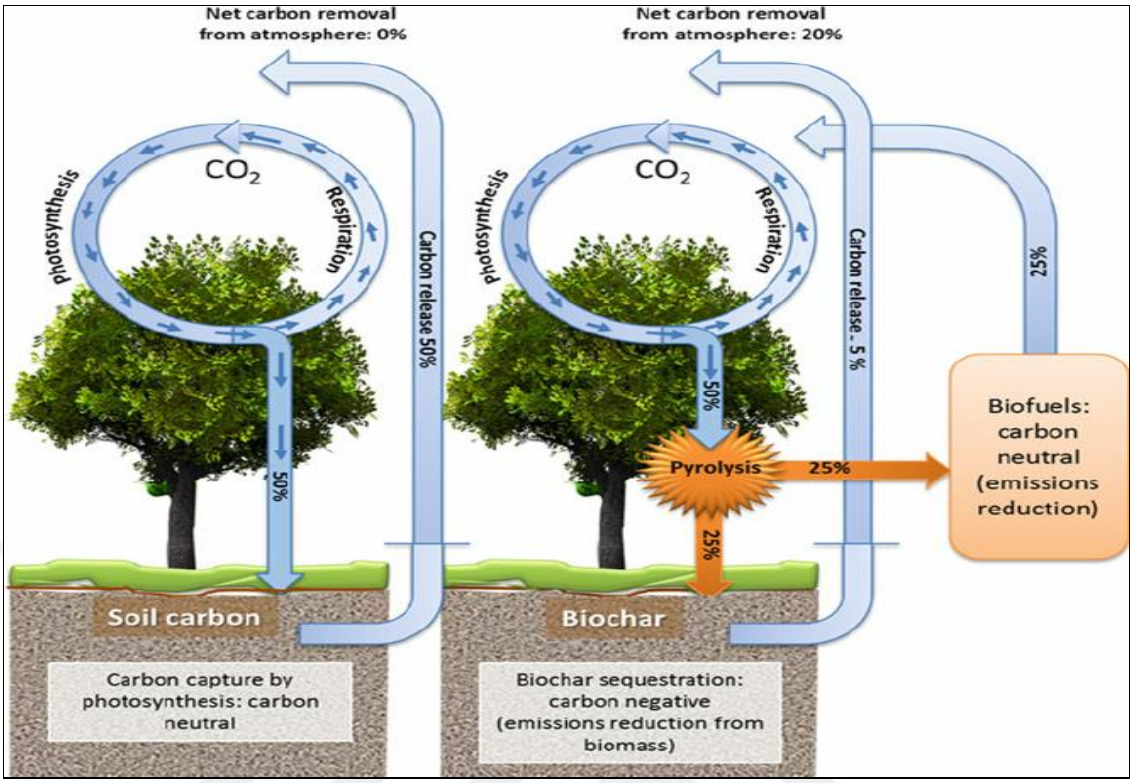

Figure 1.8. Role of biochar in carbon sequestration [69]

A conservative assumption is that biochar is capable of offsetting worldwide carbon emissions and 2.2 gigatons of carbon can be stored annually by 2050.or probably by 2030. Hence, sustainable biochar produced for all types of bio-wastes is a powerful, simple tool for land and waste management while combating climate change by providing negative carbon system.

1.3.2. Biochar as Soil Modifier

Biochar is produced of organic by-products heated under an oxygen-limited environment (pyrolysis) and is eminent from charcoal because of its use as a soil conditioner [72]. Agricultural benefits of using biochar are well documented in many studies demonstrating the significant betterment in overall soil quality by changing soil physiochemical and biotic properties, thereby enhancing agriculture productivity [82, 83].

Figure 1.9. Agricultural benefits of using biochar [69]

Biochar is a highly porous organic material with variable charge and high surface area that when applied to soil increases its cation exchange capacity (CEC) [84, 85]. It is evident from the fact that even in comparison with whole soil, clay or organic matter, the inherent cation exchange capacity of biochar alone is consistently higher [86]. Biochar is highly stable, and its mineralization is very slow within the soil compared with plant litter that shows its stability towards degradation [87].

The biochar has been highly recognized as a way to minimize changes in climate by playing a role in improving sequestration of carbon, the fertility of the soil and other roles for the betterment of the ecosystem [85, 88-93]. Biochar application's positive impacts on soil fertility include better nutrient retention through adsorption of cations [94] and an increase in acid soil pH [95-97]. It has also been observed that the addition of biochar shifts the biological community's structure and relative abundance within the soil [98-101]. Biochar not only changes the soil microbial population, but also regulate the plant growth, organic matter and nutrient cycling patterns by improving soil fertility [96, 102-104]. Stability of biochar depends upon the composition of commodities used for its production, metabolic processes involved and sort of organisms going to do its degradation [105].

Another signification of improved nutrient stability is enhanced fertilizer use efficiency (FUE). As with nitrogen, higher FUE results in either reduced farmers' costs or better yields for a given application rate of fertilizer. Nitrogen availability is a major crop-limiting factor, with N fertilizers that represent a high cost to farmers. In the Amazon of Brazil, Steiner *et al*. observes greater crop N use efficiency in the modification of acidic

soils when amended with 11 tha^{-1} wood biochar over two years [106]. The yield of wheat could be improved at lower application rates of fertilizers, with the band application of biochar at 1 tha^{-1} [107]. Additionally, nutrient retention also reduces runoff and nitrous oxide emissions, hence minimizing some of the detrimental environmental risks associated with fertilizer use.

Studies regarding biochar supplements to the soil have shown betterment in soil physical properties like aggregate stability, hydraulic conductivity, bulk density, porosity volumetric water content and water holding capacity [197, 103, 108-110]. For example, an increase in the macro aggregation of soils with 5 percent biochar was reported by Jien and Wang [110] as a consequence, the saturated hydraulic conductivity of extremely weathered soil (Ks) improved. Furthermore, a twofold increase in soil Ks is reported upon biochar addition at 16 tha^{-1} [111]. Hardie *et al*. also concluded a rise in the field saturated hydraulic conductivity with increased soil macro aggregation when treated with biochar [112]. Improved soil aggregation upon biochar additions is also documented by some other scientists [113-118].

Until today, most studies in non-saline soils have confirmed the potential of biochar addition. Research on its use in reclaiming degraded soils, primarily salt-affected soils are scarce, however. Improvements in physical properties of these soils are believed to be very important for saline-sodic soil reclamation. Additionally, augmenting divalent cations such as Ca^{2+} and Mg^{2+} in a saline-sodic soil is crucial to negate Na^{+} on the exchange sites. Major *et al*. in an experiment observed increased Ca^{2+} and Mg^{2+} availability when biochar was applied to a Colombian savanna oxisol at 20 tha^{-1} [119]. Furthermore, in an Iowa study Laird *et al*. observed a substantial rise in soil Ca^{2+} status, when an oak-derived biochar at 20 g Kg^{-1} of soil was amended to an agricultural soil in the west [90]. The potential of biochar in increasing divalent cation concentrations in the soil is also well documented by some other researchers [120, 121]. Thus, biochar could be a significant source of these cations [122] potentially helping to remediate saline-sodic soil.

Various scientists have revealed that addition of biochar to soils is highly beneficial, it not only improved the yield but also helped plants to withstand stresses induced by pesticides [123] heavy metals [124] and toxic compounds or drought [125, 126]. The lastingness of biochar in the soil is highly advantageous for cleanup of contaminated soils in contrast to other organic amendments that undergo degradation more promptly [127].

As a significant factor of plant stress, salinization adversely restricts plant growth and productivity [128-130]. Charcoals have long been known to sorb a variety of salts [131] and is also used in processes of industrial desalination [132].

A two-year field experiment was conducted by Lashari *et al*. to assess the potential of using biochar poultry manure compost (BPC) and pyroligneous solution (PS) to modify salt stressed soil and to improve crop production in dry croplands [133]. Results showed a substantial reduction in soil salinity by 3.6 g kg^{-1}, soil pH by 0.3 and soil bulk density by 0.1 g cm^{-3} after a first crop year. However, an increase of 2.6 g kg^{-1} and 27 mg kg^{-1} was noted in term of SOC and available phosphorus, respectively. The yield was enhanced by many folds (38 percent) over the control in both years. Moreover, the plots treated for two years exhibited a greater decrease in soil salinity, soil pH, and bulk density over those treated for one year. Yield, however, did not differ significantly between the two successive years with the drought in the second year.

Similarly, Wu *et al*. in a 56d incubation experiment evaluated the effectiveness of furfural and its biochar on the saline soil's overall characteristics [134]. Their research concluded that both furfural and its biochar markedly lowered pH and soil exchangeable sodium percentage (ESP), enhanced soil organic carbon (SOC) content and cation exchange capacity (CEC), and increased the phosphorus availability (P) in the soil.

Thomas *et al*. in a study addressed the strength of biochar to alleviate salt stress on two broadleaved herbaceous plant species (Abutilon theophrasti and Prunella vulgaris). Results of their study concluded that biochar application at 50 tha^{-1} as a top dressing, perfectly mitigated salt-induced mortality in A. theophrasti and prolonged survival of P. vulgaris [135].

These results confirm the use of biochar in ameliorating salt-induced stress on plants through salt sorption, thereby recommending the biochar use to mitigate adverse effects of salinization on productive lands effectively. Thus, to reclaim a salt-affected soil, it is essential to assess whether biochar can be an impending organic soil modification. Also, it is imperative to know the functioning system of the biochar when applied to a salt-affected soil, either physiochemical or biological, given its high recalcitrant C content.

1.4. HALOTOLERANT AND THEIR ROLE IN SOIL SALINITY/SODICITY AMELIORATION

Strategies to minimize adverse impacts of salt stress on crops include soil reclamation, development of salt resistant genotypes, growing halophytes and later removal of salt accumulating aerial parts of lower salt contents of the soil as well as leaching down salts from the surface to downward [136].

Another approach minimizing salt stress is to inoculate seedlings or seeds of a crop with plant growth promoting bacteria (PGPBs). Both intracellular and endocellular microbes colonize the plants in their natural habitats [137]. Rhizosphere microbial community, including both bacteria and fungi, is better known for their ability to enhance crop output under salt stress environments [138].

Use of microbial inoculants to counteract salinity stress is a sound option over developing salt-tolerant crops, which is not only time-consuming, but a difficult and uneconomical strategy for sustainable agriculture [139]. Various studies have shown that the halotolerant PGPBs have beneficial effects under saline conditions on production and physiological efficiency of maize, wheat, rice, peas, tomato, pepper, canola and other agronomic and horticultural crops [140-142].

Plant growth promoting bacteria increases plant efficiency under salt stress by the production of Osmoprotectants, hydraulic conductance and presence of Aminocyclopropane-1-Carboxylate (ACC). Thus, lowering ethylene production and translocation of Na^+ ions, increasing the biosynthesis of antioxidative enzymes, stomatal conductance and photosynthetic activities (Figure 1.10) [143].

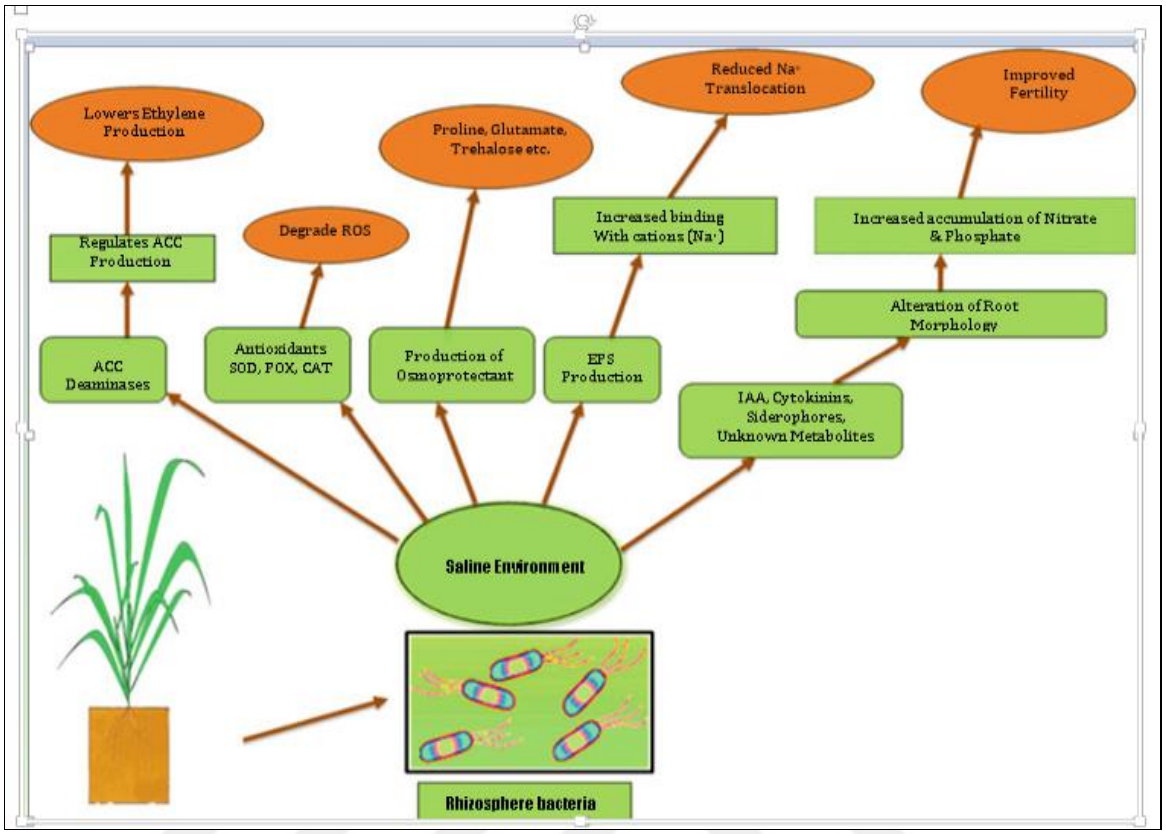

Figure. 1.10. Improvement in salt tolerance and survival of plants in saline environment induced by plant growth promoting rhizobacteria [143]

Increased salinity results in the decreased reduced osmotic potential of soil solution in the rhizosphere leading to limited availability of water to crops [144]. Under salinity stress, most of the plant's energy is consumed to make osmotic adaptations resulting in limited plant growth and yield [2].

Moreover, excess production of ethylene as a result of salinity stress limits root proliferation and, in turn, plant growth. The use of Rhizobium strains having ACC deaminase activity is considered as one of the best strategies to minimize the impact of salt-induced ethylene on crops. These plant growth promoting bacteria capable of producing ACC deaminase, regulate accelerated ethylene production to counteract biotic and abiotic stresses and encourage the seedling growth under stress environments.

Mayek *et al*. noticed that salt tolerant Achromobacterpiechaudii, capable of producing ACC deaminase improved the growth parameters of tomato plants grown in a solution of 172 mM NaCl [145]. Samiyappan concluded from his experiment that Pseudomonas

fluorescent having ACC deaminase activity led to an increased salt resistance in groundnut plants [146]. Moreover, it also improved yield in comparison to those inoculated with Pseudomonas strains without ACC deaminase activity. Likewise, Cheng *et al*. have confirmed increased yield of canola in a saline environment, when inoculated with bacteria producing ACC deaminase, via lessening the salt stress-induced ethylene synthesis [147].

To survive successfully under continuously changing conditions, a microorganism must be able to sense this change and act accordingly. Gutierrez-Manero *et al*. in his study found that a PGP bacterium, Chryseobacterium balustinum promoted germination rate, enhanced the root surface area and improved nitrogen uptake and nitrogen fixation in Lupinus Albus seedlings under saline environment [148]. Ramadoss *et al.* evaluated the potential of five halotolerant strains to alleviate the salt stress in wheat seedlings [149]. The study concluded that the strains increased root elongation of wheat seedlings by 71.7 percent compared to uninoculated control.

Seed co-inoculation with different species of PGPB, such as *Rhizobium* and *Azospirillum* might be a worthwhile approach to alleviate the harmful effects of salt stress on crops. Binary inoculation of *Rhizobium* and *Azospirillum*, as well as plant growth promoting bacterial strains exhibited increased total nodule mass of various leguminous plants, acetylene reduction activities, and macro and micronutrient contents when compared to the sole inoculation with *Rhizobium* [150-152]. Similarly, a marked improvement in Osumi soybean, in term of root growth and number of nodules has been observed in the saline environment, with dual inoculation of *Sinorhizobiumfredii* and *C. balustinum* [153].

To overcome the adverse effect of salinity stress on crops, soybean cultivar was inoculated with *Bacillus subtilis* and *Sinorhizobium proteamaculans* along with *Bradyrhizobium japonicum*. Under salinity stress, total dry weight was increased by 10 percent in all pots receiving PGPB isolates compared to the control where an increase of 3.5–4.5 percent was observed [153]. An almost same trend was noticed by Han and Lee, in their study, where seed inoculation with PGPB strains, *Serratia sp*. and *Rhizobium sp*., resulted in an enhanced antioxidant status, photosynthesis, mineral content and lettuce crop growth under salt stress [154].

Ashraf *et al.* documented the potential of native salt resistant bacterial isolates *Aeromonas hydrophilia*, *Bacillus insolitus*, and *Bacillus sp*) capable of producing exopolysaccharide in

providing a “blanket salt-tolerant cover” to inoculated wheat roots, via modifying roots surrounding soil [155]. An experiment was carried out by Ahmad *et al*. to assess the potency of auxin producing PGPR carrying ACC deaminase activity for enhancing osmotic stress tolerance index in mung bean [156]. Results revealed up to 1.4- and 1.9-fold increase in the total dry matter with a single application of *Rhizobium* and *Pseudomonas strains*, respectively. Whereas co-inoculation of *Rhizobium* and *Pseudomonas* strains, on the other hand, resulted in up to 2.2-fold increase in the total dry matter.

A good number of halotolerant bacterial isolates, *including Rhizobium, Bacillus, Azospirillum, Pseudomonas, Flavobacterium, Vibrio, Micrococcus, Alteromonas, Escherichia coli* and certain *Acetobacter* can successfully sustain their plant growth potential even at elevated salinity levels, thereby allowing plants to overcome stress effects [157, 158].

Approximately 4.3 million hectares of farmland area in Turkey is deteriorated, with 1.5 million hectares as arid and 2.8 million hectares as saline-alkaline. Saltwater intrusion from seawater as in case of Bafra plain and other coastal line of Turkey is also an important cause for soil salinity which increases the groundwater EC from 4.3 to 8.1 dSm^{-1} [159], resulting in the salinization of 53 percent of coastal regions throughout the globe [160].

Taking into account the positive impacts of rhizosphere microbes on a variety of agricultural crops, planted under salinity environment, and the significance of biochar in improving overall soil quality, subsequently enhancing agriculture productivity, we propose that combined use of halotolerant PGPBs and biochar might be an economical and effective strategy to ameliorate salt-affected soils and to improve crop productivity.

1.5. THE STUDY OBJECTIVE

This research was carried out to assess the effectiveness of two plant materials (Cotton stalk and Olive pulp) processed as biochar in the redemption of saline-sodic soil and their subsequent impact on soil nutrient status, crop growth, yield and physiological processes when used in conjunction with halotolerant PGPBs.

2. MATERIALS

2.1. CHEMICALS AND REAGENTS

- Phosphate buffer solution (PBS) (Sigma Aldrich, Germany)
- Tryptic soy agar (TSA) (Sigma-Aldrich Corporation, Germany)
- Tryptic soy broth (TSB) (Sigma-Aldrich Corporation, Germany)
- Nutrient agar (NA) (Sigma-Aldrich Corporation, Germany)
- Minimal salts (M9) medium (Sigma-Aldrich Corporation, Germany)
- 1-Aminocyclopropanecarboxylic acid (ACC)
- Crystal violet (Sigma-Aldrich Corporation, Germany)
- Gram's iodine (Sigma-Aldrich Corporation, Germany)
- Ethyl alcohol (Sigma-Aldrich Corporation, Germany)
- Safranin O (Sigma-Aldrich Corporation, Germany)
- Starch agar (Thermo Fisher Scientific)
- Iodine (PubChem)
- Skim milk agar (Sigma-Aldrich Corporation, Germany)
- Peptone water (Sigma-Aldrich Corporation, Germany)
- Nessler's reagent (Sigma-Aldrich Corporation, Germany)
- Christensen's urea agar (Sigma-Aldrich Corporation, Germany)
- Modified pikovskaya's agar (Sigma-Aldrich Corporation, Germany)
- Hydrogen peroxide solution (Sigma-Aldrich Corporation, Germany)

- Ammonium phosphate monophosphate ($NH_4H_2PO_4$) (Sigma-Aldrich Corporation, Germany)
- Ferrous sulphate (PubChem)
- Diphenylamine (PubChem)
- Sodium acetate trihydrate ($CH_3COONa.3H_2O$) (PubChem)
- Acetic acid (CH_3COOH) (PubChem)
- Ethanol (C_2H_5OH) (PubChem)
- Ammonium hydroxide (NH_4OH) (PubChem)
- Kjeldhal tablets (PubChem)
- Calcium chloride ($CaCl_2$) (PubChem)
- Urea (PubChem)
- Diammonium phosphate (DAP) (PubChem)
- Potassium sulphate (K_2SO_4) (PubChem)
- Acetone (PubChem)
- Nitric acid (HNO_3) (PubChem)
- Perchloric acid ($HCLO_4$) (PubChem)
- Tween 80 (PubChem)

2.2. INSTRUMENTS

- Centrifuge (Hettich micro 22R and Sigma 2-5 centrifuge, Germany)
- Vortex (Stuart, UK)
- Laminar flow cabinet (ESCO Lab culture Class II Biohazard Safety Cabinet 2A, Singapore)

- pH meter (Hanna, Germany)
- Incubator
- Shaker
- Hot plate
- ICP
- Conductivity meter
- Spectrophotometer
- ED-5 Wiley mill (Arthur H. Thomas Co)
- Digestion chamber
- Kjeldhal apparatus

3. METHODS

3.1. EXPERIMENTAL SETUP

The study comprised of two sets of experiments involving the biochar types, application rates and their combination with halotolerant (Table 3.1, 3.2), and was conducted in Genetics and Bioengineering Department, Engineering and Architecture Faculty, Yeditepe University, Istanbul Turkey.

Table 3.1. Cotton stalk biochar treatments

	Treatments	**Description**
Experiment 1-A	T1	No biochar (Negative control)
	T2	Cotton stalk @10t ha^{-1} (Positive control)
	T3	Cotton stalk @20t ha^{-1} (Positive control)
	T4	Cotton stalk @10t ha^{-1} + Halotolerant (Positive control)
	T5	Cotton stalk @20t ha^{-1} + Halotolerant (Positive control)
	T6	Cotton stalk biochar (CB) equivalent to @10t ha^{-1}
	T7	Cotton stalk biochar (CB) equivalent to @20t ha^{-1}
	T8	No biochar + Halotolerant (Halotolerant control)
	T9	Cotton stalk biochar (CB) equivalent to @10t ha^{-1} + Halotolerant
	T10	Cotton stalk biochar (CB) equivalent to @20t ha^{-1} + Halotolerant

Table 3.2. Olive pulp biochar treatments

	Treatments	Descriptions
Experiment 1-B	T1	No biochar (Negative control)
	T2	Olive pulp @10t ha^{-1} (Positive control)
	T3	Olive pulp @20t ha^{-1} (Positive control)
	T4	Olive pulp @10t ha^{-1} + Halotolerant (Positive control)
	T5	Olive pulp @20t ha^{-1} + Halotolerant (Positive control)
	T6	Olive pulp biochar (OB) equivalent to @10t ha^{-1}
	T7	Olive pulp biochar (OB) equivalent to @20t ha^{-1}
	T8	No biochar + Halotolerant (Halotolerant control)
	T9	Olive pulp biochar (OB) equivalent to @10t ha^{-1} + Halotolerant
	T10	Olive pulp biochar (OB) equivalent to @20t ha^{-1} + Halotolerant

3.1.1. Soil Collection/Selection

The soil to be used in the project has been collected from the Konya plain saline-sodic soil. Top 0-15 cm soil was collected and was brought to the laboratory. In order to make the soil free of coarse rock and plant material, soil was sieved through 4 mm mesh screen, mixed well to make it uniform and stored at 4 °C until use (not more than two weeks' time). About ½ kg of soil sample was taken and sieved through 2 mm mesh screen to analyze for physicochemical properties of the soil (Table 3.3). The rhizospheric soil sample was also collected in a sterilized polythene bag and was then transported to the laboratory and stored at 4 ˚Ċ in the fridge until use.

Table 3.3. Pre-soil physiochemical properties

Soil parameters	
Soil total N (g kg^{-1})	7.2
Soil available P (mg kg^{-1})	1.18
Soil available K (mg kg^{-1})	98
Organic matter (%)	0.68
Soil Ph	10.26
EC (dSm^{-1})	10.3
Sand (%)	40.3
Silt (%)	34
Clay (%)	25.7
Texture class	Sandy loam

3.2. ISOLATION, IDENTIFICATION AND BIOCHEMICAL ANALYSIS OF BACTERIAL STRAINS FROM THE RHIZOSPHERE OF SALT AFFECTED SOIL

3.2.1. Bacterial Isolation and Purification

Bacterial isolation was conducted by thoroughly mixing about 1g soil in sterile 1x PBS solution in order to prepare the suspension. Serial dilutions were prepared up to 10^{-7}, 100 µL of each dilution was spread on tryptic soy agar (TSA + 10 g NaCl) plates. Serial dilutions up to 10^{-7} were prepared, 100 µL aliquot of each dilution was spread on tryptic soy agar (TSA + 10g NaCl) plates and incubated at 28 ± 2 °C for 24 hours (Figure 3.1). For further purification, individual bacterial colonies were selected and streaked on the same media (Figure 3.2). Based on their morphological properties, 5 different colonies were isolated and were screened for their biochemical characters.

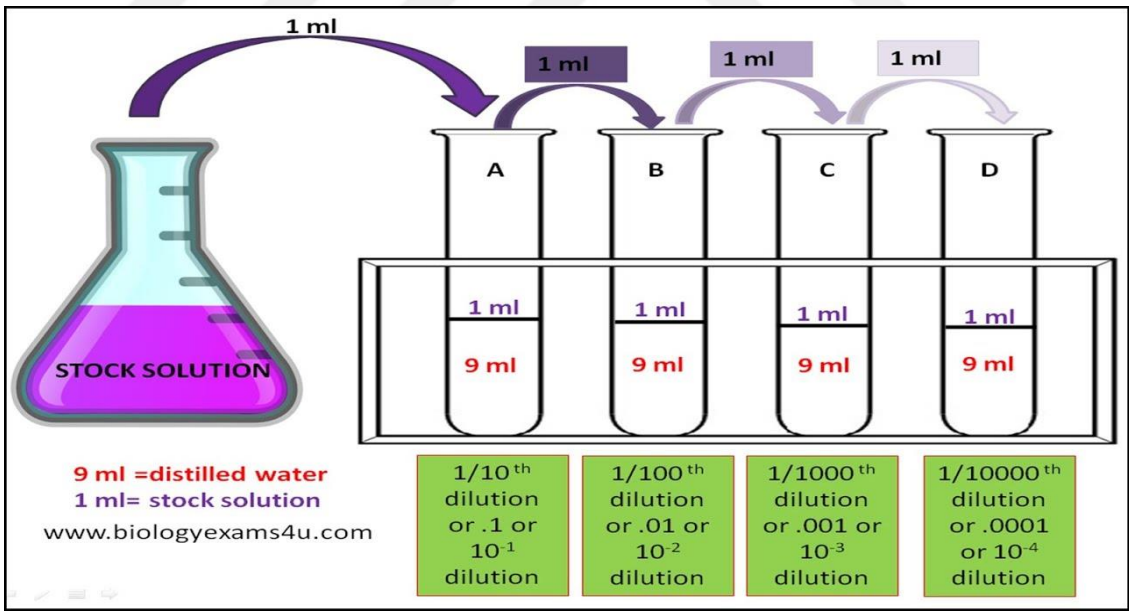

Figure 3.1. Serial dilution method [162]

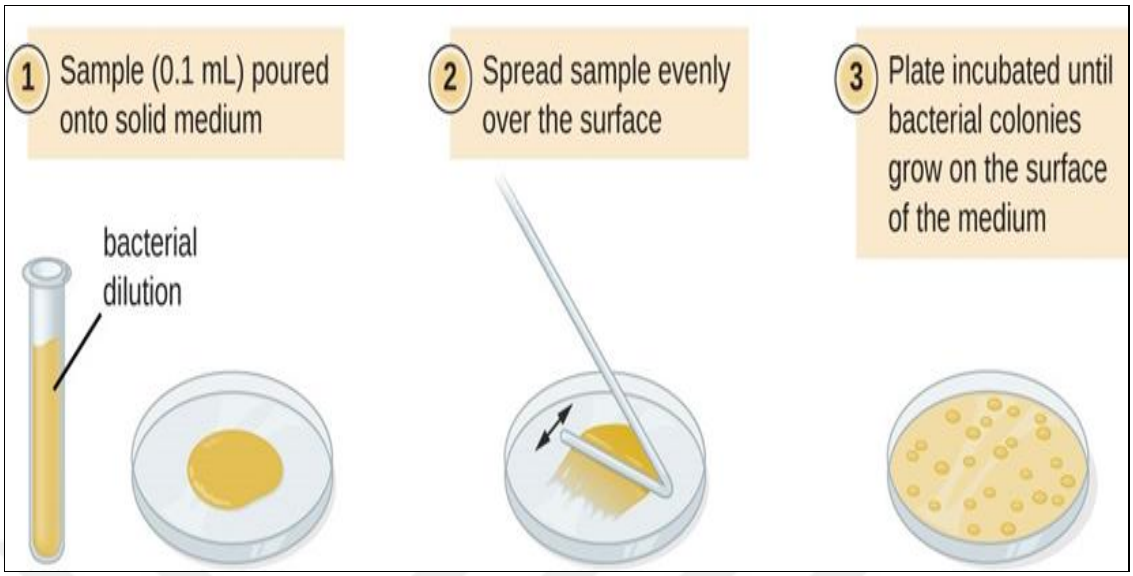

Figure 3.2. Spread plate method [162]

3.2.2. Gram Staining of Bacteria

For gram staining, a uniform heat fixed bacterial smear was transferred to a glass slide and was left to dry. The slide was flooded with crystal violet for a min and was drained with water thereafter. The smear was then flooded with iodine mordant in order to fix the crystal violet dye in the cells. A decolorizing agent, 95 percent ethanol was used to destain the smear. Ethanol rinsed away the crystal violet from the gram - negative cells, but not gram - positive cells. Safranin was used to counterstain the cells to view gram - negative bacteria. Gram-positive cells appears as purple while gram-negative cells are red when observed under light microscopy [162].

3.2.3. Bacterial Identification by Fatty Acid Methyl Ester Analysis (FAME)

3.2.3.1. Harvesting

Using a sterile 4 mm plastic inoculating loop, cultured cells from the five freshly grown plates were harvested by gently softly scraping the culture medium's surface. A clean, dry

13 mm x 100 mm screw cap culture tube was taken and the loop was wiped off the cells inside the tube.

3.2.3.2. Saponification

About 1.0 ± 0.1 ml of the methanolic base, reagent 1, was added into each of the batch culture tubes. Tubes were sealed with clean screw caps and after vortexing for 5-10 seconds, the batched sample tube rack was put in a water bath at 95 ºC -100 ºC. The tubes were removed after five minutes, vortexed again and then continued heating for another 25 minutes in the water bath. After the set time, the tubes were removed and cooled in a cold water pan.

3.2.3.3. Methylation

Each tube in the batch was uncapped, then 2.0 ± 0.1ml of the methylation reagents, reagent 2, was added to each tube. Tubes were capped tightly, the solution was vortexed for 5-10 seconds, and heated for 10 minutes in an 80 ± 1 ºC water bath. The tubes were then quickly removed and cooled down to room temperature following the process mentioned earlier in step 2.

3.2.3.4. Extraction

In order to remove fatty acid methyl esters from the acidic aqueous phase and transfer it to an organic phase, 1.25 ± 0.1 ml of Reagent 3, the extraction solvent was added to each tube. Then tightly sealed tubes were placed in a rotator and for 10 minutes gently mixed end-over end.The lower aqueous phase of the sample was removed and discarded using a clean Pasteur pipette for each sample.

3.2.3.5. Base Wash

Approximately 3.0 ml of Reagent 4, a dilute base solution was introduced to the sample preparation tubes to remove free fatty acids and residual reagents from the organic extract.

Tubes were tightly capped and end-over-end rotated for 5 minutes. A brief three-minute centrifugation was performed at 2000 rpm to clarify the interface between the phases of an emulsion. Sampler vials were labelled for extract identification. Approximately 2/3 of the organic (upper) phase was taken separately from all the tubes and transferred to clean GC sample vials each time using a clean Pasteur pipette. It was taken great care not to transfer any of the lower (aqueous) phase to the auto sampler vial. Sampler vials were then capped tightly and loaded to the automatic liquid sampler [163].

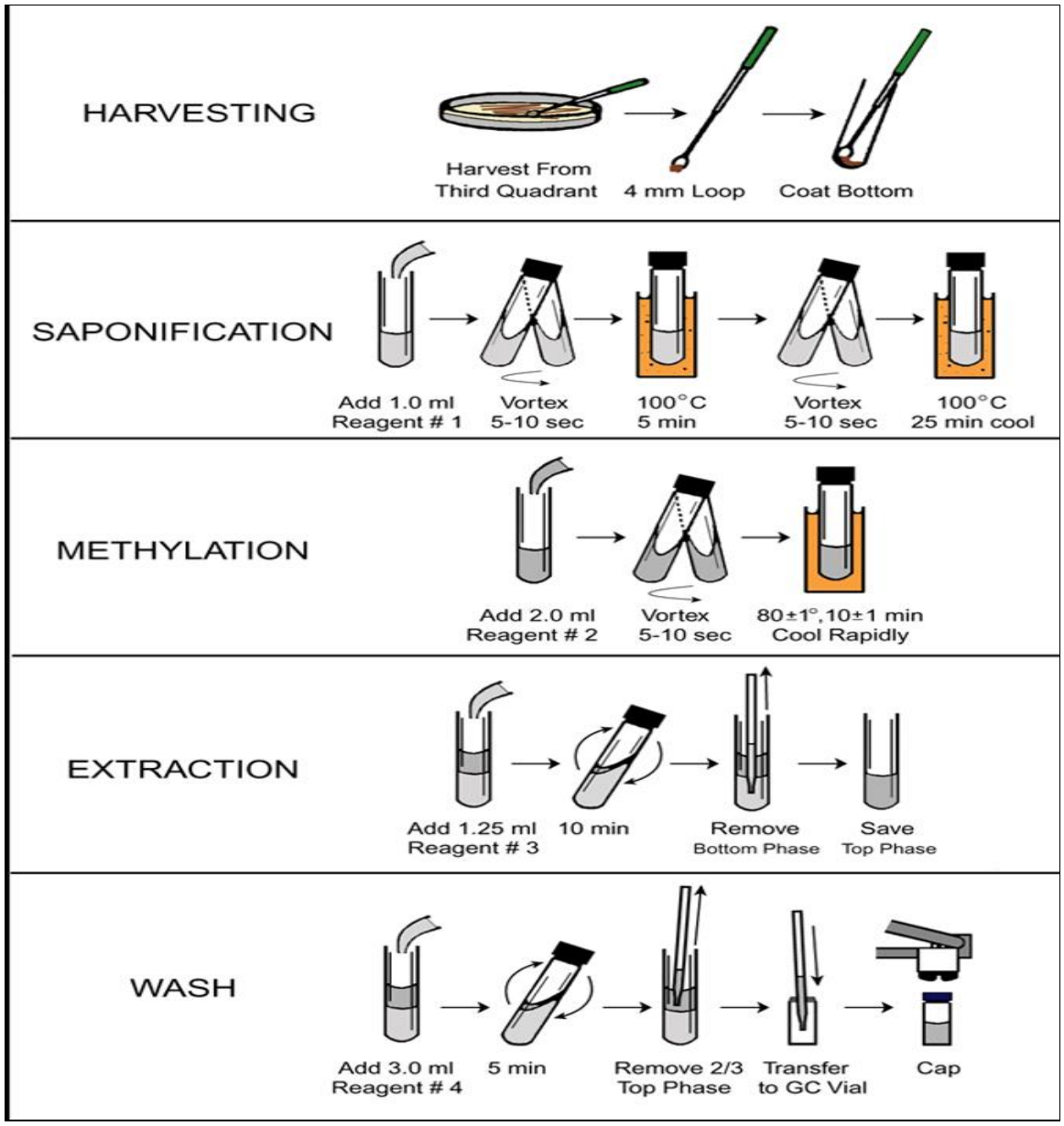

Figure 3.3. FAME, bacterial identification method [163]

3.2.4. Extracellular Enzyme Production

3.2.4.1. Amylase Production

Based on the colour, morphology and appearance, a total of five bacterial isolates designated as S1 to S5 were individually screened for their amylase production ability [162]. Starch agar medium used for this purpose was streaked with each bacterial strain and incubated for 24 hours at 37 °C. After incubation, each plate was saturated with Gram's iodine solution to determine the starch hydrolysis potential of the strains. Presence of colorless zone surrounding of colonies confirms the amylase production on addition of iodine.

3.2.4.2. Casein\Protease Hydrolysis

The relative potential of casein hydrolysis of bacterial isolates was measured on a milk agar plate, containing 20 percent of sterilized skimmed milk powder as basal salt of substrate casein [162]. Bacterial isolates were spot inoculated in the middle of the milk agar plates and incubated for 5-6 days at 37 °C at an interval of 24 hrs. Halo zones around the growing colonies indicates positive results.

3.2.4.3. Ammonia Production

Bacterial isolates (S1, S2, S3, S4 and S5) were screened for the production of ammonia in peptone water by following the method described by Cappucino and Sherman, [164]. For this, in each tube, freshly grown culture was inoculated in 10 ml peptone water and incubated at 28±2 °C for 48-72 hrs. Development of brown to yellow colour upon the/an addition of Nessler's reagent (0.5 ml) was an indication of ammonia production.

3.2.4.4. Urease Test

The test was carried out to determine the ability of bacterial colonies to produce urease enzyme using urea as a source of media. Urea agar medium were inoculated with five

cultures (S1, S2, S3, S4 and S5) and incubated at 37 ºC temperatures for 48-72 hours. Urease Positive (S7) and negative (S6) bacteria were also used in the study. After incubation, colour change from orange yellow to pink confirms the presence of urease enzyme.

3.2.4.5. Solubilization of Phosphates

Isolates phosphate solubilization was evaluated for their inorganic phosphate solubilization ability. The modified agar medium of Pikovskaya containing tricalcium phosphate was used in the assay [165]. Bacterial cultures (S1, S2, S4 and S5) were spot inoculated on the plates and incubated for 4-5 days at 28 °C. Phosphate solubilization is confirmed by the appearance of halo zones surrounding colonies.

3.2.4.6. Test for Indole Acetic Acid (IAA)

The indole test is intended to determine whether an organism can cleave the tryptophan molecule into Indole. The colorimetric method was performed to determine indole acetic acid (IAA) production by each isolate using the Salkowski method [166]. Bacterial strains were grown in LB medium supplemented with and without L-tryptophan (100 mg/L) and placed for 48 hrs in an incubating shaker at 28±2 °C. The broth was then centrifuged for 15 minutes at 3000 rpm and 1ml of the resulting supernatant was blended with 2ml Salkowski's reagent (2 percent 0.5 $FeCl_3$ in 35 percent $HCLO_4$ solution) and held in the dark for 20-30 minutes at room temperature. The development of a pink colour indicates the presence of IAA in the medium, and then the optical density was measured spectrophotometrically at 530 nm [167]. The quantity of IAA generated was calculated using the standard curve with known IAA concentration.

3.2.4.7. Catalase Test

Bacterial strains were screened for catalase enzyme production ability by taking 2.0 ml. of hydrogen peroxide (H_2O_2) on a clean glass slide. Using a sterile loop, an organism colony

was picked and allowed to come into contact with hydrogen peroxide. The bubble appearance shows a positive reaction.

3.2.4.8. Cellulose Hydrolysis

Pure cultures of bacterial isolates (S_1, S_2, S_4 and S_5) were streaked on CMC agar plates. The plates were flooded with 1 percent Congo red after 48 hours of incubation and allowed to stand at room temperature for 15 minutes. The plates were carefully stained with one molar NaCl. Clear zones around bacterial colonies indicates hydrolysis of cellulose [168].

3.2.4.9. Siderophore Production

Siderophore is a major factor influencing the survival and growth of bacteria in the soil and aqueous environment [169]. Chrome azurole S (CAS) method was used to identify bacterial isolates producing siderophores [170]. CAS agar plates were spot inoculated with test strains and incubated for 48-72 hrs at 28±2 °C. Appearance of yellow-orange halos around the colonies due to the removal of iron from the dye complex was an indication of siderophore production.

3.2.4.10. Hydrogen Cyanide Production (HCN)

Bacterial isolate production of HCN was screened using the technique described by Bakker and Schippers [171]. All isolates were streaked on TSA medium (10 percent) amended with glycine (4.4 g L^{-1}). An autoclaved filter paper saturated with a solution of picric acid (0.5 percent) and Na_2CO_3 (2 percent) was put on the inside of the petri dish. Petri plates were incubated at 28 °C for 48 hours after sealing with parafilm. A shift in the color of the filter paper from yellow to orange brown indicates the HCN production. A strain of Pseudomonas sp. was used as a positive control.

3.2.4.11. Lipase Production

To test extracellular lipase activity, nutrient agar plates containing vegetable oil were prepared. After inoculating the test organisms, plates were incubated at 37 °C for 24h. Appearance of bluish green colour upon the addition of $CuSO_4$, confirms the hydrolysis of fat in glycerol and fatty acid.

3.2.4.12. Exopolysaccharide Production (EPS)

The exopolysaccharide production ability of the screened isolates was done by inoculating the bacterial colony into 250 ml conical flasks containing 100 ml of Zobell marine broth supplemented with 7.5 percent NaCl. At a temperature of 37 ºC, inoculated flasks were incubated on shaker for 4 days at 100 rpm. Thereafter, the centrifugation was done at 5000 rpm for 30 minutes to make the Zobell marine broth cell free. Chilled ethanol was added to the supernatant in the 1:3 (v/v) ratio and kept for exopolysaccharide precipitation at 4 °C for 24 hours. By centrifugation, the precipitates were recovered and purified by washing with Milli Q water. Precipitated EPS was filtered and dried to constant weight on pre-weighed Wastman No. 1 filter paper. Amount of EPS in culture broth was calculated in terms of dry weight [28].

3.2.5. Salt Tolerance of Bacterial Strains

Salinity tolerance of the bacterial strains was tested by growing each bacteria in TSB medium supplemented with different concentration of NaCl (0, 5.0, 7.5, 10.0, 15.0, 17.5 and 20.0 percent). Sterilized 250 ml erlenmeyer flasks containing bacterial strains were placed in the incubator shaker for 72 hrs at 180 rpm and 32±2 °C. Optical density of the culture flasks were measured after every 24 hours.

3.3. INOCULUM PREPARATION

Bacterial strains were grown in sterilized DF minimal salt medium, containing ACC as substrate (N source) with a working volume of 150 mL in Erlenmeyer flasks of 250 mL

and incubated for 72 hrs at 28 ± 1 °C and 100 rpm After incubation, a spectrophotometer was used to measure the optical density of the medium and uniform population (OD_{540} = 0.45; 10^7-10^8 cfu mL^{-1}) was achieved by diluting with sterilized water before use.

3.4. BIOCHAR PRODUCTION FROM PLANT MATERIALS

As Harran region has the plentiful amount of plant residues, two types of plant residues biochar i.e. Cotton biochar (CB) and Olive biochar (OB) were prepared. The raw materials of Cotton stalk and Olive pulp were collected from commercial operations/field units. These samples were air-dried upon receipt and milled to > 2 mm. Sample grinding was followed by overnight oven drying at 105 °C before carbonization process. The moisture content of "as carbonized" material was maintained less than 5 percent. Carbonitic runs were carried out at 300 °C in triplicate per plant residue. The resulting biochar was allowed to cool and was removed from the retort. A composite sample was prepared from a homogeneous sub-sampling of each run based on equal weight. This was performed to take into consideration the possible variability in pyrolytic production [172]. Triplicate samples from each of the biochar type was analyzed for pH, EC, CEC, total N, total C, P, K, and micronutrients (Table 3.4, 3.5 and 3.6). Biochar production process was done in Harran University, Faculty of Agriculture.

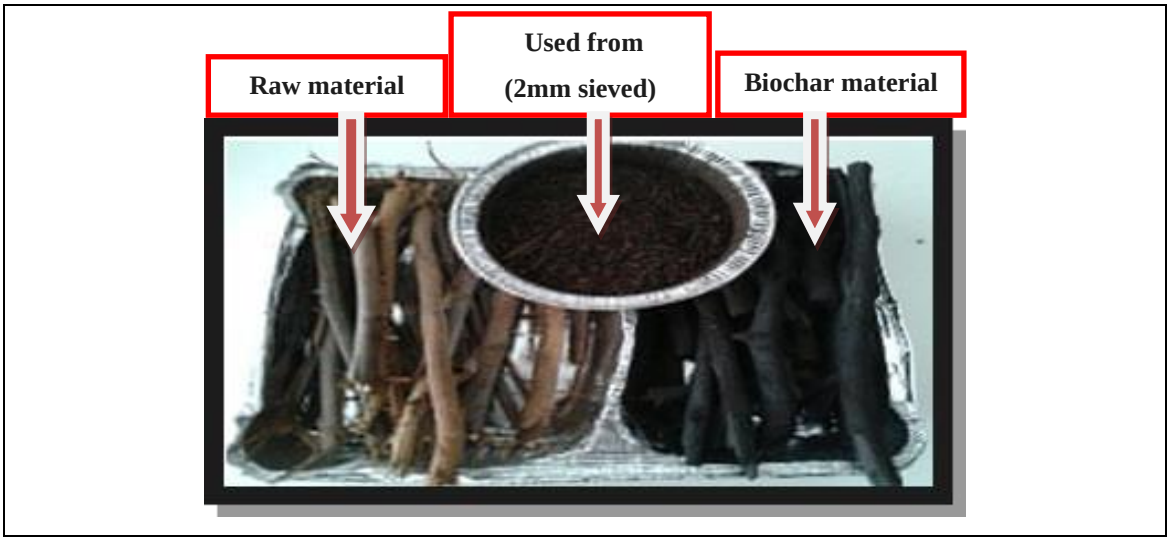

Figure 3.4. Biochar preparation from Cotton stalk

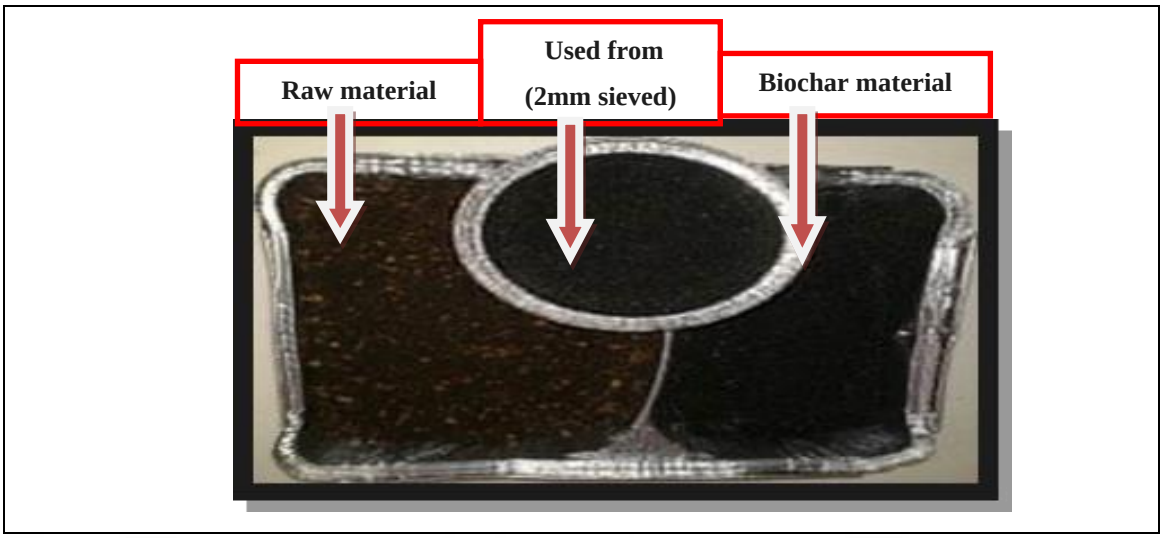

Figure 3.5. Biochar preparation from Olive pulp

Table 3.4. Various properties of Cotton and Olive biochar

Biochar Type	pH (1:2.5)	EC (dSm^{-1})	%C	%N	%H	$\%NH_4^+$-N	$\%NO_3^-$-N
Cotton Biochar	7.63	0.48	58.06	1.44	4.94	0.741	0.553
Olive Biochar	8.94	0.29	67.91	1.86	4.97	0.600	0.473

Table 3.5. Macronutrients analysis of biochar

Biochar Type	K	P	Na	Ca	Mg
			mg/kg		
Cotton Biochar	26590	3727	812.00	17870	5654
Olive Biochar	11780	11570	821.00	52520	6445

Table 3.6. Micronutrients analysis of biochar

Biochar Type	Al	B	Cu	Fe	Mn	Ni	Pb
			mg/kg				
Cotton Biochar	966.10	58.70	14.80	797	53.00	1.50	6.50
Olive Biochar	251.50	90.60	61.90	485	167.70	2.90	7.70

3.5. BIOCHAR CHARACTERIZATION IN IMPROVING THE QUALITY OF SALINE-SODIC SOIL SUPPLEMENTED WITH HALOTOLERANT PGPRS UNDER AN INCUBATION STUDY.

About 900 g of fresh soil sample, previously stored in the fridge was taken and transferred to 1000 mL storage glass jars. By adding deionized water, soil was held at 60 percent of its water holding ability (WHC). For each biochar type there were ten treatments (Table 3.1, Table 3.2), seven incubation periods: 0, 7, 17, 32, 51, 73, and 105 days and three replications, in total, 210 jars for each biochar type. Biochar were applied at 4.5 and 9.0 g/900g for 10 and 20 tha^{-1} biochar, respectively. While halotolerant application was made according to 30 L/hac basis. The soil was well mixed after amending the jars with a desired treatment and each jar's weight was noted. In order to ensure the exchange of natural gas, Jars were covered with perforated parafilm and kept for a total of 105 days in an incubator at 25±2 ^{0}C. The arrangement of jars in the incubator was done according to completely randomized design (CRD). Jars were regularly weighed to check the moisture content after every second day, and the amount of distilled water needed was added when the loss exceeded 0.05 g. Special care was given while handling the jars in order not to unsettle the soil through agitating.

3.5.1. Soil Sampling and Analysis

At each interval, soil samples were taken and analyzed for modifications in chemical parameters (Nitrogen, organic matter, EC, pH, ESP, CEC and exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}). Zero day (0) sampling was done immediately after mixing the amendments.

3.5.1.1. Soil pH Measurement

Soil pH was measured in 1:2.5 soils: water suspension. For this, 25 mL of distilled water has been added to 10 g soil. After 5 minutes of stirring, the suspension was kept at room temperature for 30 minutes. The pH was recorded with a pH meter [173].

3.5.1.2. Soil EC Measurement

For the determination of electrical conductivity (EC), 1:5 soil: water suspension was prepared by adding 50 ml deionised water in a bottle containing 10 g soil. The mixture was vigorously stirred at mechanical shaker for 30 min at 180 rpm. Total salt content in the suspension was measured using conductivity meter [174].

3.5.1.3. Soil Cation Exchange Capacity (CEC)

In order to measure cation exchange capacity (CEC) about 5 g of finely ground soil was weighed in a centrifuge tube and 33-mL 1 N sodium acetate trihydrate solution was added in it. After 5 minutes shaking tube was centrifuged at 3000 rpm and supernatant was decanted as cleanly as possible and was discarded. The process was repeated four times with 33-mL, 1 N sodium acetate trihydrate and each time supernant was discarded. The sample was subsequently swept with 33-mL 95 percent ethanol and process was repeated thrice while discarding the supernatant each time. In order to replace the adsorbed sodium from the sample, 33-mL 1 N ammonium acetate was used for washing. The Process was repeated thrice and each time supernatant was decanted into a volumetric flask of 100 mL [175]. Amount of sodium in the supernatant was determined via ICP.

3.5.1.4. Soil Exchangeable Cations (Na^+, K^+, Ca^{2+} and Mg^{2+})

About 2.0 g of fine ground dried soil was transferred to a 50 ml centrifuge tube and 20 mL 1M NH_4OAc solution was added in it. The tube was tightly sealed with screw cap and placed in the rotor shaker for 2 hrs. The sample was then centrifuged for 10 minutes and clear supernatant was used for analysis by ICP.

3.5.1.5. Exchangeable Sodium Percentage

Exchangeable sodium percentage was calculated by using the following equation:

$$ESP = (Na^+ / CEC) \times 100 \quad (3.1)$$

Where: ESP = Exchangeable sodium percentage (%)

Na^{+} = Measured exchangeable Na (cmol (+) kg^{-1})

CEC = Cation exchange capacity (cmol (+) kg^{-1})

Or;

$$ESP = \text{Exchangeable } \{(Na^{+}) / (Ca^{2+} + Mg^{2+} + K^{+} + Na^{+})\} \times 100 \quad (3.2)$$

3.5.1.6. Soil Organic Matter

Soil organic matter content (OM) was determined by using Nelson and Sommers method [47]. For this purpose, 1 g finely and uniformly ground soil sample was weighed and transferred to a 500 mL Erlenmeyer flask. Thereafter, 10 mL of l N potassium dichromate ($K_2Cr_2O_7$) was added to it, followed by 20 mL conc. Sulfuric acid (H_2SO_4). The contents were well mixed with manual stirring and allowed the flask to cool at room temperature for 30 minutes. The mixture was added approximately 175-200 mL of distilled water. Furthermore, 10 mL of concentrated orthophosphoric acid (H_3PO_4) and 15-20 drops of diphenylamine indicator was also added. Then, 0.5 N $FeSO_4.7H_2O$ was used for titration until a sharp green endpoint appeared. In the same way, blank was prepared and titrated.

The content of soil OM was calculated as below:

$$\%OM = \frac{\text{blank (mL)} - \text{soil sample (mL)} \times \text{N } FeSO_4.7H_2O \times 0.69}{\text{soil sample wt (g)}} \quad (3.3)$$

3.5.1.7. Total Nitrogen in Soil

Soil total N was analyzed using the method of Kjeldahl as Bremmer and Mulvaney (1982) described. The process comprises three steps.

3.5.1.7.1. Digestion

The soil sample of 0.5 g was transferred in a digestion tube and 1.25 g of digestion mixture

(K_2SO_4-$CuSO_4.5H_2O$–Selenium powder 100:10 w/w ratio) and 4 mL of conc. H_2SO_4 was added in it. Samples were digested at 365 °C in a block digester for 3 hours or until the digestive mixture was found to be transparent.

3.5.1.7.2. Distillation

All digestion tube content was rinsed into a distillation flask and 20 mL of 40 percent NaOH was added to the flask. About10 mL of 2 percent boric acid solution was then collected in a conical flask and up to 30 mL of distillate was harvested in it.

3.5.1.7.3. Titration

The distillate was titrated against 0.01 N HCl until the finishing point of light pink colour. Also, two blanks were digested, distilled, and by subtracting them from other sample readings, reading was noted. The sample nitrogen content was determined by applying the formula below.

$$\%\mathrm{N} = 14.0067 \times [\mathrm{TS} - \mathrm{TB}] \times \mathrm{NA} \qquad (3.4)$$

Where: N= Nitrogen

TS= Titrant of sample (mL)

TB= Titrant of blank (mL)

NA= Normality of Acid

3.5.1.8. Soil Textural Class Determinarion

Soil textural class determination was done by the Boyoucos hydrometer method. For this, fifty grams (50 g) of 2 mm soil sample was collected in 600 mL beaker and 20 mL of 2 percent sodium hexametaphosphate solution was added to it. About, 200 mL of water was then added and the soil-water blend was stirred with a glass rod. The mixture was left for 24 hours over night. The suspension was dispersed for 15 minutes in an electric dispersion cup and the entire material was then transferred to a 1000 mL cylinder. The final volume

of each sample was adjusted and Buyoucus hydrometer and temperature measurements of the suspension were recorded and fixed. Calculations were done and a soil texture triangle was used to determine the soil texture class [176].

3.5.1.9. Soil $CaCO_3$ Determination

Calcimeter estimated the percentage of calcium carbonate ($CaCO_3$). For this purpose, 0.1 N HCL was used to treat 2 g of soil sample, the volume of CO_2 from pure calcium carbonate and the samples was recorded. Then, according to Balázs *et al*., 2005, the percentage calcium carbonate was calculated [177].

3.6. BIOCHAR AND HALOTOLERANT IN THE IMPROVEMENT OF SALINE-SODIC SOIL HEALTH AND WHEAT GROWTH UNDER CONTROLLED CONDITIONS

An experiment was conducted in the glass-house of Department of Genetics and Bioengineering, Yeditepe University to assess the interactive impact of biochar and halotolerant on crop growth and yield as well as changes in physiochemical properties of saline-sodic soil. The treatment plan was the same as followed in experiment 2, except that only cotton biochar was used in this study (Table 3.1). Plastic pots with a capacity of about 5 kg were taken and filled with the already collected saline-sodic soil. After labeling with respective treatments, potts were arranged in a completely randomized order and irrigated with distilled water.

3.6.1. Germination Assay

To access wheat seed germinability under natural soil salinity conditions a germination test was conducted. For this, plastic pots filled with the sampled soil were thoroughly mixed with respective ammendments (25 and 50 g biochar/5kg soil was used for 10 and 20 tha^{-1}, respectively) (Table 3.1). Eight wheat seeds were sown in each pot and each treatment was replicated thrice. Because of salt toxicity, soil compaction, crusting and soil cracking non of the seeds were able to germinate. However, seeds were in good condition when checked

even after 3 weeks. As natural soil was unable to support seed germination, perlite was carefully mixed with soil to improve aeration and to reduce cracking.

3.6.2. Leaching

As soil was high in EC and pH, leaching was done to decrease the salinity of the soil. Good quality irrigation water was applied for this purpose. Leaching was continued until the EC reached to the level that crop to be grown could tolerate. The post leaching electrical conductivity was 5.1 dSm^{-1}.

3.6.3. Experimental Procedure

All cotton biochar treatments (were well mixed into their respective pots, except the control and the one receiving solely halotolerant as an inoculum. A basal dose of NPK (175:60:90 kg ha^{-1}) was also applied depending on the requirement of the crop. Afterwards, pots were incubated for almost 3.5 months in order to facilitate the release of nutrients from the amendments.

3.6.4. Sowing

Wheat seeds to be inoculated according to the respective treatments were first surface sterilized, moistened with cool concentrated sugar solution and coated with the inoculum, thereafter. Depending on the treatments plan, six healthy inoculated, as well as non inoculated seeds were seeded in pots. Where necessary, all regular cultural practices and irrigation/watering were performed.

At vegetative stage, the crop was sampled to measure growth parameters (shoot and root length, fresh and dry weights). Soil was sampled in the pots after harvesting the crop for post-harvest soil analysis including, EC, pH, N, OM, Ca, Mg, Na, K etc.

3.6.5. Post Harvest Soil Analysis

After crop harvest soil in the pots was used to analyze some chemical properties (EC, pH, N, OM, Na, K, Ca and Mg).

3.7. STATISTICAL ANALYSIS

All data from laboratory and greenhouse studies were analyzed according to the completely randomized design (CRD), using statistix 10 software. LSD values were used to indicate the significant variance between the mean values of both treatments and time intervals. The probability value ($P \leq 0.05$) given in the text indicated the significance of treatments, types of biochar, time intervals and their correlation .

4. RESULTS AND DISCUSSION

4.1. CHARACTERIZATION AND SCREENING OF HALOTOLERANT BACTERIAL STRAINS

4.1.1. Gram Staining and Cell Morphology

Gram staining of the isolates was done to determine their morphological characters. Strains were designated as S1, S2, S3, S4 and S5. It was found that all the isolates were gram positive rod, motile and spore-forming, however S2 was non-motile and non-spore forming (Table 4.1).

Table 4.1. Morphological characters of isolated strains

	Gram Stain	Shape	Motility	Endospore
S1	+	Bacilli	+	+
S2	+	Bacilli	-	-
S3	+	Bacilli	+	+
S4	+	Bacilli	+	+
S5	+	Bacilli	+	+

4.1.2. Extracellular Enzyme Production

4.1.2.1. Amylase Production

Bacterial strains were analyzed for Amylase production by starch hydrolysis method. Results indicated that out of five, two strains (S_1 and S_2) were able to hydrolyze the starch (Figure 4.1, 4.2).

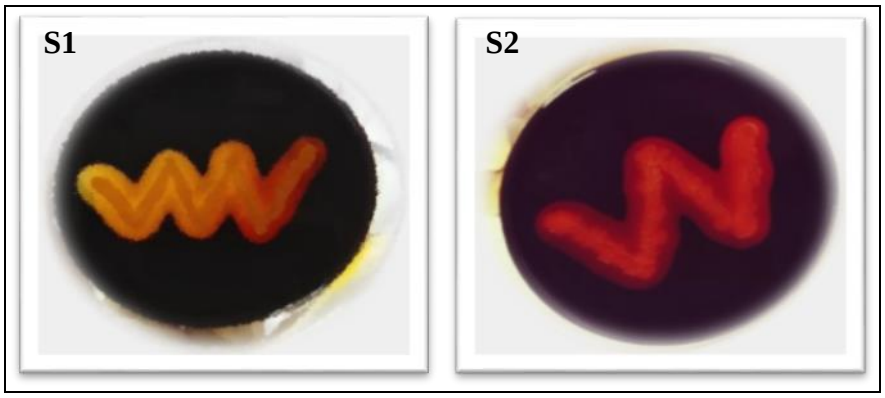

Figure 4.1. Bacterial strains after the addition of iodine, clearing zone surrounding colonies indicates the presence of starch hydrolysis: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2)

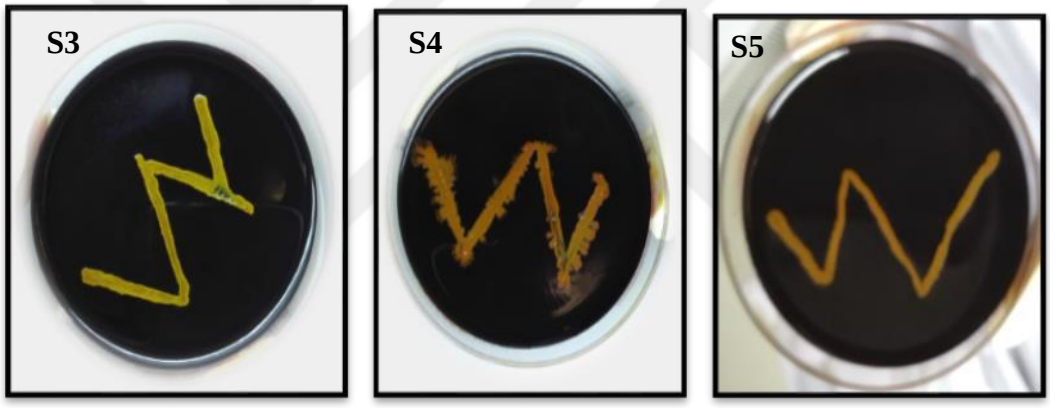

Figure 4.2. Bacterial strains after the addition of iodine, no zone formation surrounding colonies indicates the absence of starch hydrolysis: *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) and *Virgibacillus pantothenticus* (S5)

4.1.2.2. Casein\Protease Hydrolysis

Bacterial isolates were spot inoculated on casein media containing skim milk powder as substrate caesin. According to the results obtained, all the strains were able to greatly hydrolyze caesin except S_3 (Figure 4.3). Two bacterial strains S_4 and S_5 showed complete caesin hydrolyze within 8-12 hrs.

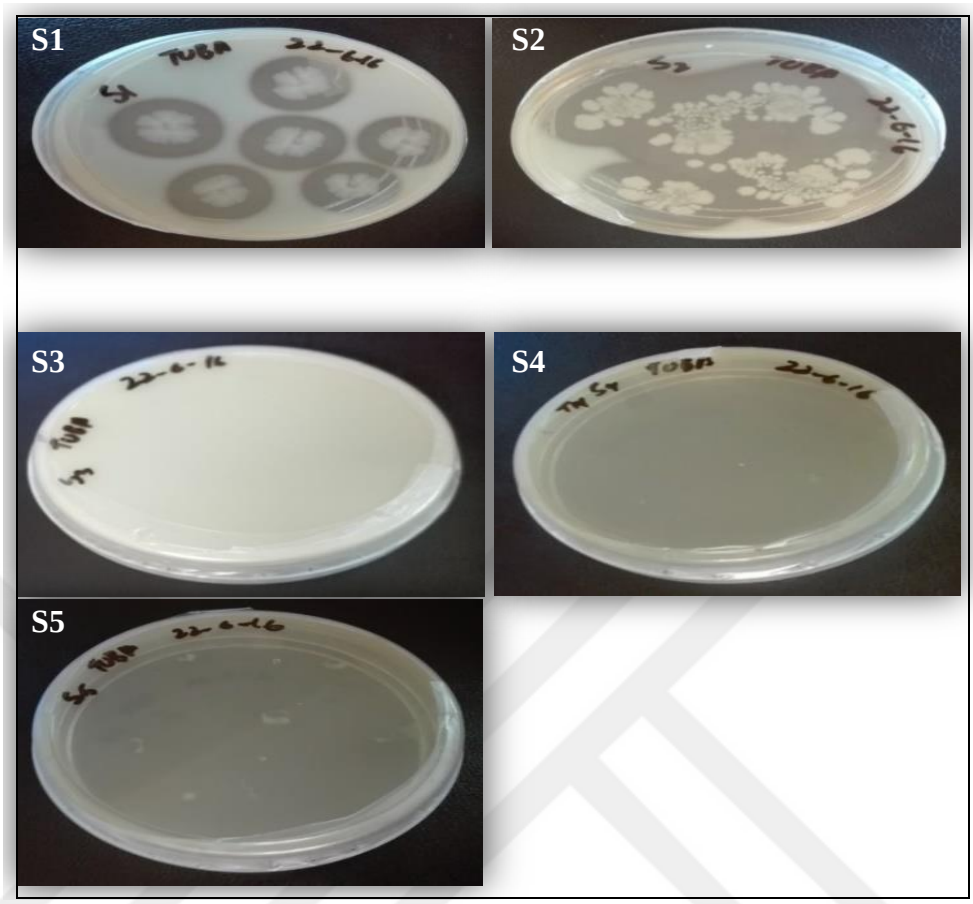

Figure 4.3. Casein hydrolysis by islolated strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) and *Virgibacillus pantothenticus* (S5)

4.1.2.3. Ammonia Production

Bacterial isolates (S_1, S_2, S_3, S_4 and S_5) were screened for ammonia production in peptone water. The results clearly indicated ammonia production by all five bacterial strains upon addition of Nessler‘s reagent (0.5 ml) when compared to the control (Figure 4.4). Bacterial isolates S_2 and S_3, however, were able to produce more ammonia when compared to the other three.

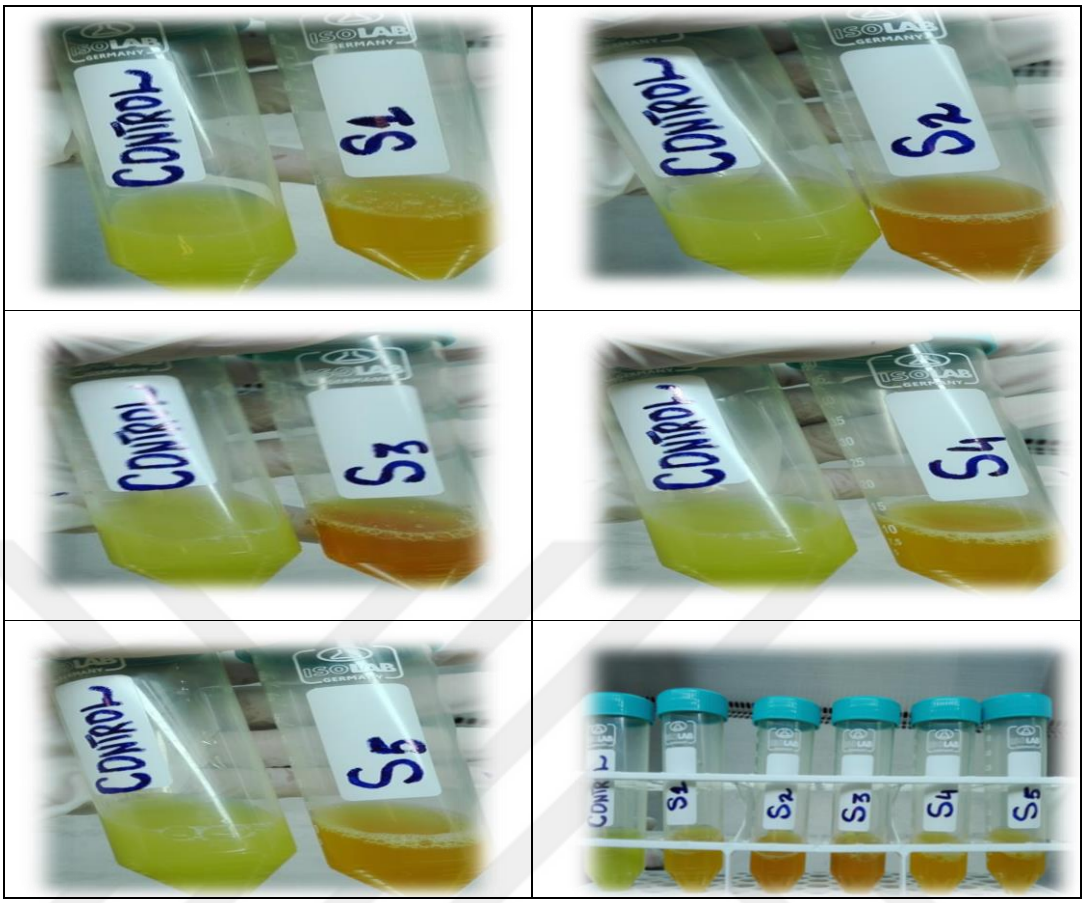

Figure 4.4. Ammonia production by isloated strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) and *Virgibacillus pantothenticus* (S5)

4.1.2.4. Urease Test

The test was carried out to determine bacterial colonies ability to produce Urease enzymes by the use of urea as a media source. Urease positive (S_7) and negative (S_6) bacteria were also used in the study. The appearance of a pink color confirmed the presence of uresae enzymes after incubation in all the bacterial strains except S3 which did not show any colour change (Figure 4.5).

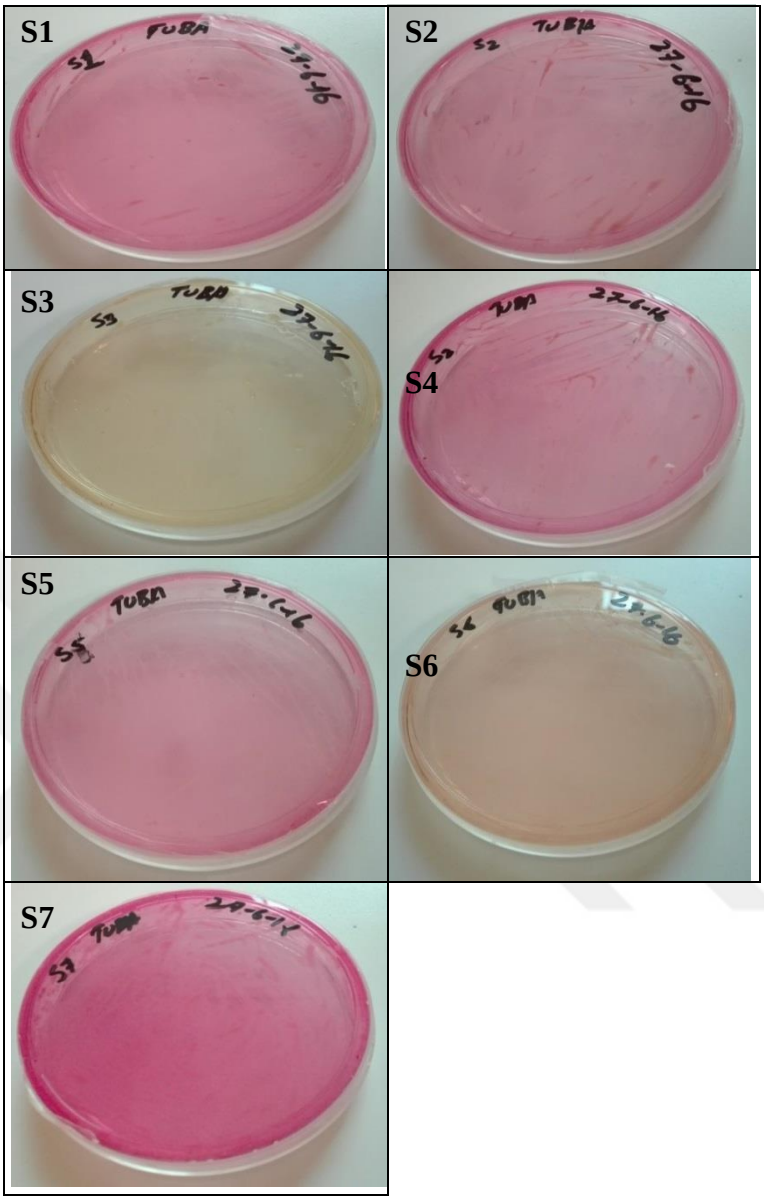

Figure 4.5. Urease enzyme production by isloated strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) *Virgibacillus pantothenticus* (S5), urease negative (S6) and urease positive (S7) bacteria

4.1.2.5. Solubilization of Phosphates

Isolates phosphate solubilization was evaluated for their inorganic phosphate solubilization ability. According to the results obtained, only S_4 and S_5 bacteria were able to slightly hydrolyze the insoluble phosphate (Figure 4.6).

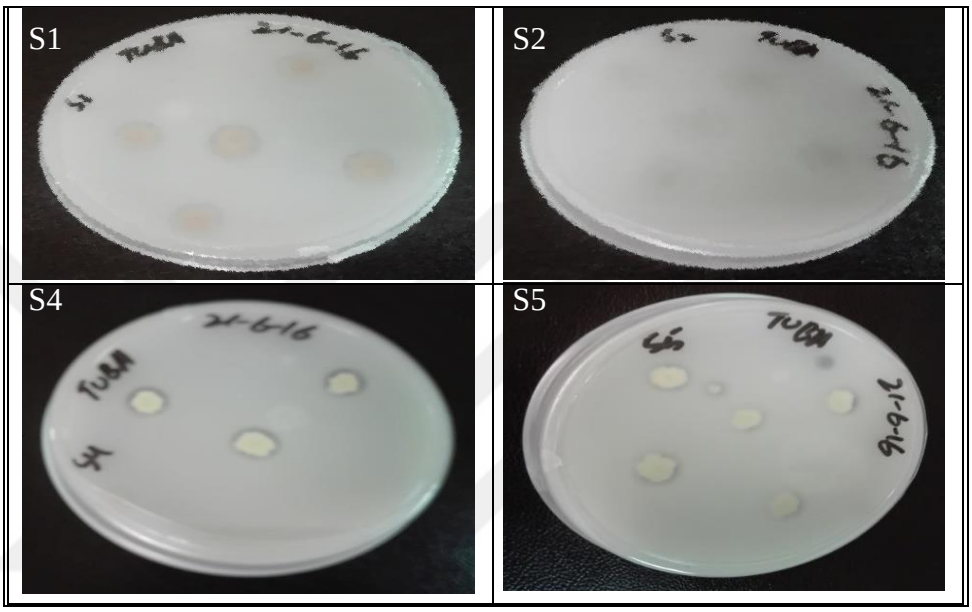

Figure 4.6. Phosphate solubilization by isloated strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus amyloliquefaciens* (S4) *Virgibacillus pantothenticus* (S5)

4.1.2.6. Catalase Test

Bacterial strains for catalytic production were tested and the results confirmed the presence of catalase enzyme in all the tested isolates (Figure 4.7).

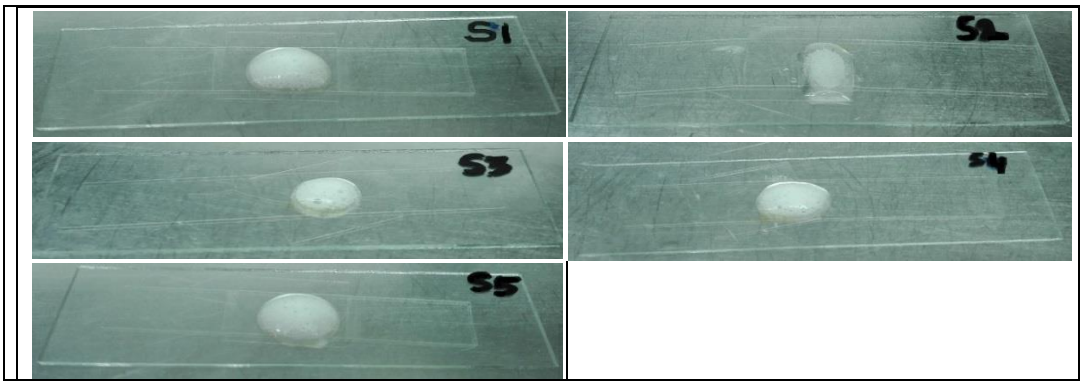

Figure 4.7. Catalase production by isolated strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) *Virgibacillus pantothenticus* (S5)

4.1.2.7. Cellulose Hydrolysis

Bacteria were screened for their potential to degrade cellulose. According to our results none of the bacteria were able to hydrolyze cellulose, thus confirming the absence of cellulase enzyme (Figure 4.8).

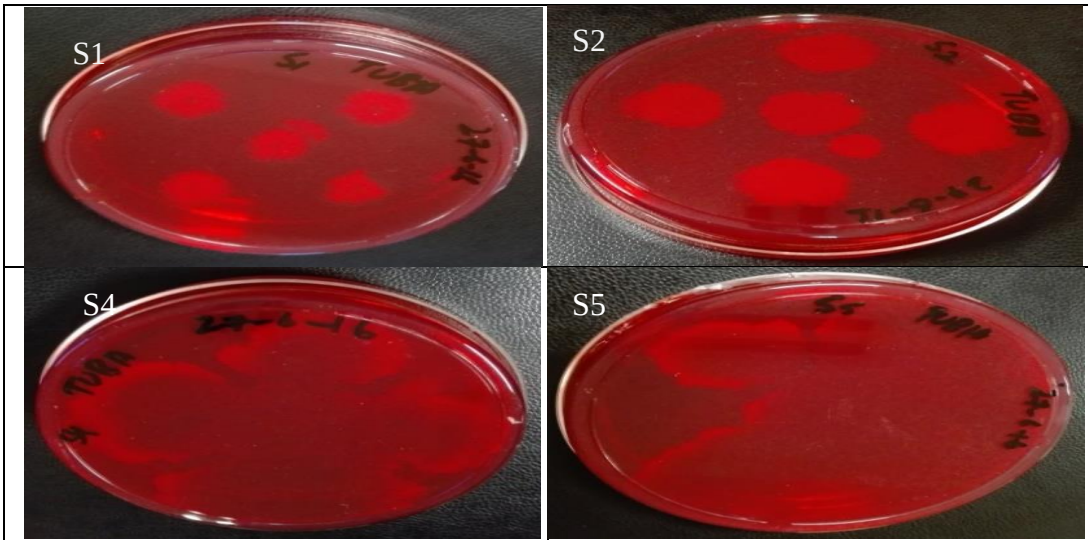

Figure 4.8. Cellulose hydrolysis by isolaoted strains: *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus amyloliquefaciens* (S4) *Virgibacillus pantothenticus* (S5)

4.1.2.8. Siderophore, HCN and EPS Production

Out of 5 isolates S1, S4 and S5 were able to produce both HCN and siderophore, while EPS was synthesized by all the strains.

4.1.2.9. Lipase Produdction

Lipase enzyme play an important part in the biodegradation of oil and bioremediation of contaminated soils. All the tested strains were positive for lipase activity.

4.1.2.10. Test for Indole Acetic Acid

Strains were tested for their IAA production ability. All the strains produced IAA and the concentration varied among different isolates. However, S1, S2 and S5 made the greatest concentration of IAA.

Table 4.2. Some extracellular enzyme production by isolated strains

Strains	HCN	EPS	Siderophore	Lipase	IAA
S1	+	+	+	+	++
S2	-	+	-	+	++
S3	-	+	-	+	+
S4	+	+	+	+	+
S5	+	+	+	+	++

4.1.3. Salinity Tolerance Test

Isolates were tested for their ability to withhstand salinity by growing them with varying concentrations of NaCl in the TSB medium. An increase in tolerance to the higher rates of NaCl was seen over time. After 72 hrs of incubation, strains S1, S2 and S5 showed the ability to grow well at 15 percent NaCl solution, while S3 and S4 exhibited halotolerance at concentration of 17.5 percent NaCl, however, each strain was able to withstand 20 percent NaCl (Figure 4.9)

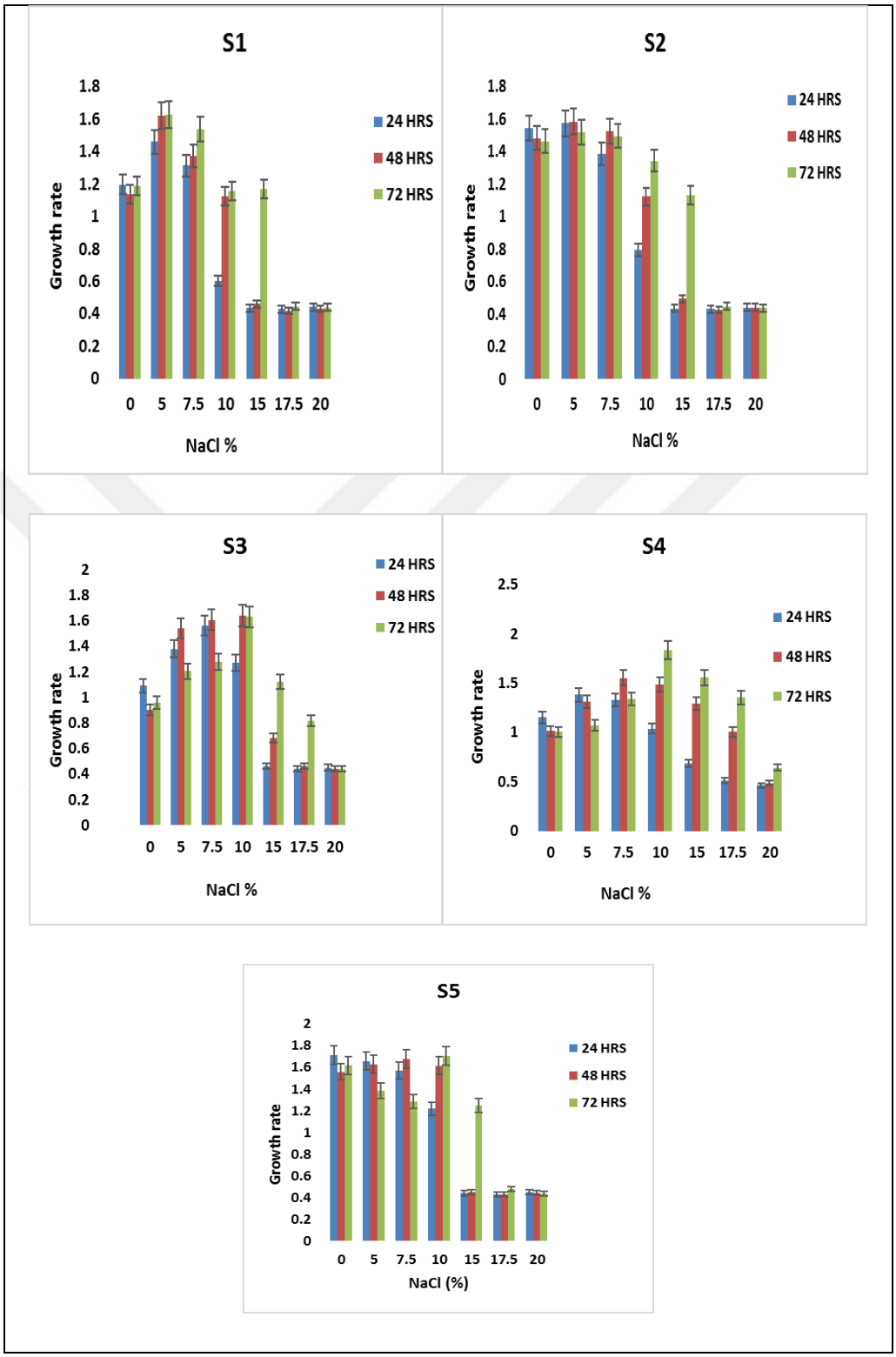

Figure 4.9. NaCl tolerance of isolated strains at different time intervals. Bar represents mean ± SE of three replicates. *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) *Virgibacillus pantothenticus* (S5)

4.1.4. Bacterial Identification Using Fatty Acid Methyl Ester Analysis (FAME)

Fatty acid methyl ester analysis (fame) of bacteria suggested that four of the halophiles belonged to Bacillus while one to Mycobacterium species. The isolated bacterial strains most closely resembled to *Bacillus subtilis* (S1), *Microbacterium flavescens* (S2), *Bacillus atrophaeus* (S3), *Bacillus amyloliquefaciens* (S4) and *Virgibacillus pantothenticus* (S5).

Results of the research has shown that all the strains were able to survive 20 percent NaCl and most of them showed best growth in the range of 10-15 percent at 37 ˚Ċ, and were classified as moderate to extreme halophiles according to the definition referred in several reports [179, 180]. Ability of halotolerant to withstand high concentration of NaCl (10-20 percent) has also been reported by other researchers [181-184].

High salt tolerant bacteria isolated in our study were found positive for IAA production, a plant hormone which is active in seed germination, root initiation, cell enlargement and cell division, therefore crucial to stimulate growth of plants in stressful circumstances. Bacterial IAA stimulates the proliferation of lateral roots resulting in greater root surface area thereby enabling the plants to access more water and nutrients from the soil [185, 186].

All the tested isolates showed potential for EPS production, polymers that protect bacteria from desiccation and salt stress by acting as a boundary between cells and surrounding environment thus helping their survival under stress conditions [187]. The EPS plays a great role in improving overall soil quality and soil fertility by significantly increasing soil aggregation, stabilizing soil structures, and increasing water holding and cation exchange capacity [187, 189]. Previous studies have demonstrated that bacterial EPS under salinity stress can bind sodium ions and alleviate its toxic effect in the soil [190]. Salt free soil thus favors the plant growth by providing the sufficient nutrients in the soil [191].

Production of exopolysaccharide can also be beneficial in attachment of bacterial cells to biotic surfaces like plants. Inoculation of Chickpea seeds with EPS producing bacterial strains increased plant growth at elevated salt stress [192]. Studies conducted by many researchers concluded that inoculation of EPS-producing bacteria would be an appreciated approach for amelioration and improving crop productivity of the salt-affected soils [189, 193, 194].

Bacterial volatile compounds (VCs) play an important role in suppressing plant pathogens [195] signifying their importance for biological plant diseases control. Isolated halophiles in our study were able to produce ammonia and HCN which may participate in the inhibition of many plant pathogens and metalloenzymes under salinity stress [196]. Saline-sodic soils are usually deficient in iron (Fe), a micronutrient that plays a major role in plant physiology and biochemistry including synthesis of chlorophyll and maintenance of chloroplast structure. Three bacterial strains in this study (S1, S4 and S5) were very effective to produce siderophore which can assist plant growth by binding iron in its available form (Fe^{3+}) [197]. Siderophores are considered biocontrol agent as they sequester the iron from the pathogen needed for their growth [198], thus protecting the plants from numerous fungal or bacterial diseases [199, 200].

Halophilic bacteria isolated in this study were able to produce extracellular enzymes including catalase, an important antioxidant enzyme that helps in maintaining plant reactive oxygen species (ROS) levels during oxidative stress [201].

As salt affected soils are highly degraded and need special attention for their reclamation, the application of halophilic bacteria could be a sensible approach to help restore the soil's fertility and productivity. Halophilic microbes are found to remove salt from saline soils [202-204]. According to Arora *et al*. two halophilic bacterial strains efficiently removed the sodium ions from the soil at higher concentration of NaCl (10 percent) [204]. Reduction in soil electrical conductivity (EC) and total dissolved salts (TDS) by halophiles is also reported by researchers. Hence, these salt tolerant bacteria loaded with plant growth promoting traits help in bio-remediation of salt affected soils and thereby improves the crop yields.

4.2. INCUBATION STUDY RESULTS

4.2.1. Effect of Cotton Stalk (CS) Its Biochar (CB) and Combinations With Halotolerant on Soil pH and EC

The effects of cotton stalk (CS), its biochar (CB) and combinations with halotolerant on changes in soil pH and EC over 105 day incubation period is displayed in Table 4.2 and 4.3. Analysis of variance had significant ($p \leq 0.05$) effect on the amendments, incubation time and their interactions on soil pH and EC.

According to the results obtained, amendments applied either singly or together with halotolerant at both rates considerably ($P \leq 0.05$) increased the pH with time, except T6. By the end of incubation at day 105, the same pH values were recorded in T1 and T4 (10.35) and followed by T5 and T9, respectively as 10.33 and 10.34.

Different rates of cotton stalk applied either alone or in combination with halotolerant had almost the same effect on pH for all the treatments. However, treatment, receiving cotton stalk @ 20 tha^{-1} + Halotolerant had a lower soil pH among them.

When comparing the various treatments of CS and its biochar, biochar applied @ 20 tha^{-1} had the lowest pH value, followed by T9 (CB @ 10 tha^{-1} + halotolerant) and T5 (CS @ 20 tha^{-1} + halotolerant). Sole application of halotolerant also increased the pH values.

Averaged across different treatments, the data presented in Figure 4.10 indicated that cotton stalk biochar applied @ 20 tha^{-1} had the lowest pH value (10.25) while the highest (10.33) was recorded in the control.

The EC values have also been significantly affected by application rates and incubation timings in all amendments. A significant variation in soil EC among different amendments was observed immediately after the addition of amendments (Day 0). In general, all the treatments displayed lower EC compared to the control. The lowest EC of 9.54 dSm^{-1} was recorded under T2, followed by 9.77, 9.80 and 9.81 dSm^{-1} under T9, T5 and T3, respectively. There was no significant difference between these treatments. At Day 7, a sudden fall in EC values was observed for all the treatments including control. The EC among different amendments at Day 7 varied between 6.17-7.82 dSm^{-1} compared to 9.54-

10.32 dSm^{-1} on Day 0. Thereafter, a gradual increase in soil EC for all the treatments was recorded with time, reaching their highest values after 3.5 months of incubation (Table 4.3).

Averaged across different amendments, CS applied @ 10 tha^{-1} (T2) showed the minimum EC value (9.58 dSm^{-1}) while the highest (10.52 dSm^{-1}) was recorded in the control (Figure. 4.11).

Table 4.2. Cotton stalk biochar effect on soil pH

pH								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	10.26b	10.36a	10.34a	10.34a	10.34ab	10.35a	10.35a	**0.006**
T2	10.24cd	10.28e	10.33a	10.32bc	10.30cde	10.30bc	10.31d	**0.006**
T3	10.24cd	10.26f	10.34a	10.31cd	10.32abcd	10.28cde	10.32cd	**0.006**
T4	10.25bcd	10.22g	10.33a	10.33b	10.34ab	10.24e	10.35a	**0.008**
T5	10.25bcd	10.32c	10.27b	10.22f	10.27e	10.33ab	10.33b	**0.020**
T6	10.29a	10.32d	10.31a	10.30e	10.31bcde	10.29bcd	10.29e	**NS**
T7	10.24cd	10.19h	10.18c	10.30e	10.29de	10.25e	10.32cd	**0.029**
T8	10.24cd	10.34b	10.32a	10.32b	10.36a	10.29bcd	10.32c	**0.032**
T9	10.26bc	10.29e	10.19c	10.30e	10.33abc	10.31abc	10.34b	**0.045**
T10	10.24cd	10.32c	10.33a	10.31de	10.31bcd	10.25de	10.32cd	**0.012**
LSD	**0.016**	**0.005**	**0.035**	**0.012**	**0.037**	**0.045**	**0.010**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.3. Cotton stalk biochar effect on soil EC

EC (dSm^{-1})								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	10.32a	7.82a	10.10a	10.78a	11.26d	11.65a	11.76b	**0.067**
T2	9.54g	6.51g	9.13e	9.75g	10.81g	10.95e	10.37g	**0.058**
T3	9.81ef	7.75b	9.85b	10.11f	11.71c	10.56f	9.38h	**0.086**
T4	10.08c	6.75f	10.05a	10.55d	12.15a	11.41bc	11.44d	**0.073**
T5	9.80ef	6.57g	9.30d	10.30e	11.90b	11.24d	11.94a	**0.070**
T6	10.17b	6.85e	10.07a	10.67b	10.30i	11.26d	11.57c	**0.078**
T7	10.11bc	7.55d	9.39c	10.57cd	10.12e	11.30cd	11.42d	**0.069**
T8	9.85e	7.77ab	9.29d	9.56h	10.08j	10.89e	10.93e	**0.090**
T9	9.77f	6.17h	8.69f	9.33i	10.71h	11.48b	11.51c	**0.075**
T10	9.93d	7.67c	9.33cd	10.62bc	11.03f	10.44g	10.77f	**0.068**
LSD	**0.058**	**0.065**	**0.076**	**0.055**	**0.060**	**0.112**	**0.084**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

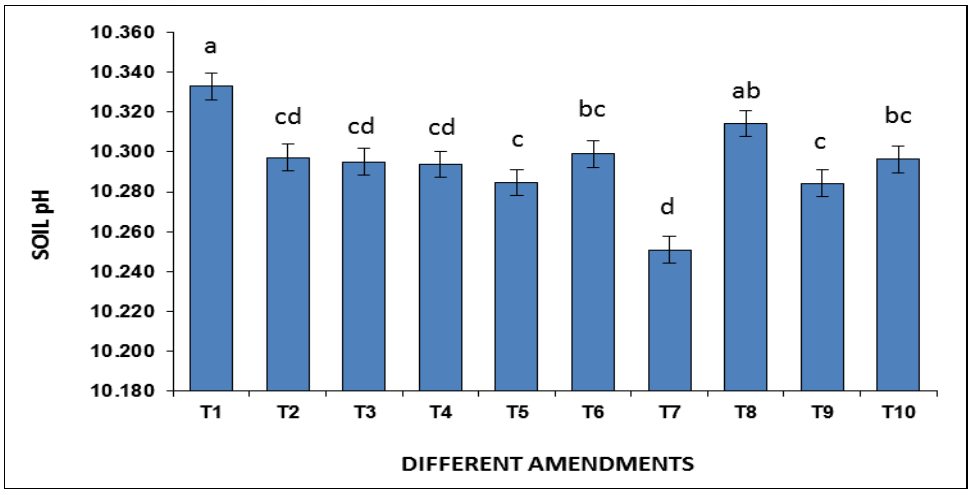

Figure 4.10. Modifications in the pH of saline-sodic soil with Cotton feedstock (CF), its biochar (CB) and combinations with halotolerant (average over incubation period) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1 – control; T2 – CF @ 10 tha^{-1}; T3 – CF @ 20 tha^{-1}; T4 – CF @ 10 tha^{-1} + Halotolerant; T5 – CF @ 20 tha^{-1} + Halotolerant; T6 - CB @ 10 tha^{-1}; T7 – CB @ 20 tha^{-1}; T8 – Halotolerant; T9 – CB @ 10 tha^{-1} + Halotolerant; T10 – CB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

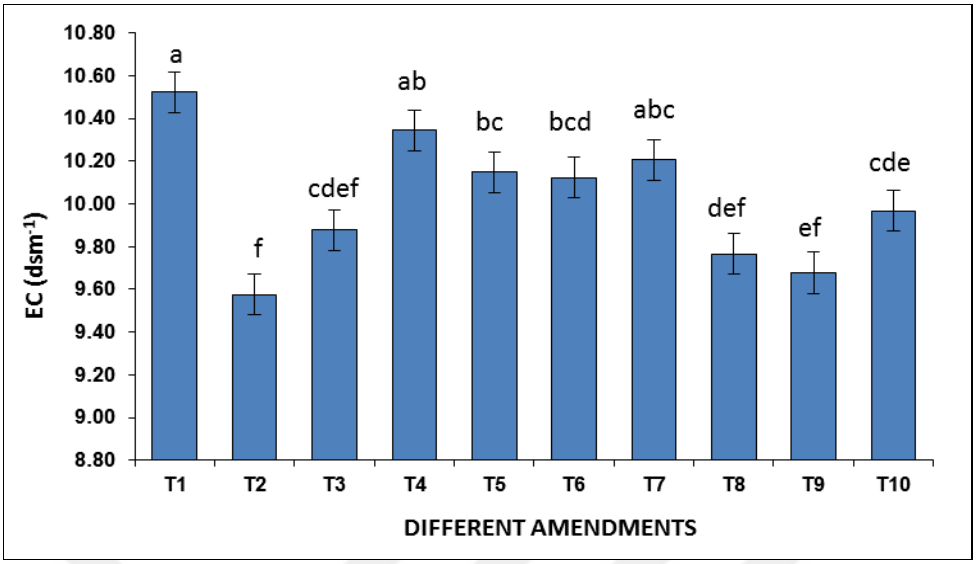

Figure 4.11. Modifications in the EC of saline-sodic soil with Cotton feedstock (CF), its biochar (CB) and combinations with halotolerant (average over incubation period) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1 – control; T2 – CF @ 10 tha^{-1}; T3 – CF @ 20 tha^{-1}; T4 – CF @ 10 tha^{-1} + Halotolerant; T5 – CF @ 20 tha^{-1} + Halotolerant; T6 – CB @ 10 tha^{-1}; T7 – CB @ 20 tha^{-1}; T8 – Halotolerant; T9 – CB @ 10 tha^{-1} + Halotolerant; T10 – CB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

4.2.2. Effect of Olive Pulp (OP) Its Biochar (OB) and Combinations With Halotolerant on Soil pH and EC

Data shown in the Table. 4.4 displays a significant impact of added amendments on pH of the soil. According to the results, T6 and T7 increased soil pH from 10.23 at Day 0 to 10.34 and 10.32 at Day 51, respectively. Likewise, T9 and T10 increased the pH values from 10.25 and 10.24 at Day 0 to 10.33 to10.34 at Day 105. Olive pulp applied @ 10 and 20 tha^{-1} also tended to increase soil pH ranging from 10.23 and 10.25 at day 0 to 10.33 at day 51 and day 105, respectively. Almost same increasing trends in soil pH was observed when OP was applied with halotolerant.

Olive pulp biochar @ 20 tha^{-1} had pH slightly lower than @ 10 tha^{-1} when applied alone, but variable results were seen when applied in combination with halotolerant. Whereas, OP

in both cases displayed lower pH with the higher application rate (20 tha^{-1}). The day 0 pH of halotolerant applied soil (10.24) was increased to a maximum of 10.34 at day 7, but dropped to 10.29 at day 73, and increased again (10.32) at day 105. Though, all amended treatments significantly increased pH during incubation time, control soil still had a relatively higher pH than the rest of the amendments at the end of the experiment (Figure 4.12.).

The soil EC has also been significantly affected by the added amendments and incubation timings (Table 4.5). A significant decrease in EC was seen soon after the incorporation of amendments in the soil (Day 0). Among various amendments the lowest soil EC was recorded in T7 (9.44 dSm^{-1}) while the highest was in the control (10.30 dSm^{-1}). Same as cotton stalk biochar an unexpected drop in soil EC under all the amendments was recorded at day 7. However, as the incubation progressed a steady increase in EC values was seen reaching the highest values at day 51. In contrast to CB, a declining trend of soil electrical conductivity started towards the later part of the incubation from day 73 to day 105. At the end of incubation, the lowest soil EC of 9.60 dSm^{-1} was recorded in T7 compared to the 10.53 dSm^{-1} in the control (Figure 4.13).

An increase in pH for all the amendments was recorded with time varying from 10.23 at day 0 to 10.35 at day 105, however the pH of amended soils was still lower than that of the control. Rise in pH with biochar addition to saline-alkaline soils is also confirmed by some other researchers. Application of sugarcane bagasse biochar @ 10 tha^{-1} after 120 days of incubation increased the pH from 7.78 to 8.04, which was 3.3 percent more than the control [205]. Similarly, Abdullaeva *et al.* in their study of physio-chemical parameters of saline-alkaline soil modified with 25 g kg^{-1} apple-wood biochar reported a substantial rise in the pH of biochar amended soil relative to unamended soil [206]. A research conducted by She *et al*. also confirms a rise in the pH of soil affected by salinity with the use of wheat straw biochar [207]. These findings are in accordance with the results of our study, except that the pH of the amended soils in our study was still lower than that of control. According to the reports, presence of organic ions and inorganic carbonates in biochar ashes makes it highly basic, thus its application would result in increased soil pH [208-211].

An increase in the soil pH due to the biochar application has been reported by many researchers [92, 205, 212-215], but most of these soils had lower pH compared with the

biochar pH [216-218]. According to some researchers the findings could be contrary if low-pH biochar is added to high-pH soils, particularly saline-sodic and sodic soils [217, 219-221]. However, the results of our study disagreed with many of the recent studies [135, 136, 185, 222-227], which showed a significant reduction in pH of salt-affected soils with biochar addition.

Not only both biochar but feedstocks also resulted in increased pH with time. Increased soil pH with cotton straw application has also been confirmed by other researchers. For example, Huang *et al.* reported an increase in soil pH ranging from 6.8 to 7.3 with the addition of cotton straw [228]. Likewise, Yu *et al*. performed an experiment to assess the effectiveness of cotton straw and its biochar on nitrogen use efficiency and cotton yield, as well as soil nutrient status [229]. Both cotton straw and biochar increased the soil pH, however, this increase was remarkably higher for biochar treatments. Regarding the effect of olive waste, Gougoulias *et al*. in their study of chemical and biological characteristics of sandy loam soil modified with waste from olive oil mills documented a little boost in soil pH [230]. In contrast, Hamed *et al*. reported a reduction in soil pH upon adding solid olive mill waste on calcareous soil [231]. The increase in pH values with cotton stalk and olive pulp over time in our study could be the consequence of the mineralization of proteins, amino acids and peptides to ammonia [229, 232].

Both biochar types resulted in increased electrical conductivity (EC) at the end of incubation compared to the values at day 0, however, same as pH, again control soil had the highest EC. The EC of CB amended soils was higher than that of OB ones, and that may be ascribed to the higher EC of CB. Results obtained in our study are supported by the research work conducted by some scientists on saline or alkaline soils, reporting an increase in EC after addition of biochar [205, 218, 220, 233]. The rise in EC can be credited to the elevated ash content of the biochar. Type, quantity and aging of biochar, initial salt content of biochar, soil and irrigation water, and experimental conditions are other possible contributing factors to modify the EC of salt-affected soils.

Amendments applied at higher rates had lower EC. Similar results are observed by Amini, who concluded considerable decreases in EC with an increased biochar application rate across the range of saline-sodic soils [224].

Table 4.4. Olive pulp biochar effect on soil pH

pH								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	10.26a	10.35a	10.34a	10.35a	10.34a	10.33a	10.35a	**0.009**
T2	10.25b	10.29c	10.27fg	10.26e	10.33bc	10.309c	10.32d	**0.008**
T3	10.23d	10.24g	10.26h	10.31c	10.32c	10.305cd	10.33bc	**0.009**
T4	10.238cd	10.27def	10.31c	10.30c	10.34a	10.32b	10.335bc	**0.007**
T5	10.23d	10.27def	10.27f	10.31c	10.33ab	10.28g	10.29f	**0.008**
T6	10.23d	10.28d	10.31d	10.31c	10.34a	10.29ef	10.31e	**0.009**
T7	10.23d	10.26f	10.27f	10.29d	10.32c	10.297de	10.31e	**0.008**
T8	10.24c	10.34b	10.32b	10.32b	10.30d	10.296ef	10.32d	**0.009**
T9	10.25b	10.29c	10.26gh	10.31c	10.31d	10.32b	10.328cd	**0.007**
T10	10.24c	10.27ef	10.29e	10.29d	10.32c	10.29fg	10.337b	**0.010**
LSD	**0.0065**	**0.0066**	**0.0084**	**0.0076**	**0.0083**	**0.0080**	**0.0095**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.5. Olive pulp biochar effect on soil EC

EC (dSm^{-1})								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	10.30a	7.79c	10.06a	10.80a	11.36b	11.75a	11.69a	**0.116**
T2	9.93c	5.83g	9.87b	10.42c	11.78a	10.38f	10.23d	**0.099**
T3	9.68e	6.87e	9.15f	9.96fg	11.20c	11.09d	9.82g	**0.112**
T4	10.04b	7.01d	9.73c	10.44c	11.77a	11.56b	10.14e	**0.065**
T5	9.63e	6.93e	9.31e	10.19e	11.03e	10.10g	10.07e	**0.108**
T6	9.81d	8.02a	9.81bc	10.30d	11.05d	10.74e	9.95f	**0.131**
T7	9.44f	7.93b	9.34e	9.95g	10.58f	9.93h	9.69h	**0.097**
T8	9.85d	7.79c	9.16f	9.54h	10.15g	10.84e	10.76c	**0.107**
T9	9.92c	6.79f	9.59d	10.04f	10.82e	11.24c	10.89b	**0.092**
T10	9.97bc	6.74f	8.62g	10.53b	11.10cd	11.04d	10.68c	**0.065**
LSD	**0.073**	**0.083**	**0.112**	**0.083**	**0.100**	**0.114**	**0.095**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

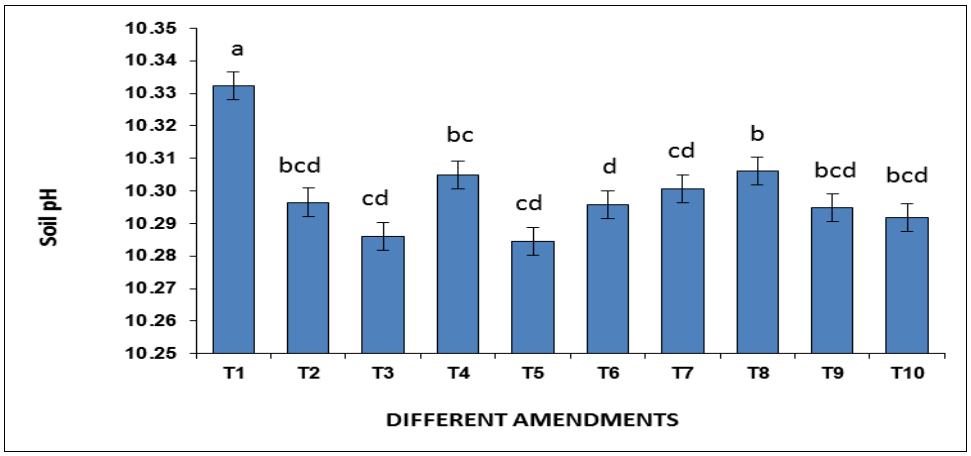

Figure 4.12. Modifications in the pH of saline-sodic soil with Olive pulp (OP), its biochar (OB) and combinations with halotolerant (average over incubation period) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1 – control; T2 – OP @ 10 tha^{-1}; T3 – OP @ 20 tha^{-1}; T4 – OP @ 10 tha^{-1} + Halotolerant; T5 – OP @ 20 tha^{-1} + Halotolerant; T6 – OB @ 10 tha^{-1}; T7 – OB @ 20 tha^{-1}; T8 – Halotolerant; T9 – OB @ 10 tha^{-1} + Halotolerant; T10 – OB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

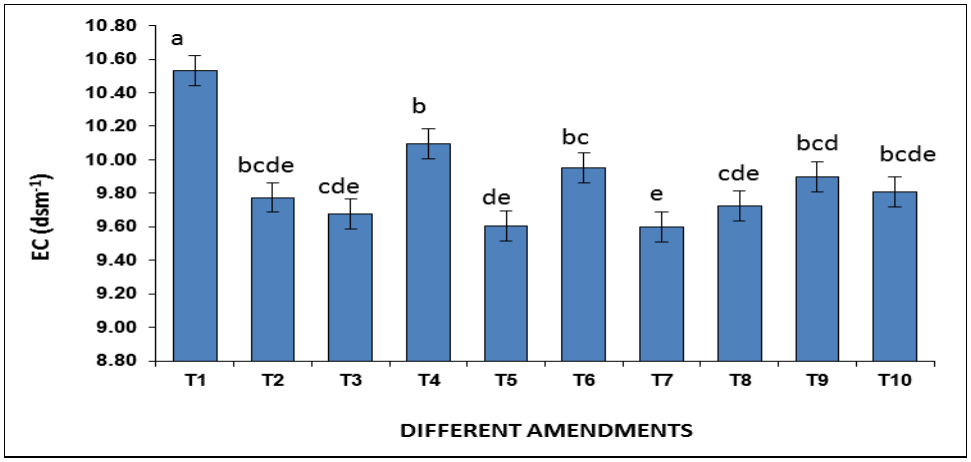

Figure 4.13. Modifications in the EC of saline-sodic soil with Olive pulp (OP), its biochar (OB) and combinations with halotolerant (average over incubation period) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– OP @ 10 tha^{-1}; T3– OP @ 20 tha^{-1}; T4– OP @ 10 tha^{-1} + Halotolerant; T5– OP @ 20 tha^{-1} + Halotolerant; T6– OB @ 10 tha^{-1}; T7– OB @ 20 tha^{-1}; T8– Halotolerant; T9 – OB @ 10 tha^{-1} + Halotolerant; T10– OB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

4.2.3. Effect of Cotton Stalk (CS) Its Biochar (CB) and Combinations With Halotolerant on Soil Organic Matter (OM) and Soil Total Nitrogen (TN)

Soil OM during incubation showed significant changes depending on the periods of incubation and the type of amendments added (Table 4.6). An increase in OM content was noted at the beginning of the incubation upon the addition of various amendments. In general, soil amended with CS applied in conjunction with halotolerant or alone displayed higher OM compared to the control, biochar treatments and sole application of halotolerant. A substantial increase in OM content was observed for all treatments as the incubation period progressed. However, halotolerant when applied alone was not that efficient in increasing OM content when compared to the control. Among the various treatments, the highest OM content was observed under T5, which was at par with T2 and T3 (Figure. 4.14). By the end of incubation, the OM content ranged from a minimum of 8.9 g kg^{-1} to the maximum of 16.1 g kg^{-1} with a relative increase of 58.41 percent.

When comparing the application rates, both CS and its biochar applied at higher rates (20 tha^{-1}) had the highest soil OM.

Both CS and its biochar efficiently increased the soil OM from the start of incubation, however, the release of OM under biochar treatments was quite slow in the beginning but increased favorably with time.

All the amendments significantly affected soil total N (Table 4.7). According to the results obtained, the day incubation began TN varied between 0.51 g kg^{-1} in control to 0.75 g kg^{-1} in T10. As the incubation progressed, an increase in the amount of total N for all the amendments was observed. Figure 4.15 presents the general impact of various amendments on soil TN (averaged across incubation timings). Soil TN differed significantly among different amendments. The highest soil TN was recorded in the treatment where the soil was amended with 20 tha^{-1} CB + Halotolerant, followed by the treatment receiving 10 tha^{-1} CB + Halotolerant. The magnitude of the increase was from 24 percent to 129 percent over the control.

Compared to feedstock, biochar was more efficient in raising soil TN. While, sole halotolerant application was least effective among all the amendments.

The analysis of variance revealed that application rates also had a significant effect on soil TN. Both CS and CB applied @ 20 tha^{-1} were more effective in increasing the soil TN over 10 tha^{-1}, no matter applied alone or with halotolerant.

Table 4.6. Cotton stalk biochar effect on soil OM

Organic Matter (OM) (g kg^{-1})								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	8.0ef	8.9ef	10.2cd	9.2e	8.8e	9.9de	8.9e	**0.086**
T2	12.6bc	12.3b	13.4a	14.4a	12.6bc	13.1b	12.0cd	**0.083**
T3	14.5a	14.7a	13.0a	13.3b	14.9a	14.6a	12.9bc	**0.103**
T4	11.9c	10.8c	11.6b	11.2c	12.4bc	11.7c	11.1d	**NS**
T5	13.2b	14.2a	12.7a	13.0b	15.2a	14.7a	16.1a	**0.129**
T6	9.6d	12.4b	10.8bc	10.7c	10.7d	10.8cd	11.1d	**0.107**
T7	9.4d	10.1cd	11.0bc	9.9d	11.4cd	11.3c	11.7d	**0.115**
T8	7.1f	8.6ef	8.9e	8.8e	9.1e	9.2e	11.1d	**0.078**
T9	8.3e	9.2de	9.6de	9.1e	11.6bcd	11.3c	12.1cd	**0.116**
T10	8.2e	8.2f	11.1b	9.4de	12.7b	13.4b	13.2b	**0.135**
LSD	**1.103**	**0.095**	**0.084**	**0.075**	**0.123**	**0.105**	**0.106**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.7. Cotton stalk biochar effect on soil TN

Total Nitrogen (TN) (g kg^{-1})								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	0.51e	0.47f	0.45e	0.49f	0.45h	0.46g	0.39g	**0.0062**
T2	0.66bc	0.69cd	0.81abc	0.85d	0.90ef	0.92e	0.98e	**0.0062**
T3	0.67b	0.68d	0.87a	0.96c	0.98cd	0.88e	0.92e	**0.0064**
T4	0.65bc	0.74abc	0.80bc	0.78e	0.86f	0.92e	0.95e	**0.0066**
T5	0.56de	0.59e	0.68d	0.83de	0.95de	0.99d	1.05d	**0.0059**
T6	0.63bc	0.72bcd	0.76c	0.88d	0.93de	1.07c	1.14c	**0.0072**
T7	0.69ab	0.75ab	0.84ab	0.83de	1.02c	1.10c	1.19bc	**0.0059**
T8	0.60cd	0.54e	0.50e	0.52f	0.59g	0.62f	0.62f	**0.0063**
T9	0.67b	0.75ab	0.85ab	1.02b	1.14b	1.19b	1.25b	**0.0069**
T10	0.75a	0.78a	0.81abc	1.11a	1.23a	1.30a	1.38a	**0.0075**
LSD	**0.0068**	**0.0055**	**0.0065**	**0.0060**	**0.0062**	**0.0057**	**0.0061**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

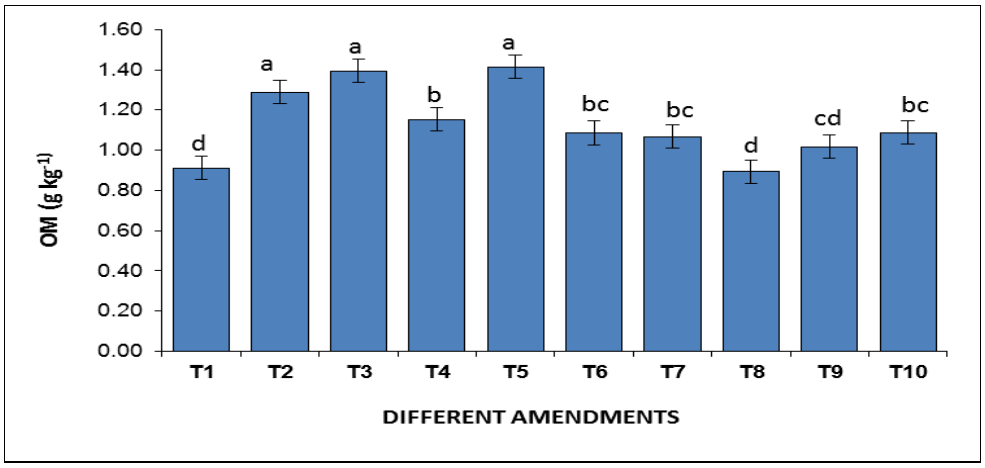

Figure 4.14. Modifications in the OM of the saline-sodic soil with Cotton stalk (CS), its biochar (CB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– CS @ 10 tha^{-1}; T3– CS @ 20 tha^{-1}; T4– CS @ 10 tha^{-1} + Halotolerant; T5– CS @ 20 tha^{-1} + Halotolerant; T6– CB @ 10 tha^{-1}; T7– CB @ 20 tha^{-1}; T8– Halotolerant; T9– CB @ 10 tha^{-1} + Halotolerant; T10– CB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

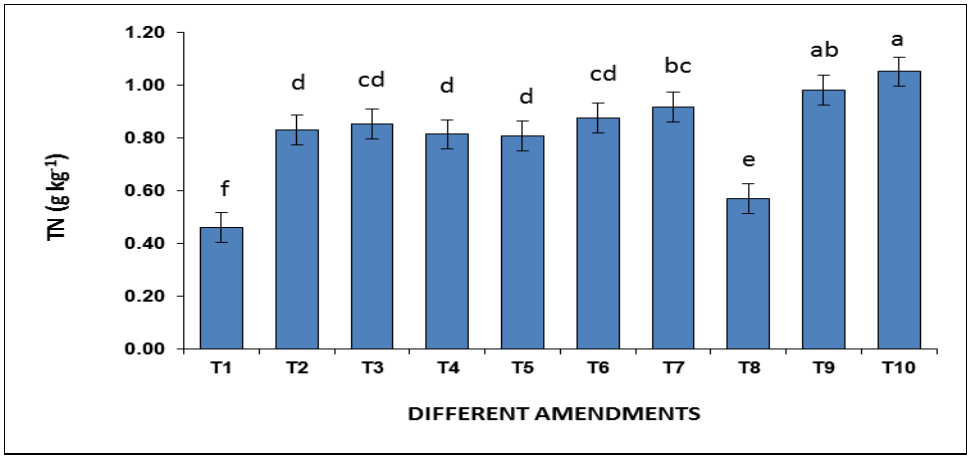

Figure 4.15. Modifications in the TN of the saline-sodic soil with Cotton stalk (CS), its biochar (CB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– CS @ 10 tha^{-1}; T3– CS @ 20 tha^{-1}; T4– CS @ 10 tha^{-1} + Halotolerant; T5– CS @ 20 tha^{-1} + Halotolerant; T6– CB @ 10 tha^{-1}; T7– CB @ 20 tha^{-1}; T8– Halotolerant; T9– CB @ 10 tha^{-1} + Halotolerant; T10– CB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

4.2.4. Effect of Olive Pulp (OP) Its Biochar (OB) and Combinations With Halotolerant on Organic Matter (OM) and Total Nitrogen (TN)

Analysis of variance had a significant effect for treatments and incubation time on soil OM. Results presented in Table 4.8 illustrate an increase in OM content for most of the amendments shortly after their addition in the soil (day 0). However, treatment that received 10 tha^{-1} OB and halotolerant did not differ significantly in its OM content from control treatment. All the amended treatments increased soil OM significantly compared to the control at the end of study. But, OP applied alone or in combination with halotolerant at both rates was more efficient than its biochar. The OM ranged from 7.9 g kg^{-1} in control to 14.3 g kg^{-1} in the treatment receiving 20 tha^{-1} OP+ halotolerant and the magnitude of increase was 81 percent (Figure. 4.16). The release of OM in OP amendments was quite speedy in the beginning of incubation, but then turned out to be slow after day 51 reaching their lowest values at the end. While a totally opposite result was observed in context of OB treatments. Halotolerant application also significantly increased soil OM from 6.5 g kg^{-1} at day 0 to 9.7 g kg^{-1} at day 105, this shows a comparative rise of 49 percent.

Soil TN has also been impacted considerably by treatment types, application rates and incubation timings (Table 4.9). Generally, an increase in soil TN in all amended treatments was seen during incubation and higher TN content was recorded at the end of the 3.5 months. However, no significant changes were seen in the treatment that received only halotolerant. Both OP and its biochar applied alone or in combination with halotolerant were almost equally effective in increasing the soil TN.

At the end of the experiment, all the treatments significantly increased the soil TN relative to the control, but the treatment receiving both 20 tha^{-1} OB and halotolerant had the highest soil TN (1.140 g kg^{-1}) (Figure. 4.17).

Each biochar type (Cotton stalk and Olive pulp), their feedstock as well as halotolerant increased the soil OM and soil TN over the control, proving the suitability of these amendments for restoring the fertility status of degraded salt affected soils. The results of our research are in line with other researchers findings [96, 135, 228, 229, 234-238]. Oladele *et al*. observed a significant rise in soil TN content with rice husk biochar on a sandy clay loam Alfisol [239]. Nigussie *et al.* documented similar results by using corn

stalk biochar in chromium polluted soil and noted a considerable increase in TN by adding 10t/ha biochar [240]. Xu *et al*. also reported an increase in TN with the application of 9.2 tha^{-1} peanut shell biochar on red Ferrosol [241]. Abbasi *et al*. recorded a substantial rise in TN and OM following the addition of white clover residue and poultry manure biochar on a loam soil [211].

Biochar could be a direct source of many nutrients, depending on the nature of feedstock and pyrolysis. Recent studies have shown significant improvements in the nutrient status of biochar modified salt-affected soils [96, 224, 242-246]. For instance, Abdullaeva et al., 2014, recorded a considerable rise in OM and TN when apple wood chip biochar was added to a saline-alkaline soil. Usman *et al*. when Conocarpus biochar was added on a saline water irrigated sandy soil, recorded an rise in soil OM [247]. Agbna *et al.* found a significant improvement in the OM status of soil with applying high amount of wheat straw biochar under salt stress [248].

All the materials added significantly improved soil TN and OM, however the combination amendments with halotolerant PGPR'S had the highest values. This could be attributed to the ability of bacteria to immobilize mineral nutrients, fix nitrogen as well as synthesis and mineralize soil organic matter (SOM), thereby enhancing the efficiency of added amendments.

Amendments applied at both rates (10 and 20 tha^{-1}) enhanced soil TN and OM significantly over the control, though higher rate (20 tha^{-1}) had the higher nutrient values. These findings have been confirmed by Njoku *et al.* who observed higher magnitude of different nutrients with the higher quantity of biochar applied [249]. Likewise, Juriga *et al*. also reported that higher dose of biochar had higher value of SOM [250].

Since biochar contains a lot of organic carbon, organic matter and different nutrients, thus its addition to the soil increases TN and organic matter [71].

Table 4.8. Olive pulp biochar effect on soil OM

Organic Matter (OM) (g kg^{-1})								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	7.8f	7.3g	7.5h	8.0g	8.4e	8.3f	8.0e	**0.032**
T2	13.1b	12.4c	12.1b	11.9b	13.2b	11.8c	11.2c	**0.058**
T3	13.4b	13.0b	14.9a	12.3b	16.0a	14.4a	12.3b	**0.049**
T4	11.8c	11.2d	10.8c	10.4d	13.0b	12.1c	10.9c	**0.050**
T5	16.5a	14.6a	11.6b	13.6a	16.4a	14.1a	13.4a	**0.055**
T6	7.8f	8.1f	9.3f	10.9c	10.2cd	10.3d	9.4d	**0.058**
T7	9.3d	8.2f	10.2d	10.0de	9.7d	10.4d	9.9d	**0.049**
T8	6.5g	8.8e	8.6g	9.3f	9.4d	9.8e	9.7d	**0.070**
T9	8.2ef	7.4g	9.5ef	9.6ef	10.0d	11.8c	11.0c	**0.071**
T10	8.7e	7.9fg	10.0de	10.3d	10.8c	13.5b	12.2b	**0.091**
LSD	**0.063**	**0.056**	**0.065**	**0.051**	**0.078**	**0.053**	**0.061**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.9. Olive pulp biochar effect on soil TN

Total Nitrogen (TN) (g kg$^{-1)}$)								
Treatments	**Day 0**	**Day 7**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	0.49e	0.46f	0.44d	0.47d	0.44e	0.42e	0.37f	**0.0056**
T2	0.69bc	0.74bc	0.78b	0.86b	0.92bc	0.95c	1.00cd	**0.0064**
T3	0.75ab	0.69cd	0.77b	0.80c	0.89bc	0.97c	1.05cd	**0.0057**
T4	0.60d	0.63d	0.84a	0.82bc	0.87c	0.96c	1.02cd	**0.0049**
T5	0.74abc	0.78ab	0.84a	0.87b	0.94ab	1.00bc	1.09ab	**0.0068**
T6	0.61d	0.77bc	0.77b	0.84bc	0.88c	0.97c	0.99d	**0.0064**
T7	0.68c	0.81a	0.82ab	0.85bc	0.98a	1.04ab	1.10a	**0.0066**
T8	0.62d	0.56e	0.54c	0.52d	0.57d	0.61d	0.62e	**Ns**
T9	0.76a	0.77ab	0.80ab	0.87b	0.91bc	1.04ab	1.09ab	**0.0069**
T10	0.71abc	0.80ab	0.86a	0.96a	0.98a	1.09a	1.14a	**0.0057**
LSD	**0.0064**	**0.0066**	**0.0064**	**0.0057**	**0.0050**	**0.0061**	**0.0050**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

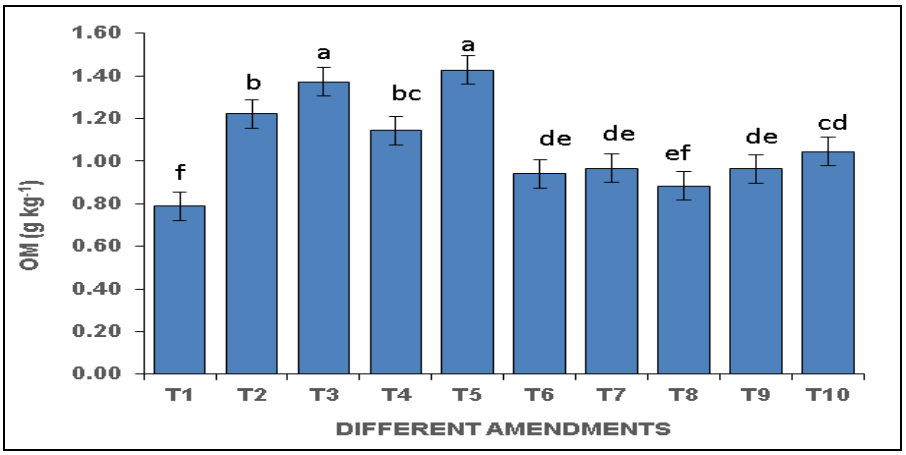

Figure 4.16. Modifications in the OM of the saline-sodic soil with Olive pulp (OP), its biochar (OB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– OP @ 10 tha^{-1}; T3– OP @ 20 tha^{-1}; T4– OP @ 10 tha^{-1} + Halotolerant; T5– OP @ 20 tha^{-1} + Halotolerant; T6– OB @ 10 tha^{-1}; T7– OB @ 20 tha^{-1}; T8– Halotolerant; T9– OB @ 10 tha^{-1} + Halotolerant; T10– OB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

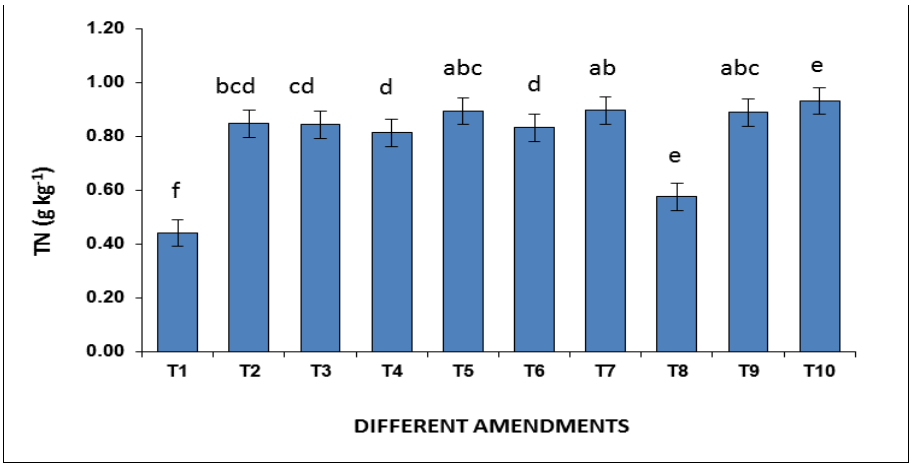

Figure 4.17. Modifications in the TN of the saline-sodic soil with Olive pulp (OP), its biochar (OB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– OP @ 10 tha^{-1}; T3– OP @ 20 tha^{-1}; T4– OP @ 10 tha^{-1} + Halotolerant; T5– OP @ 20 tha^{-1} + Halotolerant; T6– OB @ 10 tha^{-1}; T7– OB @ 20 tha^{-1}; T8– Halotolerant; T9– OB @ 10 tha^{-1} + Halotolerant; T10– OB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

4.2.5. Effect of Cotton Stalk (CS) Its Biochar (CB) and Combinations With Halotolerant on Soil Exchangeable Ions (Ca^{2+}, Mg^{2+}, K^+ and Na^+)

Table 4.10 outlines the impacts of various amendments on soil exchangeable cations. There was a substantial variation in soil Na^+ shortly after the addition of different amendments. In the initial sampling (at day 0), alone application of halotolerant showed a greater reduction in soil Na^+ (11778 mg kg^{-1}) while, the combination of halotolerant with 10 tha^{-1} biochar had the highest Na^+ content (16553 mg kg^{-1}). An increase in Na^+ content for most of the amendments was recorded as the incubation progressed, reaching its highest level at day 32. A remarkable decrease, however, was observed thereafter till the end of incubation (day 105). When analyzed the soil samples at day 105, all the amendments significantly reduced soil Na^+ content, CB, however, was more efficient than the feedstock. Application of halotolerant bacterial strains was also found effective in reducing the soil Na^+ level in comparison to the control. The greatest reduction in Na^+ content (10156 mg kg^{-1}) was observed in the soil receiving biochar @ 20 tha^{-1} + halotolerant.

Results specified in Table. 4.11 indicates a considerable increase in exchangeable soil K^+ with the added amendments. An irregular increasing or decreasing trend in soil K^+ was observed between the weeks 2-5. However, a steady rise in K^+ level was witnessed afterwards, with a gradual fall after week 11. Averaged across different treatments (Figure. 4.18) after the incubation period was ended, the highest K^+ value (1382 mg kg^{-1}) was tracked in the soil treated with 20 tha^{-1} CB + halotolerant (T10) followed by the same dose applied without bacteria (T7). Regarding application rates, amendments at higher rates had higher soil K^+ content.

Data presented in Table 4.12 shows the effect of different amendments on the Ca^{2+} status of soil. A noteworthy variation in soil Ca^{2+} level for different amendments was recorded in the first place (Day 0). Generally, all the amendments displayed significantly higher Ca^{2+} compared to the control (Figure. 4.18). Soil treated with halotolerant bacterial strain, however, had the highest Ca^{2+} level. The timings effect indicated a favorable increase in soil Ca^{2+} status for most of the amendments, reaching the highest values by the end of 3rd week. Although, a somewhat different trend was observed in case of halotolerant when applied alone and in combination with biochar at both rates. The highest values for these

amendments were seen at week five. Thereafter, all the amendments displayed a gradual decline in Ca^{2+} level over time.

At the end of the incubation, the soil Ca^{2+} under various amendments varied between 1834.7 and 2209.3 mg kg^{-1} compared to the 1420.7 mg kg^{-1} in control. The magnitude of increase among various amendments was 38 to 61 percent over the control.

According to the results obtained, soil Mg^{2+} status was also significantly affected by the added amendments. A rise in the Mg^{2+} level was witnessed till day 51, then values tend to decline slightly as incubation progressed (Table. 4.13). Among different amendments, application of 20 tha^{-1} biochar + halotolerant had considerably higher Mg^{2+} relative to the rest. Evaluating the efficiency of different amendments, combination of CB and halotolerant increased the soil Mg^{2+} status more proficiently than the sole use of CB. Whereas, in case of CS the result was totally opposite.

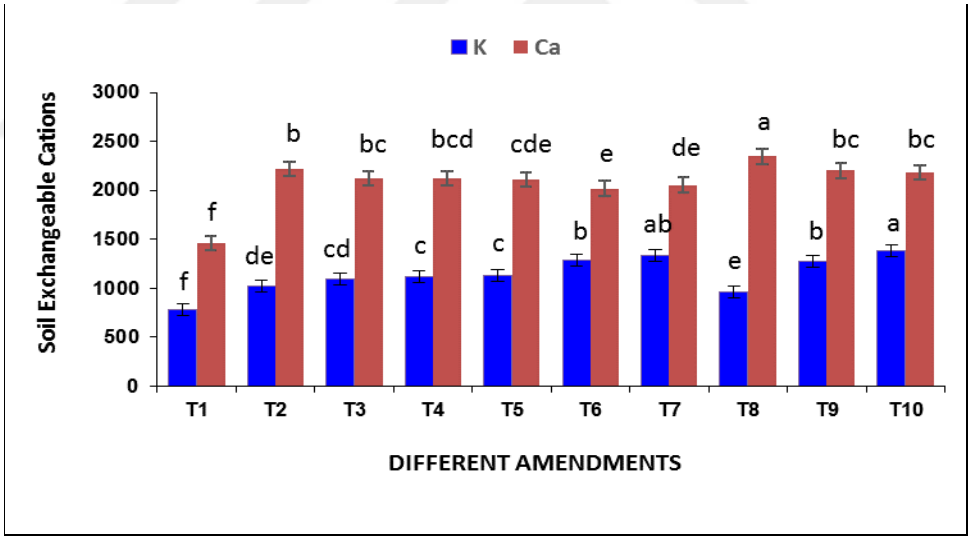

Figure 4.18. Modifications of exchangeable ions (Ca^{2+} and K^{+}) in the saline sodic soil amended with Cotton stalk (CS), its biochar (CB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– CS @ 10 tha^{-1}; T3– CS @ 20 tha^{-1}; T4– CS @ 10 tha^{-1} + Halotolerant; T5– CS @ 20 tha^{-1} + Halotolerant; T6– CB @ 10 tha^{-1}; T7– CB @ 20 tha^{-1}; T8– Halotolerant; T9– CB @ 10 tha^{-1} + Halotolerant; T10– CB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$

Table 4.10. Cotton stalk biochar effect on soil exchangeable Na^+

Na^+ (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	15555bc	16545a	17420e	17135c	15752a	14850a	**368.93**
T2	16448a	15880b	16420g	15575d	12685e	11789c	**235.49**
T3	15058d	15655c	18645ab	17868a	13280bc	11910b	**244.92**
T4	14993d	14690d	18440b	17828ab	13085cd	11895b	**175.25**
T5	15143cd	14190e	18085c	17630b	13480b	11912bb	**260.80**
T6	15865b	15835bc	18835a	15320e	13025d	11137e	**207.42**
T7	14908d	16735a	17785d	15165e	12155g	11085e	**614.67**
T8	11778e	13135-	15675h	14254g	12470ef	11645d	**78.27**
T9	16553a	14100e	17465e	14640f	12455f	10663f	**200.18**
T10	15350cd	15960b	17100f	14035h	12190g	10156g	**271.05**
LSD	**487.44**	**206.93**	**212.90**	**208.54**	**227.84**	**89.23**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.11. Cotton stalk biochar effect on soil exchangeable K^+

K^+ (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	732.83h	739.70g	722.90g	841.00j	889j	765.50j	**12.58**
T2	858.08g	840.60f	845.50e	1134i	1254.7i	1185.5h	**31.40**
T3	904.77f	874.20de	906.70c	1268.1g	1365.6g	1247.2g	**14.75**
T4	854.47g	863.40ef	881.50cd	1301.4f	1455.9f	1380e	**13.48**
T5	938.08d	896.75d	875.50d	1312.4e	1487.4e	1289.4f	**21.57**
T6	1047.0b	1103.5b	948.45b	1577.6c	1573.8d	1491.8c	**18.21**
T7	1033.3c	1154.0a	1042.5a	1653.1a	1667.6c	1432.5d	**18.61**
T8	515.42i	875.50de	792.25f	1228.4h	1286.7h	1094.7i	**17.35**
T9	931.13e	956.60c	935.10b	1515.7d	1747.2a	1572.2b	**21.50**
T10	1128.5a	1151.5a	1047.0a	1615.8b	1726.7b	1625.0a	**15.21**
LSD	**5.69**	**28.56**	**27.23**	**9.08**	**9.23**	**8.62**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.12. Cotton stalk biochar effect on soil exchangeable Ca^{2+}

Ca^{2+} (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	1202.7g	1529.7e	1596.7d	1530.3h	1479.7g	1420.7h	**28.38**
T2	1720.7b	2517.3abcd	2472.0bc	2386.7b	2141.3c	2088.3c	**112.12**
T3	1625.3d	2439.7cd	2370.7c	2280.3d	2057.7d	1971.7d	**134.35**
T4	1643.3c	2721.3a	2344.7c	2153.0e	1996.7e	1863.3e	**138.36**
T5	1723.0b	2692.7ab	2406.3bc	2210.7g	1984.7e	1834.7g	**157.03**
T6	1200.7g	2573.3abc	2477.0bc	2072.0f	1944.3f	1847.3f	**125.64**
T7	1292.7e	2475.7bcd	2445.7bc	2053.3f	2077.3d	1983.0d	**144.80**
T8	1937.0a	2326.7d	2828.3a	2534.3a	2254.7ab	2209.3a	**141.14**
T9	1272.3f	2586.3abc	2593.0b	2349.0c	2246.0b	2171.7b	**147.27**
T10	1271.7f	2455.0cd	2478.0bc	2410.3b	2277.0a	2197.3a	**143.26**
LSD	**7.98**	**230.72**	**200.46**	**37.40**	**26.91**	**12.26**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.13. Cotton stalk biochar effect on soil exchangeable Mg^{2+}

Mg^{2+} (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	95.597g	108.96g	120.24d	128.47g	110.33f	101.46h	**5.74**
T2	110.72b	183.97c	184.57b	189.65c	177.00b	129.47e	**9.43**
T3	110.32b	158.87e	167.70c	172.51e	154.20de	127.50ef	**12.03**
T4	106.87c	164.63de	170.93c	171.57e	153.17de	124.92f	**4.11**
T5	114.11a	169.87d	168.97c	163.26f	144.70e	116.86g	**7.95**
T6	100.73f	158.33e	175.53bc	180.79d	168.07bc	137.70d	**12.48**
T7	104.12d	178.50c	183.90b	188.71c	165.40bcd	144.58c	**7.04**
T8	102.84de	143.73f	173.67c	176.34de	155.83cde	139.37d	**10.22**
T9	101.56ef	206.67b	228.80a	239.93b	219.96a	194.22b	**4.39**
T10	106.54c	219.03a	235.20a	247.76a	226.83a	200.45a	**6.74**
LSD	**1.37**	**8.28**	**9.69**	**5.20**	**13.62**	**4.45**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

4.2.6. Effect of Olive Pulp (OP) Its Biochar (OB) and Combinations With Halotolerant on Soil Exchangeable Ions (Ca^{2+}, Mg^{2+}, K^+ and Na^+)

The effect of the amendments and incubation timings was significant for soil Na^+ (Table 4.14). A considerable reduction in Na^+ content was recorded after the addition of different amendments at day 0, when compared with the control. However, this reduction was not significant in certain treatments, i.e T2 and T4. Among the added amendments the minimum Na^+ level (10780 mg kg^{-1}) was observed for the treatment which was given only halotolerant bacterial strain (T8) while the maximum (15763 mg kg^{-1}) was in the treatment receiving OP @ 10 tha^{-1}. However, an increase in soil Na^+ level was seen over time, coming to the most elevated values after 4.5 weeks of incubation. From there on, the Na^+ level started to diminish towards the last part of incubation and lower Na^+ values were observed at the end of the study. All the amendments considerably reduced the Na^+ content of the soil compared to the control at the end of incubation. Olive biochar, however, regardless of whether used alone or in conjunction with halotolerant was more efficient than all OP treatments and sole application of halotolerant. The Na^+ level ranged between 14945 mg kg^{-1} in unamended control to 10030 mg kg^{-1} in the treatment which received 20 tha^{-1} OB and halotolerant bacterial strain, and the magnitude of decrease was 49 percent.

Soil exchangeable K^+ was considerably impacted by the amended treatments, incubation, days and application rates (Table. 4.15). The addition of all the amendments, except T8, resulted in increased soil K^+ compared to the control at day 0. But after that a fall in soil K^+ was started and the lowest K^+ values for most of the amendments were recorded at day 32. The soil K^+ status began to rise thereafter, reaching the highest values at day 51. At the end of the experiment, both OP and its biochar as well as halotolerant increased the soil exchangeable potassium efficiently over control. Averaged over incubation days, the highest soil K^+ values were witnessed in T7 (1173.35 mg kg^{-1}), preceded by T6 (1172.76 mg kg^{-1}) (Figure. 4.19). The magnitude of increase among various treatments was 23 to 55 percent relative to the control.

Different amended treatments considerably increased the soil exchangeable Ca^{2+} over time (Table. 4.16). The day incubation began, a rise in soil Ca^{2+} status was observed and the maximum values were recorded at week 5. Afterwards. Ca^{2+} level started to fall gradually towards the end of incubation. Compared to the level at day 0, a significant rise in soil

exchangeable Ca^{2+} was witnessed for all the amendments, including control, at the end of the experiment. Among all the amendments, sole application of halotolerant was more effective than the rest, increasing Ca^{2+} level from 1912 mg kg^{-1} at Day 0 to 2217 mg kg^{-1} at day 105. Biochar application rates (10 and 20 tha^{-1}) were almost equally effective in increasing soil Ca^{2+} level when applied in combination with halotolerant. But, higher rate displayed higher Ca^{2+} content when applied alone. Whereas, the OP application rates in both situations exhibited the similar pattern (Figure. 4.19).

Exchangeable Mg^{2+} was also significnatly affected by the amended treatments and incubation timings (Table. 4.17). Starting from the day of incubation almost all the treatments tended to increase the soil Mg^{2+} till day 31. Thereafter, a decline in soil Mg^{2+} level was seen as incubation proceeded, showing the lower values at the end of study (day 105). When compared the effectiveness of different amendments, addition of OB with and without bacterial strain showed higher Mg^{2+} levels than OP treatmnets. The highest Mg^{2+} level (189.73 mg kg^{-1}), though, was displayed by the treatmnet receiving 10 tha^{-1} OB+halotolerant (T9) displaying a comparative 79 percent rise over control.

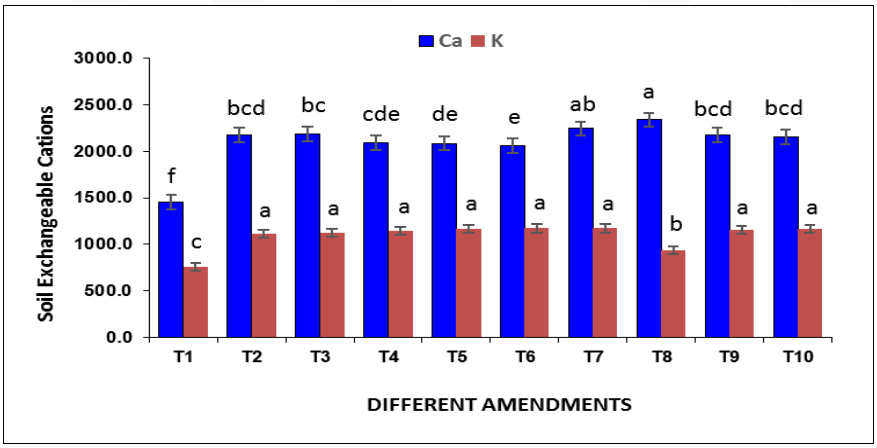

Figure 4.19. Modifications of exchangeable ions (Ca^{2+} and K^{+}) in the saline sodic soil amended with Olive pulp (OP), its biochar (OB) and combinations with halotolerant (average over incubation periods) incubated at 25˚C under monitored conditions in the laboratory. (Different treatments used are: T1– control; T2– OP @ 10 tha^{-1}; T3– OP @ 20 tha^{-1}; T4– OP @ 10 tha^{-1} + Halotolerant; T5– OP @ 20 tha^{-1} + Halotolerant; T6– OB @ 10 tha^{-1}; T7– OB @ 20 tha^{-1}; T8– Halotolerant; T9– OB @ 10 tha^{-1} + Halotolerant; T10– OB @ 20 tha^{-1} + Halotolerant. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$).

Table 4.14. Olive pulp biochar effect on soil exchangeable Na^+

Na^+ (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	15459a	16410b	17370a	16888a	15680a	14945a	**132.38**
T2	15763a	15890d	16950b	14620b	12975c	11840b	**140.79**
T3	14548b	14875f	16435d	13400f	12410e	11510d	**120.75**
T4	15378a	14780f	16485d	14110cd	13135b	11704c	**166.69**
T5	14420b	14635g	16470d	13900e	12585d	11496d	**130.71**
T6	14535b	15600d	16825bc	13945de	12290e	11075e	**336.99**
T7	14723b	15365e	15890e	12310h	11540g	10715f	**433.49**
T8	10780c	16205b	15700f	14165c	12415e	11540d	**79.103**
T9	14515b	15290e	16760c	13030g	11990f	10030h	**530.16**
T10	14710b	14365h	16825bc	13145g	12125f	10113g	**192.56**
LSD	**515.31**	**139.59**	**145.51**	**177.22**	**139.59**	**49.42**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.15. Olive pulp biochar effect on soil exchangeable K^+

K^+ (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	729.90e	737.30h	713.40g	837.30h	793.55i	727.03g	**24.90**
T2	863.08d	777.20f	820.75cd	1507.4f	1423e	1312cd	**15.64**
T3	887.27bc	845.25e	825.45bc	1496f	1410.1f	1295.1d	**7.09**
T4	869.95d	756.20g	806.05e	1569.7d	1514.8a	1354.8ab	**6.69**
T5	919.95a	875.75cd	815.50d	1534e	1504.3b	1368.1a	**18.36**
T6	871.25cd	883.10bcd	806.45e	1662.7b	1494.7c	1318.4cd	**9.83**
T7	913.63a	884.60bc	830.35ab	1637.3c	1468.9d	1305.4d	**7.75**
T8	522.17f	874.00d	796.35f	1067.1g	1218.2h	1123.2f	**31.00**
T9	861.23d	923.45a	797.80f	1677.7a	1394.9g	1264.9e	**13.85**
T10	894.03b	892.15b	837.65a	1647.5c	1404.8f	1334.8bc	**13.15**
LSD	**17.20**	**9.27**	**7.55**	**12.73**	**9.27**	**26.381**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.16. Olive pulp biochar effect on soil exchangeable Ca^{2+}

Ca^{2+} (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	1199.3i	15550.0e	1585.0d	1507.3e	1467.3g	1407.3h	**15.97**
T2	1669.0b	2495.3abc	2554.7bc	2246.3d	2098.0e	1999.7f	**102.60**
T3	1633.7c	2370.3cd	2713.0ab	2428.0bc	2028.0f	1940.0g	**96.13**
T4	1243.7h	2392.7cd	2486.7c	2366.7c	2082.7ef	1991.3f	**130.09**
T5	1264.7fg	2405.7bcd	2505.3c	2193.0d	2119.0de	2027.3e	**148.25**
T6	1257.3g	2397.7cd	2441.3c	2218.3d	2077.7ef	1988.7f	**136.18**
T7	1274.0f	2520.0abc	2571.3bc	2472.7ab	2472.7a	2154.3c	**193.98**
T8	1912.0a	2290.0d	2831.3a	2540.7a	2240.7b	2217.0a	**88.34**
T9	1379.7d	2627.3a	2516.3bc	2239.3d	2169.3cd	2106.7d	**142.19**
T10	1313.7e	2594.3ab	2410.7c	2224.3d	2204.0bc	2180.7b	**139.98**
LSD	**10.52**	**196.07**	**202.37**	**75.18**	**63.70**	**16.38**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.17. Olive pulp biochar effect on soil exchangeable Mg^{2+}

Mg^{2+} (mg/kg)							
Treatments	**Day 0**	**Day 17**	**Day 32**	**Day 51**	**Day 73**	**Day 105**	**LSD**
T1	90.54e	110.96g	118.12e	112.83h	105.16f	97.42h	**6.01**
T2	113.77a	201.94c	190.70bc	164.55e	143.21e	125.78f	**5.58**
T3	108.78ab	180.07d	194.87b	156.53f	137.86e	116.66g	**6.11**
T4	98.72cd	135.68f	165.60d	176.62d	154.62d	133.21e	**6.30**
T5	96.34de	150.13e	167.40d	164.72e	158.38d	134.19e	**11.71**
T6	100.18cd	197.87c	175.10cd	173.05d	166.39c	145.00d	**14.09**
T7	101.91bcd	231.40a	165.13d	185.81c	172.48c	151.10c	**15.75**
T8	101.66cd	144.73f	174.40cd	160.62ef	151.95d	143.33d	**6.05**
T9	102.58bcd	214.10b	226.27a	214.59a	200.00a	180.87a	**5.53**
T10	104.01bc	212.07b	218.73a	205.43b	191.12b	174.58b	**5.75**
LSD	**6.99**	**6.41**	**16.64**	**4.93**	**6.99**	**3.93**	

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

4.2.7. Effect of Cotton Stalk (CS), Olive Pulp (OP), Their Biochar and Combinations With Halotolerant on Exchangeable Sodium Percentage (ESP) and Sodium Adsorption Ratio (SAR)

All of the amendments significantly reduced the soil SAR and ESP at the end of incubation (Figure 4.20 and 4.21). Both char materials were almost equally effective in lowering soil salinity and sodicity to some extent. However, Olive pulp and its biochar exhibited lower values for both SAR and ESP. Application of salt tolerant bacterial strain were also equally effective. Regarding application methods, each material and its biochar when applied together with halotolerant lowered the SAR and ESP more efficiently than when applied alone, except for CS. Amendments applied at higher rates had a lower SAR and ESP values, however, again the result was opposite in case of CS.

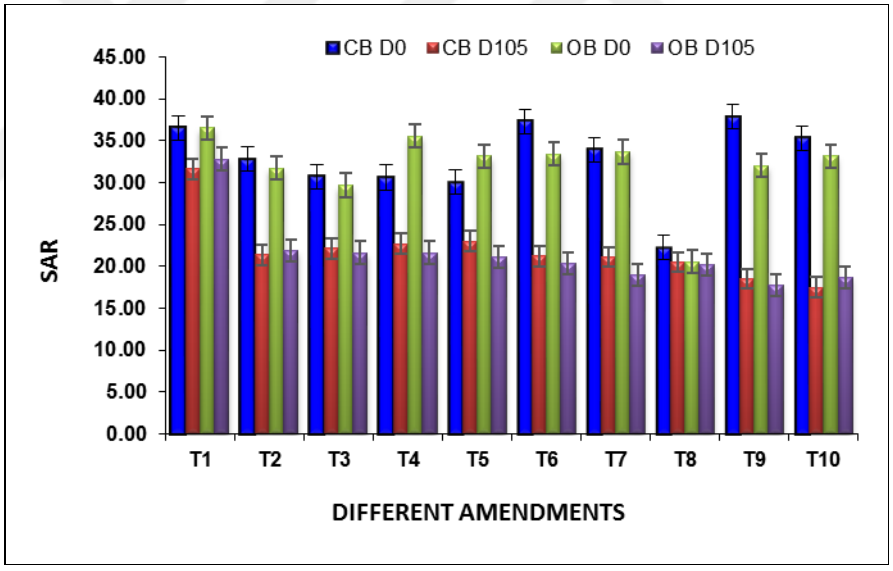

Figure 4.20. Soil SAR as affected by different amendments of Cotton stalk and Olive pulp biochar

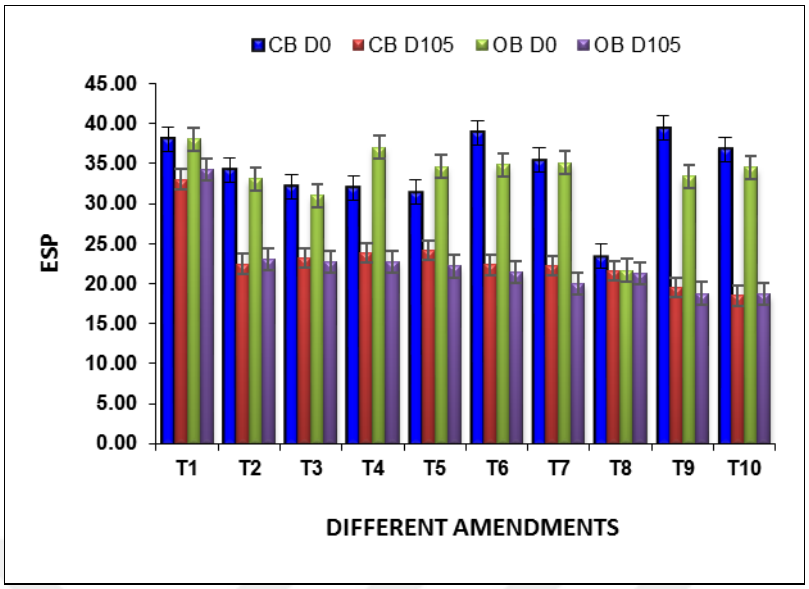

Figure 4.21. Soil ESP as affected by different amendments of Cotton stalk and Olive pulp biochar

In contrast to the control, different amended treatments significantly increased soil CEC and exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}) at the end of incubation. This rise is due to the presence of large number of cations in original plant materials (Cotton and Olive). The concentration of exchangeable cations in cotton biochar modified soils was slightly greater than that of olive biochar and the reason of difference was most probably because of unique chemical composition of the organic amendments. Results obtained in our study are confirmed by other researchers who also reported increased CEC and exchangeable cations by adding different biochar types in different soils [217, 228, 231, 234, 250]. Silva *et al.* [251] concluded that exchangeable Ca^{2+}, Mg^{2+} and K^{+} levels increased with increasing biochar application. Nguyen *et al.* reported that the addition of rice-husk and -straw biochar resulted in higher soil cation exchange capacity (CEC) as well as exchangeable Ca^{2+}, Mg^{2+} and K^{+} than no biochar [252]. Biochar application with elevated CEC can improve the soil CEC.The increase in soil CEC upon biochar addition could be the result of high porosity and high surface area of biochar [225, 228]. Additionally, the slow oxidation of biochar in soils can also improve the soil CEC and thus boost the soil's nutrient retention ability [226, 240]. As a consequence, all of these mechanisms promote the biochar restriction of the process of soil salinization.

At the end of the study, there was a significant decrease in soil exchangeable sodium (Na^+), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR). These findings are in line with the research of Alcívar *et al.* who stated that SAR, ESP and soil exchangeable Na^+ levels reduced significantly when biochar and gypsum were mixed applied [248]. After four months of incubation, Wu *et al.* noted a significant reduction in soil ESP with biochar application [231]. Similarly, Sappor *et al.* recorded the decreased exchangeable Na^+ due to the addition of saw-dust biochar [253]. According to Chaganti *et al.* incorporation of organic amendments (manures, compost, biochar and biosolids) enhance the hydraulic conductivity of saline-sodic soil, which in turn promotes salt leaching and significantly reduces soil ESP and SAR [232]. In addition, the implementation of organic amendments improves the Ca^{2+} concentration and encourages the removal of adsorbed Na^+, resulting in decreased soil SAR [253]. Chemical reactions in the soil matrix have a significant impact on the relative modifications in soil exchangeable Na^+ [222]. Another probability might be that the biochar's negative surface charges could adsorb salts from soil (e.g. Na^+) or salts could get entrapped in biochar fine pores and thus reduce exchangeable Na^+ concentration [235]. Biochar-induced decrease in saline water's upward motion can also lead to a decrease in soil salinity [247, 252].

Significantly higher concentration of soil exchangeable calcium (Ca^{2+}) was observed after combined biochar and halotolerant application. These results suggest that biochar and halotolerant PGPBs have a synergistic effect to bring more Ca^{2+} to the soil solution, in this way, sodium exchange from soil exchange sites is improved. This could likely be ascribed to the chemical composition of CB and OB (1.79 and 5.25 percent Ca^{2+}, respectively), as well as calcite dissolution ability of bacteria. Increased availability of Ca^{2+} and Mg^{2+} has been noted by many scientists after the addition of biochar in normal [210, 215, 245, 247] and saline environments [230, 247, 244, 246, 247], thereby suggesting the use of biochar as an effective ameliorant in degraded soils.

4.3. EFFECT OF COTTON STALK (CS), IT'S BIOCHAR (CB), AND HALOTOLERANT ON WHEAT GROWTH AND POST HARVEST SOIL PROPERTIES

4.3.1. Wheat Growth Parameters

A significantly possitive effect of applied amendments on wheat growth parameters (shoot and root length, shoot fresh and dry weight) was seen (Figure 4.22 and 4.23). All the amendments significantly increased the growth parameters when compared to the control, however their combinations with halotolerant were highly effective. The highest values for all the parameters were obtained with the combined application of 20 tha^{-1} biochar and halotolerant (T10). Sole application of halotolerant also had a positive effect on improving wheat growth under salinity stress.

Results obtained in our study are supported by many other researchers [134, 136, 215, 223, 253-258] . Kanwal *et al.* in their study concluded an improvement in the germination and growth of wheat under salinity stress amended with 1 and 2 percent biochar [258]. Similarly, Akhtar *et al.* documented that combined application of biochar and plant growth promoting bacteria mitigated the adverse effect of salinity on maize [254]. Again, Akhtar *et al*. in a pot experiment concluded a positive effect of biochar amendments on wheat performance under salinity stress [215]. Usman *et al.* in an experiment documented that conocarpus biochar significantly increased the tomato yield under saline water irrigation [219]. Likewise, Agbna *et al.* conducted a greenhouse experiment with biochar and concluded an overall improvement in tomato growth and yield under saline soil condition [255].

Improvements in crop germination, growth and yield with biochar under salinity stress may be due to the Na^+ sorption by biochar, thereby limiting the Na^+ uptake by the plants, hence protecting them against salt stress. Biochar, indirectly enhances crop performance by improving soil physio-chemical and biological properties of saline soil thus providing the conditions favourable for crop establishment.

Halotolerant PGPBs safeguard related crops against damaging salinity impacts. Production of exopolysaccharide (EPS) can be beneficial in attachment of bacterial cells to biotic

surfaces like plants. Inoculation of EPS-producing bacteria would be an appreciated approach for amelioration and improving crop productivity of the salt-affected soils [189, 193, 194]. EPS under salinity stress can bind sodium ions and alleviate its toxic effect in the soil [190]. Salt free soil thus favors the plant growth by providing the sufficient nutrients in the soil [191]. Production of ammonia and HCN may participate in the inhibition of many plant pathogens and metalloenzymes under salinity stress [196]. Moreover, siderophores are considered biocontrol agent as they sequester the iron from the pathogen needed for their growth [198], thus protecting the plants from numerous fungal or bacterial diseases [199, 200].

As salt affected soils are highly degraded and need special attention for their reclamation, the application of halophilic bacteria could be a sensible approach to help restore the soil's fertility and productivity, thereby improves the crop yields.

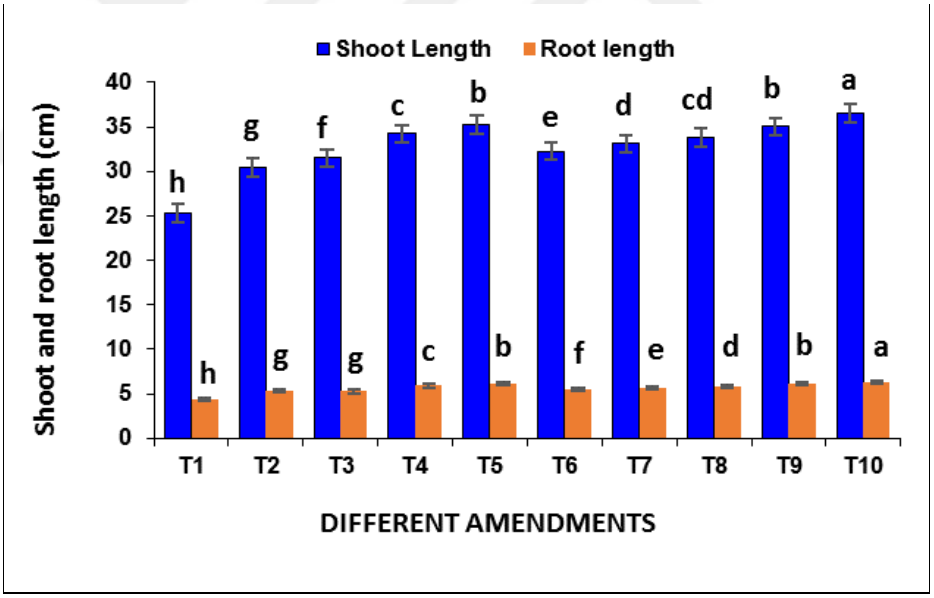

Figure 4.22. Cotton biochar effect on shoot and root length of wheat. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$

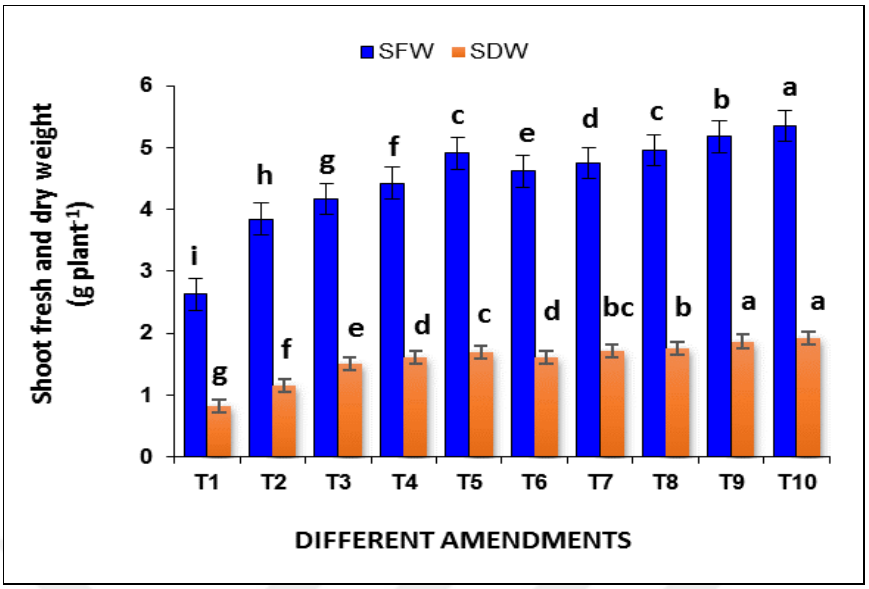

Figure 4.23. Cotton biochar effect on shoot fresh and dry weights of wheat. The letters on each bar indicate the statistical differences among the treatments with $p \leq 0.05$

4.3.2. Soil Properties After Crop Harvest

The soil in the pots was analyzed for certain chemical properties after the harvest. Analysis of variance had a substantial effect for amendments on the selected soil properties (Table 4.18).

For all treatments, a substantial decrease in soil pH was observed after crop harvest. Control soil had the highest pH (10.347) while the lowest (10.280) was recorded in T6 where 10 tha^{-1} biochar was applied. The reduction in pH varied between 10.280 to 10.342 among different amendments. Lower the application rates, lower was the pH. All treatments of biochar significantly lowered the soil pH relative to the control however, alone application of biochar was more effective than its combination with halotolerant. Almost same trend was seen for cotton stalk applications.

Soil electrical conductivity (EC) was also significantly reduced in comparison to the control. The difference among treatments was also significant and the lowest EC value (4.23 dSm^{-1}) was recorded in the treatment receiving 20 tha^{-1} CS, followed by 4.52 dSm^{-1} in halotolerant treatment. In case of CS, soils amended with higher application rate (20 tha^{-}

^{1}) had lower EC, regardless of whether used alone or combined with halotolerant. While results were opposite when coming to biochar. Again, as observed in pH, co-application of treatments with halotolerant had higher EC compared to their sole applications. Comparing biochar with its feedstock, feedstock was more effective in lowering the soil EC.

Both cotton stalk (CS) and its biochar (CB) as well as sole application of halotolerant improved the OM significantly relative to the control. Soil OM's concentration varied between 9.62-14.13 g kg^{-1} among different amendments. The OM content increased with incresing the application rates and the maximum value was observed in the treatment receiving 20 tha^{-1} CS + Halotolerant, followed by the same rate of biochar. Soil TN concentration in the control was 0.035 percent and was increased to the maximum of 0.13 percent with the combine appliaction of halotolerant PGPBs and 20 tha^{-1} biochar.

Exchangeable cations were aslo greatly affected by the amendments. An increase in Ca^{2+}, Mg^{2+} and K^{+} contents was recorded, while on the other hand Na^{+} was significantly reduced (Table 4.19). Though all the amendments significantly reduced the soil Na^{+} level, sole application of halotolerant was the most effective amongst them. Results indicated that Na^{+} contentration was 68.44 Cmol (+) kg^{-1} in control soil and was decreased to the smallest point of 51.46 Cmol (+) kg^{-1} indicating a comparative reduction of 25 percent.

Increasing the application rates led to the increased concentration of K^{+} and Mg^{2+}. Biochar at 20 tha^{-1} applied together with halotolerant had the highest values of 3.04 Cmol (+) kg^{-1} and 1.38 Cmol (+) kg^{-1} for K^{+} and Mg^{2+}, respectively. However, result was opposite in case of Ca^{2+}, where soil amended with lower rate of both CS and CB had the maximum Ca^{2+} level.

Overall, when comparing the cotton biochar with the feedstock, biochar was more efficient in improving the nutrient status of saline sodic soil. Undoubtedly, sole application of amendments had positive effect on soil health but the effect was more obvious for their combination with halotolerant.

Some scientists also noted a reduction in EC of saline sodic soils with biochar implementation [230, 234, 241, 248]. This can be ascribed to the biochar's fine pore structure which allows the adsorption of different materials by physically trapping them in the pores [238, 249). But the formation of the pore relies on the biochar's manufacturing temperature. Biochar manufactured at elevated temperatures has a larger surface area and

micro porosity [250], leading in increased salt absorption in the functional groups on the biochar surface [235, 251, 252].

Table 4.18. Cotton stalk biochar effect on post harvest soil chemical parameters

Treatments	pH	EC (dSm^{-1})	OM (g/kg)	TN (%)
T1	10.347a	5.31a	7.58 i	0.035g
T2	1.301f	4.63f	10.41f	0.090de
T3	10.308de	4.23h	11.29c	0.080e
T4	10.342b	5.05b	9.78g	0.090de
T5	10.322c	4.88e	14.13a	0.095cd
T6	10.280g	4.92de	9.83g	0.11bc
T7	10.305ef	4.94d	10.57e	0.12b
T8	10.313d	4.52g	9.62h	0.055f
T9	10.306ef	4.94cd	10.69d	0.12b
T10	10.326c	4.97c	11.62b	0.13a
LSD	**0.0053**	**0.0462**	**0.062**	**0.012**

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

Table 4.19. Cotton stalk biochar effect on post harvest soil exchangeable cations

Treatments	Na	K	Ca	Mg
		Cmol +/kg		
T1	68.44a	1.88h	7.05j	0.86e
T2	63.29b	2.22f	9.87e	1.14bc
T3	60.84c	2.41d	8.32g	1.12c
T4	60.15e	2.26f	8.24h	1.04d
T5	60.93c	2.35e	8.17i	1.06d
T6	59.37f	2.89b	9.75f	1.15bc
T7	56.49h	2.56f	9.93d	1.17b
T8	51.46i	2.41d	11.14b	1.07d
T9	60.62d	2.65c	11.81a	1.35a
T10	57.10g	3.04a	10.34c	1.38a
LSD	**0.14**	**0.047**	**0.062**	**0.043**

Note: Different letters in each column show significant differences among treatments with $p \leq 0.05$

5. CONCLUSION

Biochar (Cotton and Olive) application slightly increased the already high pH and EC of the saline-sodic soil after 3.5 months of incubation. Therefore, applying biochar under such circumstances would not be a sensible approach, unless coupled with other filed practices including the use of gypsum or leaching. However, on the positive side both biochar types significantly increased the OM and TN content of the soil, especially when applied in combination with halotolerant. Salt affected soils are highly depleted in nutrients and have almost negligible microbial activity. Biochar as a pure carbon source improves soil carbon stock when added to the soil, providing a rich source of energy to the microbes, hence increasing biological activities and microbial biomass carbon. High biological activity is one of the key factors responsible for healthy soil.

Moreover, biochar application considerably increased the CEC and exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}), which in turn reduced soil exchangeable Na^{+}, SAR and ESP, thereby improving the soil physical properties including soil structure, aggregate stability, bulk density and hydraulic conductivity.

Each biochar type greatly affected the soil properties when compared to the control; however, more promising results were seen for their combine application with halotolerant. Thus, co-application of biochar and halotolerant PGPBs is strongly suggested for the reclamation of deteriorated saline-sodic soil, as well as improving the nutrient status.

High pH and EC of saline-sodic soils is a limiting factor for crop growth, as it creates an environment unsuitable for them to absorb enough nutrients for healthy plant growth. Therefore, it is critical to consider the biochar characteristics such as biochar type (acidic or alkaline) and feedstock source before using it for salt-affected soil reclamation. Soil properties, including soil texture, salinity and sodium levels, levels of nutrients and soil native C content, are also essential to consider before reclaiming.

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