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

THE EFFECTS OF HAZELNUT HUSK COMPOST WITH *Bacillus megaterium* var. *phosphaticum* ON PHOSPHORUS DYNAMICS OF SOIL AND WHEAT YIELD

Master's Thesis

Md Mahfuzur RAHMAN

Supervisor
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II Supervisor
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SAMSUN
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.ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **“THE EFFECTS OF HAZELNUT HUSK COMPOST WİTH *Bacillus megaterium* var. *phosphaticum* ON PHOSPHORUS DYNAMİCS OF SOİL AND WHEAT YİELD ”** prepared by **Md Mahfuzur RAHMAN**, and supervised by **Prof. Dr. Rıdvan KIZILKAYA** and **Assoc. Prof. Dr. Andrey V. GOROVTSOV**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 17.6.2022 .

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ÖZET

Bacillus megaterium var. *phosphaticum* İLE AŞILANMIŞ FINDIK ZURUFU KOMPOSTUNUN TOPRAK FOSFOR DİNAMİKLERİ VE BUĞDAY VERİMİ ÜZERİNE ETKİSİ

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Kompost, toprağın mevcut fosfor (P) durumunu iyileştirebilen ve ürün gelişimini teşvik edebilen sürdürülebilir bir toprak değişikliği olarak ortaya çıkmaktadır. Fındık zurufu uygulamaları, daha iyi toprak yönetimi ve bitki gelişimi için etkide bulunduğu önceki çalışmalarla belirlenmiştir.Buna karşın, sürdürülebilir buğday verimi için fındık zurufu kompostunun (HHC) fosfat çözücü bakteriler (PSB) ile birlikte uygulanması hakkında bilimsel bir bulgu bulunmamaktadır. Bu nedenle, bu çalışmanın temel amacı, fındık zurufu kompostunun ve *Bacillus megaterium* var *phosphoticum* ile aşılamanın topraktaki P dinamikleri ve buğday üretimi üzerineetkilerinin belirlenmesidir. Deneme, Ondokuz Mayıs Üniversitesi (OMÜ) Toprak Bilimi ve Bitki Besleme Bölümü serasında gerçekleştirilmiştir. Deneme toprağı OMÜ'nün tarımsal deneme arazisinden alınmıştır. Alınan toprak, kil bünyeli olup sadece artan dozlarda kompost (C) uygulaması (%0, %1C, %2C, %4C), sadece bakteri (B) uygulaması ve kompost ve bakterinin beraberce uygulaması şeklinde (%1C+B, %2C+B ve %4C+B) üç tekerrürlü olarak tesadüf parselleri deneme desenine göre yürütülmüştür. Deneme sonunda bitki gelişimi ve buğday bitkisinin tane verimleri belirlenmiştir. Buğday hasadından sonra, alınan toprak örneklerinde toprakların bazı kimyasal ve biyolojik özelliklerindeki değişimler araştırılmıştır. Elde edilen sonuçlar, fındık zurufu kompostunun artan dozlarda tek başına uygulanmasıyla hem de kompostun bakteri aşılaması ile topraklara uygulanması sonunda bitkideki ürün verimini artırdığı ve toprak özelliklerini iyileştirdiği belirlenmiştir. Ayrıca elde edilen sonuçlar, daha yüksek kompost uygulama dozlarının bitkinin su kullanım verimliliğini arttırdığını da göstermektedir. Bununla birlikte, daha yüksek buğday verimi için, %1 kompostun tek seferde uygulamasının yeterli olduğu saptanmıştır. Ayrıca, kombine işlemler göz önüne alındığında, %4 C+B uygulamaının en yüksek verimi gösterdiği saptanmıştır. Yapılan uygulamaların toprak pH'sını, EC'yi, organik karbonu ve değişebilir katyonları olumlu yönde etkilediği belirlenmiştir. Ayrıca, bitkideki P ile toprak alkalen fosfataz enzim aktivitesi arasında önemli bir negatif ilişkiler saptanmıştır. İyi bir bitki gelişimi için P'yi kaldırmakta ve toprakta eksilen bu besin maddesi bakterilerin P'nin çözünürlüğünü artırmak için fosfataz enzimini salgılayarak organik P'u parçalamakta ve topraktaki alınabilir P'nin miktarını artırmaktadır. Yapılan uygulamaların toprak solunumu ve mikrobiyal biyomas C'nu da artırdığı belirlenmiştir.

Anahtar Sözcükler: Fındık zurufu kompostu, *Bacillus megaterium* var. *phosphoticum*, Fosfor, Buğday verimi

ABSTRACT

THE EFFECTS OF HAZELNUT HUSK COMPOST WITH *Bacillus megaterium* var. *phosphaticum* ON PHOSPHORUS DYNAMICS OF SOIL AND WHEAT YIELD

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Compost has been revealed as a sustainable soil amendment, which can improve soil available phosphorus (P) status and stimulate crop development. Hazelnut husk applications have already displayed their performance for better soil management and plant growth. Interestingly, there is no clear information about implementing hazelnut husk compost (HHC) in conjunction with phosphate solubilizing bacteria (PSB) for sustainable wheat yield. Therefore, in this study, the prime objective was to evaluate the effect of hazelnut husk compost and *Bacillus megaterium* var. *phosphaticum* on soil P improvement and wheat production. The experiment was conducted under the Department of Soil Science and Plant Nutrition, Ondokuz Mayis University (OMU). The experiment was arranged in a completely randomized design with four single (1%, 2%, 4% compost and bacteria) and three combined treatments (1%C + bacteria, 2%C + bacteria, and 4%C + bacteria) with three replications and three controls. Plant development and grain yield were monitored carefully. The results demonstrate that both individual and merged applications significantly promoted plant growth and increased soil health. The results suggest that higher compost doses increase plant water-use efficiency. However, for better wheat yield, single application of 1% compost is sufficient, as this is significantly similar to 2% and 4% applications. On the other hand, considering the combined treatments, the 4% compost + bacteria illustrates the highest yield. It recommends that for single HHC implementation, a low dose is enough, and for HHC-bacteria integrated application, a higher dose is better to get the maximum grain production. Organic amendments positively influenced the soil pH, EC, organic carbon, and exchangeable cations. The plant-available P and alkaline phosphatase enzyme showed a significant negative relationship. It indicates that when plants uptake P for their development and soil continues to lack this nutrient, the bacteria become more functional to release phosphatase enzyme to solubilize P. The treatments also represented improvements in respiration and microbial biomass carbon in the soil. The outcomes of this research can assist in understanding how soil chemical and biological activities are influenced by hazelnut husk compost and PSB applications, which will be valuable in accomplishing appropriate soil management in sustainable agriculture.

Keywords: Hazelnut husk compost, *Bacillus megaterium* var. *phosphaticum*, Phosphorus, Wheat yield

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CONTENTS

SYMBOLS AND ABBREVIATIONS

BSR	: Basal Soil Respiration
C	: Compost
Ca	: Calcium
EC	: Electrical Conductivity
HHC	: Hazelnut Husk Compost
K	: Potassium
MBC	: Microbial Biomass Carbon
Mg	: Magnesium
P	: Phosphorus
pH	: Power of Hydrogen
PGPR	: Plant Growth Promoting Rhizobacteria
PSB	: Phosphorus Solubilizing Bacteria
SOM	: Soil Organic Matter
WUE	: Water Use Efficiency

FIGURES LEGENDS

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1. INTRODUCTION

The dramatic increase of the world population coupled with the growing demand for food and reduction of cultivable land has raised massive challenges for tomorrow's agriculture. Moreover, the covid-19 pandemic with locust crisis and political conflicts have generated additional threats to our global food system. Most agricultural lands are suffering from severe soil fertility loss due to soil organic matter depletion and nutrient imbalances which ultimately decline productivity (Lal, 2015; Sanchez, 2002). Land degradation has already affected more than half of the African population and created the continent's most focusing issue with an enormous cost. Because of this land degradation and falling crop productivity, each year around 42 billion US dollars in income and 6 million hectares (ha) of cultivable land are being lost (Bationo et al., 2006). Food insecurity and soil nutrient deficiency are directly linked to each other due to extended land use (Henao & Baanante, 1999). To fight against these challenges, the United Nations has set the second most important sustainable development goal (SDG), which is "Zero Hunger", and to achieve this goal, there is no other way except "Sustainable Agriculture" (Food Security and Nutrition and Sustainable Agriculture | Department of Economic and Social Affairs, n.d.). This concept has prompted research on developing regenerative resources by using modern techniques with less environmental impact (Tang et al., 2020). This theme emphasizes moving away from synthetic chemical uses and embracing biological approaches to ensure the production of quality food for all with managing soil health properly.

To make a safer future, the world is now moving towards the production of quality food and as the highest crop production will take place in the coming ages, phosphorus (P) is getting more consideration as a nonrenewable resource (Cordell et al., 2009; Gilbert, 2009). Because of its slow diffusion and strong fixation characteristics, it is considered one of the least available macronutrients in the soil. This essential element governs fundamental roles in plants' biological activities like photosynthesis, respiration, cell membrane formation, and nucleic acid synthesis (Wu et al., 2005). P assists in energy storage and transfer as this is a part of organic molecules, for example, adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Optimum P supplying level at the root zone is essential to maximize the efficiency of plant roots to mobilize and acquire P from the rhizosphere (Neumann &

Römheld, 2002). It is also important to support stem development, improve seed formation, and enhance nitrogen (N) fixation.

In spite of the abundant amount of total P stock (approx. 170 different phosphate contributing minerals) in soil, just a small portion (which is less than 1%) remains in available form (Bünemann, 2015). Besides, P loss in the surroundings is getting higher, which is about 2.6% per year (Pearce & Chertow, 2017). To fulfil the low phytoavailability of P and gain a high yield, chemical fertilizer application has been considered as a global response (Weber et al., 2014). However, the P use efficiency (PUE) is relatively low, and the recovery rate usually is less than 20% in one year of fertilizer application (McBeath et al., 2012; Simpson et al., 2011). Additionally, the injudicious application of this fertilizer and the lack of knowledge of the applicator can seriously affect soil health, especially the biological properties of soil. As a pollutant, phosphate was put in the same class as nitrate in the last century. Dissolved phosphates can stay in the soil for a long time and a part of it can also mix with the seawater through erosion, where it can be confined for hundreds of years (Correll, 1998; Daniel et al., 1998). Furthermore, the implementation of P fertilizers may form heavy metal pollution both in soil and plant tissue (Atafar et al., 2010; Huang & Jin, 2008).

In these circumstances, as an alternative, the use of rock phosphate could have brought a good outcome. But the drawback is that rock phosphate is characteristically very less soluble with high residual effects, and its release rate is also quite limited. Several aspects are responsible for governing the optimum utilization of raw phosphates by cultivated crops, especially the soil pH. This factor highly regulates the solubility of rock phosphate in soil (Bolan & Hedley, 1990; Kanabo & Gilkes, 1987). It has been found that when the soil pH remains high (alkaline condition), the use of raw rock phosphate does not bring any convincing change to the P nutrition of plants (Çağatay et al., 1973). On the contrary, the raw rock phosphate application increased crop production in the soil with less pH (acidic condition) (Chien & Menon, 1995). Hence, this is a pretty critical solution to deal with.

Therefore, we need to move forward to decide on sustainable ways by using an efficient agroecosystem that can intensify crop production and boost soil fertility status (Timmusk et al., 2017). The compost application in the soil is a sustainable strategy that has a high positive impact on nutritional values. It can also enhance soil structure and microbial activities (Candemir & Gülser, 2010; Martens, 2000). Compost is considered as a comprehensive source of different beneficial microbes, like bacteria, fungi, and actinomycetes (Boulter et al., 2002). These microbes embrace enormous significance through regulating plant hormonal biosynthesis, boosting the accessibility of soil macro and micronutrients, alleviating drought and salinity stresses, and supporting the bioremediation of heavy metals to increase plant development and yield (Ahemad & Kibret, 2014; Jacoby et al., 2017). Besides, composting is a promising technology for waste management that can restore soil fertility for improving crop production. It is a well-known process for recycling and humification of organic matters (Feng et al., 2014; Hande Ajinkya & Deshpande, 2014), which reduces the implementation of inorganic fertilizers in soil (Eneji et al., 2001). Wu et al. (2007) described that organic amendments increased both the microbial biomass P and the transformation of P in soil. This large microbial biomass is capable of averting the fixation or adsorption of soluble P (Khan & Joergensen, 2009). In addition, compost application can improve soil structure and reduce soil erosion (Diacono & Montemurro, 2010).

Again, the application of biofertilizers comprising plant growth-promoting rhizobacteria (PGPR), especially phosphate solubilizing bacteria (PSB), was found as an excellent alternative to increase plant nutrient availability and yield (Vessey, 2003). PSB characteristically secrete organic acids, like carboxylic acid (Deubel & Merbach, 2005), thus pH gets lower in the root zone area (He & Zhu, 1998), which ultimately can dissociate the complex forms of phosphate like $Ca_3(PO_4)_2$ in soil and boost the available form of P to the plants. *Bacillus and Pseudomonas* are the two commonly isolated genera of PSB found in the environment (Chen et al., 2006; Peix et al., 2004). Considering the fact that the bacterial community is essential in the biological transformation of organic molecules because of their specific metabolic activities, a better understanding of PSB functional role with compost application is quite necessary.

Here, it was hypothesized that: (a) wheat plant growth, soil chemical properties and microbial community will be enhanced by the hazelnut husk compost (HHC) application, and (b) the inoculation of PSB (*Bacillus megaterium* var. *phosphaticum*) to HHC might have intense the adaptability to increase the P status in soil with other soil chemical and biological factors, which could ultimately magnify wheat production.

Finally, this study aimed to evaluate:

- the effect of hazelnut husk compost, *Bacillus megaterium* var. *phosphaticum* and their integrated application on improving the availability of P in soil.
- the influence of these applications on the wheat production.

2. REVIEW OF LITERATURE

2.1. Composting and compost

Composting is an organized bio-oxidative process where the organic wastes are transformed into homogenous and plant-available forms (Azim et al., 2018). This process requires proper moisture and temperature also. Another way, this can be explained as a complex metabolic procedure where in aerobic condition different microbes act to generate their biomass through using N and carbon (C) (Azim et al., 2018). In this operation, microbes initiate to produce heat and a specific substrate in a more stable form, which is known as compost (Roman et al., 2015). Antízar-Ladislao et al. (2006) described composting facilitates the aerobic transformation of organic matter into humic substances with the action of various microorganisms, and by following this procedure; we get the stable product named compost. So, in a word, composting is the direct outcome of diversified microbial activities in an aerobic environment (V. K. Sharma et al., 1997). During composting, the most dominant microorganisms are bacteria and fungi (Galitskaya et al., 2017). Besides, actinomycetes have been found to be highly active in this biodegradation process, where they release a broad range of extracellular enzymes capable of metabolizing rigid molecules (Limaye et al., 2017).

Two major phases are involved in composting process (Adani et al., 1999). The first is the decomposition phase, where maximum biodegradable substances decompose, and the organic residues become stable through microbiological activities. This phase includes the first three stages of the composting process: mesophilic, thermophilic, and second mesophilic or cooling stage. The second phase is the humification phase, which corresponds to the maturation stage, where organic matter is reorganized into the stable matter. The composting process starts with the mesophilic stage, where mesophilic microorganisms break down C and N. The composting process starts with the mesophilic stage where mesophilic microorganisms break down C and N. Here, the temperature lies between 20 to 45^0C, and pH becomes low because of the production of organic acids (Hafeez et al., 2018). In the thermophilic stage (40 to 70^0C), the mesophilic microorganisms are replaced with thermophiles, actinomycetes, and fungi, and these microbes can break down the more complex C sources like cellulose and lignin (Chennaoui et al., 2018). In the cooling stage, the temperature is lowered down to 35 to 45^0C, and finally, in the maturation stage, the mixture stays at the environmental temperature (Makan et al., 2013). Moreover, different factors are responsible for regulating the composting process, which include temperature, moisture content, pH level, carbon-nitrogen (C/N) ratio, and raw materials texture (Gonawala & Jardosh, 2018; Hafeez et al., 2018; Ameen et al., 2016; Yan et al., 2015; Misra et al., 2003).

2.2. Hazelnut husk compost

In the world, Turkey is considered the highest hazelnut producer, contributing almost 75% of the world's total hazelnut production (500,000 to 650,000 tons per year) (Guney, 2013). Around 621,000 ha area in the Black Sea Region of Turkey is under hazelnut cultivation (Kızılkaya et al., 2015). After harvesting, nearly the same quantity of hazelnut husk remains as a waste product of agriculture. However, this waste can be turned into valuable compost, which contains a high amount of organic matter, approximately 93.16% (Kızılkaya et al., 2015). Besides, this hazelnut husk compost (HHC) application can improve the other physical, chemical and biological properties of soil that eventually can enhance the crop production (Petropoulos et al., 2020; Zeytin & Baran, 2003; Özenc & Çalışkan, 2001). Kızılkaya (2016), in his study, described the soil quality parameters viz. microbial carbon biomass, basal respiration, total organic C, total N, C/N ratio, NO_3^- N, aggregate stability, electrical conductivity, and hydraulic conductivity were remarkably enhanced due to HHC implementation in soil. At the same time, HHC application increased the exchangeable cations, aggregate stability, and initial infiltration rate and reduced the bulk density and penetration resistance significantly.

2.3. Influence of compost application and microorganisms on the phosphorus (P) dynamics of soil

As an essential macronutrient, P is extremely necessary to be present in the soil for completing the plant's life cycle as it governs a crucial role in its metabolic functions. Several P pools in soil, like Fe or aluminum (Al) bound P or calcium (Ca) bound P, are responsible for reloading the P when it is absorbed from the soil solution by plants (Saleque et al., 2004). The intense reaction of P with the soil colloids creates a critical situation where the diffusion mechanism is suppressed by the action of mass flow (Hinsinger, 2001). Another factor that is highly responsive to impacting the accessibility of Ca, Fe and Al and that eventually contributes to P fixation is the soil pH. On the one hand, in the soil with high pH (alkaline soil), the Ca^{2+} ion binds with phosphate ions and lessens the availability of P by generating insoluble phosphate materials. On the other hand, soil with low pH (acidic soil) produces hydroxyl phosphate precipitates by the action of Fe and Al with phosphate ions, and finally, the P becomes inaccessible to plants (Harris et al., 2006). Moreover, cation and anion on the exchange sites can influence the phosphate adsorption in soil. As diverse factors are responsible for P fixation, it is reasonably hard to find out the exact reason.

Compost is a big source of P (Nelson & Janke, 2007). The application of this organic amendment can increase the bioavailability of P in soil (Takeda et al., 2009). To develop soil productivity, the use of manure is a good option. However, as it has a high amount of moisture contents and low nutrient availability, a large mass is needed to fulfil the requirement. This can generate an excess problem of high labor costs and transport expenses for marginal farmers (Ibrahim et al., 2008). Composting offers one of the best solutions to escape this problem, as decomposition and humification processes can minimize these bulky properties. Compost has the ability to add much organic matter to soil with a low release of N and P to plants (Sullivan et al., 2002). At the same time, compost can help progress the physical properties of soil. Meanwhile, compost can improve the overall microbial status in soil.

Compost is an excellent source of diversified microorganisms characterized by the specific endogenous microflora (Saison et al., 2006). With the implementation of compost, different microbes are incorporated into the soil, which are vital to solubilize fixed P by producing organic acids. These organic acids can reduce the soil pH, control the chelation activities, fight with P adsorption sites, and form soluble complexes with different ions to release P. In acidic conditions, the highest soluble form of phosphate, $H_2PO_4^-$ is formed. This is a monovalent anion. When pH gets increased, the formation of divalent and trivalent forms become prevalent that are insoluble in soil solution. Therefore, the formation of organic acids can reduce pH level, which assists in the conversion of divalent and trivalent to monovalent ions. Many studies have found that phosphate solubilizing microorganisms produce oxalic acid, gluconic acid, tartaric acid, lactic acid, indol acetic acid, and gibberellins which can help maintain the acidic condition of the soil and enhance the crop yield

(Gutiérrez-Mañero et al., 2001). The phosphate solubilizing microorganisms have a very exclusive characteristic of releasing three specific enzymes, viz. phytase, C–P lyase and phosphatase. These three enzymes are remarkably active in mineralizing organic P in soil (Othman & Panhwar, 2014). Among the three mentioned enzymes, phosphatase is the widely released enzyme that can remove P from its surface by hydrolyzing phosphoric acid into phosphate ions (Billah et al., 2019).

Phosphate solubilizing microorganisms comprise the capability to solubilize insoluble P into plant-available form (Chen et al., 2006; Rodriguez et al., 2004). For example, the phosphate solubilizing fungi, viz. *Mucor ramosissimus*, *Penicillium expansum*, and *Candida krissii* were found adequate to enrich wheat development and P availability in soil solution (Xiao et al., 2009). The wheat productivity was also reported to be increased in both pot and field conditions through the inoculation of two specific PSB: *Bacillus megaterium* and *Planococcus rifietoensis* (Erman et al., 2010; Rajput et al., 2013). In another experiment, *Bacillus megaterium* was confirmed to improve the bunch size, weight and number of bananas (Attia et al., 2009). Chandra & Kumar (2008) conducted a field study with three microbes: PGPR, *Bacillus megaterium* as a PSB, and *Rhizobium leguminosarum*, to evaluate their influence on lentil plants. They found that the combined application of these three microorganisms significantly enhanced the nodulation and grain yield of lentils.

2.4. Can the application of compost create issues?

Several studies have represented the versatile benefits of composts in soil health improvement. Soil fertilized by the compost application maintains the nutrients in forms which are easily obtainable for plant root uptake and utilization (Razaq et al., 2017; Khater, 2015; Loks et al., 2014). At the same time, many researchers feel wary of the large-scale application of compost in agriculture as compost may induce some negative consequences, such as accumulation of heavy metals, soil salinization, and alkalization with groundwater pollution (Sharifi & Renella, 2015; Carbonell et al., 2011). This can also affect the remediation capacity in contaminated soil. Because of changes in soil external environment (pH), decomposition of organic matter and other factors due to compost application, fixed heavy metals can be slowly released as time passes (Huang et al., 2016). To overcome this complicated situation, implementing compost with beneficial microorganisms can bring an effective solution (Rahman et al., 2021; Ahmad et al., 2018). Besides, this co-application strategy in crop fields can also enhance nutrient (N, P, K) availability in soil, accelerate bioremediation of hydrocarbons, and initiate better protection against many soil-borne pathogens (Osei-Twumasi et al., 2020; Arif et al., 2017).

2.5. Combined application of compost and microorganisms to improve available nutrient status in soil

2.5.1. Improvement of nitrogen in soil

One of the most common issues during composting is the ammonia (NH_3) gas emission. This emission is highly responsible for N loss (about 21 to 77% of total N) in compost and adversely affects the environment (Yang et al., 2019). Interestingly, Zhao et al. (2020) described that the combinatory application of the thermotolerant nitrifying bacteria (TNB) and sewage sludge compost reduced ammonia emission, which is about 29.7%. It also decreased the N loss and enlarged the nitrifying bacteria population in soil with overall enhancing the performance of compost (temperature, pH, C/N ratio, organic matter status, and germination index). In another study, it has been found that inoculation of phosphorus-mobilizing rhizobacterial strain *Bacillus cereus* GS6 with food and fruit waste compost significantly improved nodulation and nodule efficiency (21.80 ± 0.81 mg N/ gram nodule dry weight) as well as ensured a greater accumulation of NPK contents in shoot, grain, and nodule biomass of soybean (Arif et al., 2017). Progress of N in soil has also been informed by Jiang et al. (2015), where they used some specific bacterial agents (nitrobacteria, *Azotobacter*) and pig manure compost together. The N status of compost can also be improved by adding N-fixing bacteria (*Azotobacter chroococcum* and *Azotobacter lipoferum*) (Kumar & Singh, 2001). Furthermore, they stated in addition to enhancing P status, mixing phosphate-solubilizing bacterium (*Pseudomonas striata*) in compost also enhanced N accessibility in soil.

2.5.2. Improvement of phosphorus in soil

Application of compost can cause organic matter stabilization and humification (Zhao et al., 2017). This increasing trend of humic matter can cause biofixation of phosphates which directly affect the P status in soil (Borggaard et al., 2005). This issue can be solved completely by using the PGPR, especially phosphate solubilizing bacteria (PSB) in compost (Vessey, 2003). Estrada-Bonilla et al. (2021) in their study showed that the application of sugarcane waste compost inoculated with *Bacillus* sp. BACBR04, *Bacillus* sp. BACBR06, and *Rhizobium* sp. RIZBR01 increased the phytate-degrading enzyme activity that was interrelated with the improvement of organic P content in the soil. Besides, they also found that this co-application increased the N and K content in sugarcane plant tissue. Zhang et al. (2021) inoculated *Bacillus* sp. P6 with kitchen waste compost where they reported the improvement of Olsen P status and enhancement of P-mobilizing bacteria activities in soil. In another experiment, phosphor compost was applied in a combination with Arbuscular Mycorrhizal Fungi (AMF): *Acaulospora scrobiculata*, *Glomus deserticola*, *Glomus intraradices* and *Glomus versiforme*, and Bacteria: *Myroides odoratus*, *Alcaligenes feacalis*, *Alcaligenes sp.*, and *Alcaligenes sp*. in a tomato pot experiment. It was revealed that this merged application enhanced alkaline phosphatase enzyme activity in the soil and at the same time, improved plant P utilization (El Maaloum et al., 2020). The integrated application of fruit and vegetable waste compost with *Bacillus* sp. CIK-512 had also been recorded to boost the P availability in soil (Ahmad et al., 2018). Likewise, Arif et al. (2017) in their soybean pot study found that the food and fruit waste compost with *Bacillus cereus* GS6 significantly improved the nodule formation in plant root and also soil phosphomonoesterase and dehydrogenase activity were increased because of high microbial P cycling. Available P content in soil and maize growth had also been reported to be accelerated when rock phosphate enriched compost was applied together with *Burkholderia cepacia* and *Klebsiella pneumoniae* (Iqbal et al., 2016).

2.5.3. Improvement of potassium in soil

Like N and P, potassium (K) is another essential plant nutrient that governs the activities of a plant's protein synthesis, carbohydrate metabolism and adaptive responses under stressed conditions (Anschütz et al., 2014; Chérel, 2004). Co-application of compost and bacteria has been proved to improve the K content in soil and plant parts. Chi et al. (2020), in their research demonstrated that the inoculation of *Streptomyces griseorubens* JSD-1 inoculants with swine manure and rice straw composting increased the K availability in composts. Another recent study suggests that K enriched compost with *Klebsiella oxytoca* KSB-17 significantly improved the

K status in soil, which ultimately intensified maize growth and production in semi-arid agroecosystems (Imran et al., 2020). Improvement of potassium status had also been recorded by Abdel-Rahman et al. (2016), where they showed the two thermotolerant bacterial isolates, *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v with rice straw was quite faster in mature compost production. At the same time, this aggregation decreased the C/N ratio highly and enhanced the K level (607.1 ± 10.1 ppm) in compost.

2.5.4. Improvement of other soil nutrients

The synergistic application of compost and bacteria also demonstrated positive outcomes in strengthening the other properties of soil. Aggregated use of compost and some specific agriculturally beneficial microorganisms (*Candida tropicalis*, *Phanerochaete chrysosporium*, *Streptomyces globisporus*, *Lactobacillus* sp., and photosynthetic bacteria) was found effective to improve soil organic C status and microbiological activities. In addition, the lycopene and carotenoid contents of tomato were also stated to be raised significantly in a work conducted by Verma et al. (2015). Similarly, the accessibility and utilization of some micronutrients like iron (Fe), manganese (Mn), and zinc (Zn) have been identified to multiply by the collective application of compost and bacteria (Shahzad et al., 2008a). The oleic acid percentage (up to 40%) and oil contents (up to 51%) of sunflower were also observed to enrich due to the merged practice of farmyard manure compost with PGPR (including *Azotobacter* and *Azospirillium*) (Shoghi-Kalkhoran et al., 2013). In other research, Ahmad et al. (2018) suggested the aggregated use of *Bacillus* sp. CIK-512 and compost can improve certain physical properties of soil (expanded the surface area of the root, increased water absorption ability).

3. MATERIALS AND METHODS

3.1. Soil

3.1.1. Soil collection site

The research soil was collected on 10 November 2021 from the experiment field area at the Faculty of Agriculture, Ondokuz Mayis University, Atakum, Samsun - Türkiye. The average altitude of this area is 190m. Figure 1 demonstrates the specific coordinates of the soil collection site. I used ArcMap version 10.5 to generate the map.

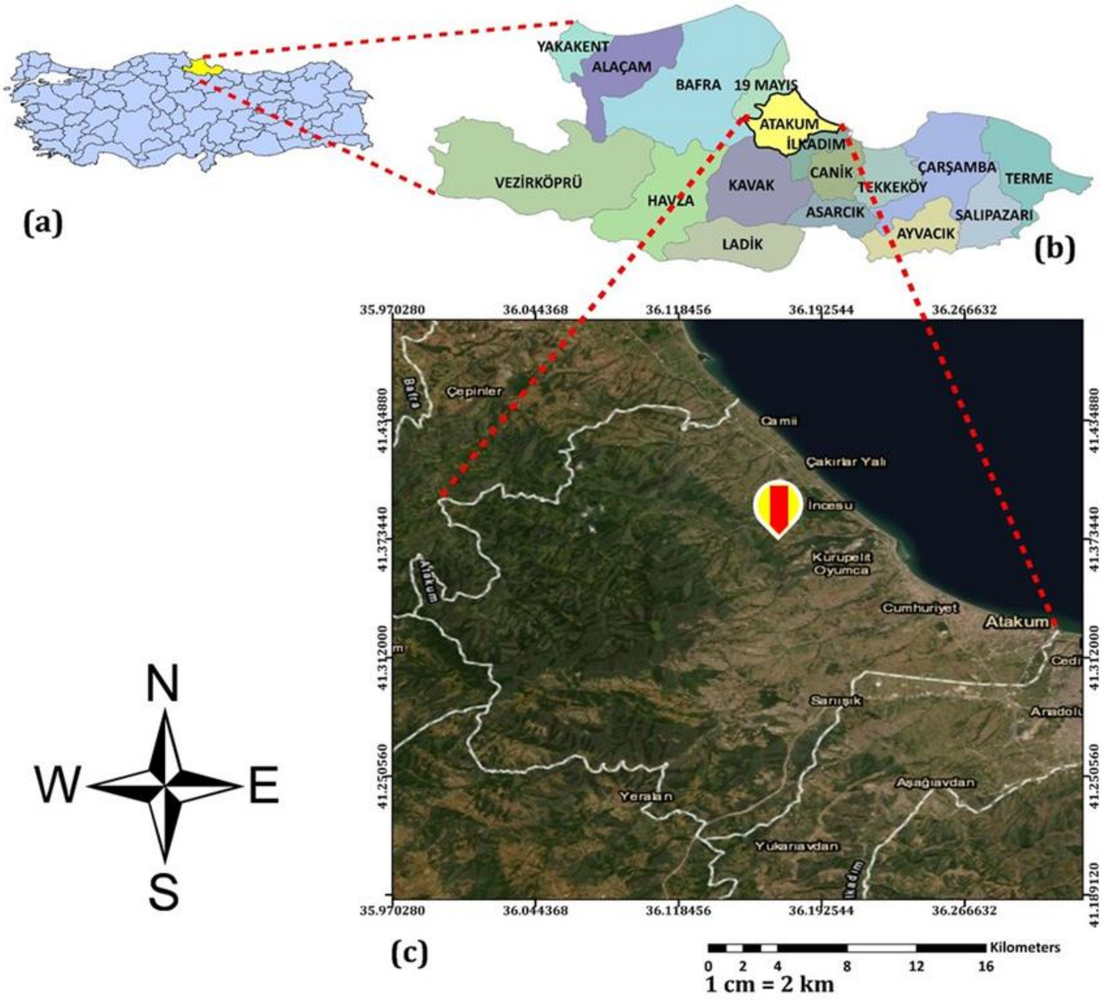

Figure 1. Map of the study area. (a) Location of the study site in Türkiye, (b) Samsun province with the study site, and (c) the specific area of soil collection

3.1.2. Collection and preparation of the soil

Using a shovel around 200 kg of soil was collected from 0 to 20 cm depth. The soil was air dried for ten days, then crushed and passed through a 4 mm sieve to homogenize and mixed very well. An aliquot of that soil was sieved to 2 mm and stored in a Ziploc bag for determining the basic physical and chemical properties.

3.1.3. General properties of soil

The stored soil samples' physical and chemical properties (2 mm sieved) were determined by following different methods. These properties are shown in Table 1.

Table 1. Some physio-chemical properties of the experimental soil

Properties	Values	Methods
Clay (%)	57.22	Hydrometer method (Day, 1965)
Silt (%)	21.95	
Sand (%)	20.83	
Textural class	Clay	
pH (1:1)	6.58	pH meter (glass electrode) (Rowell, 1994)
EC (µS/ cm)	451.50	EC meter (Rowell, 1994)
Organic matter (%)	4.17	Walkley and Black method (Walkley & Black, 1934)
Lime content ($CaCO_3$)	1.39	Scheibler calcimeter method
Available P (ppm)	14.64	Olsen method (Olsen et al., 1954)
Exchangeable Ca (me/ 100g soil)	26.96	1 N NH_4OAc method (Rowell, 1994)
Exchangeable Mg (me/ 100g soil)	21.68	

3.2. Collection of PSB agent for study

The PSB, *Bacillus megaterium* var. *phosphaticum*, is a rod-shaped and gram-positive bacterium which is also known as phosphobacterium (Cooper, 1959; Menkina, 1963). The indigenous strain of the bacteria (*Bacillus megaterium* var. *phosphaticum* strain RK1) were identified and collected from the Soil Microbiology Lab of Department of Soil Science and Plant Nutrition, Ondokuz Mayis University.

3.3. Collection and preparation of compost for application

Approximately 5 kg of hazelnut husk compost was collected from the greenhouse of the Department of Soil Science and Plant Nutrition, Ondokuz Mayis University. It was sieved with a 4 mm sieve to homogenize. First, 2.5 kg of compost was kept in the greenhouse as a fresh condition for individual application. The other 2.5 kg was taken to the Soil Microbiology Lab to mix with *Bacillus megaterium* var. *phosphaticum*. We sprayed the inoculant suspension using a sprayer at a rate of 400 ml in 1 kg compost under laminar airflow. Then the mixture was kept inside the incubator for three days at 25^0C temperature. Some basic properties of compost are represented in table 2.

Table 2. Some common properties of hazelnut husk compost

Properties	Values	Methods
pH (1:10)	7.32	pH meter (glass electrode)
EC (µS/ cm)	644.00	EC meter
Moisture (%)	58.70	
Nitrogen (N) (%)	1.84	Kjeldahl method
Carbon (C) (%)	55.40	Dry combustion method
C/N ratio	30.11	

Figure 2. Hazelnut husk compost after sieving with 4 mm sieve

Figure 3. Mixing *Bacillus megaterium* var. *phosphaticum* inoculant suspension with the hazelnut husk compost under laminar airflow

Figure 4. The mixture of Hazelnut husk compost and *Bacillus megaterium* var. *phosphaticum* after incubation

3.4. Pot selection and greenhouse setup

The pot study was conducted at the greenhouse of the Department of Soil Science and Plant Nutrition, Ondokuz Mayis University, Samsun. The greenhouse was set to a 16-hour daylight photoperiod. The temperature was kept around 25 to 28^{0}C during the day and 16 to 20^{0}C at night temperature. The selected plastic buckets or pots were 18 cm in height, 17.5 cm in the bottom and 20.2 cm in top diameter. The pots did not have any drainage holes.

3.5. Treatment selection, experimental design and pot preparation

For this study, 3 kg of homogenized soil was weighed in each pot. We followed a completely randomized design (CRD) with factorial arrangement of treatments to study the individual and combined application effects of compost and bacteria. We selected seven different treatments with three replications for each and three controls. So, a total of 24 (twenty-four) pots were prepared for treatment application. The detailed specifications of the selected treatments are as follows:

Table 3. Specification of treatments for individual and combined application

Treatments	Doses	Measurements
T1	Control	Without any treatment
T2	1% compost (wt/wt)	30 g compost per pot
T3	2% compost (wt/wt)	60 g compost per pot
T4	4% compost (wt/wt)	120g compost per pot
T5	*Bacillus megaterium* var. *phosphaticum*	10 ml inoculant suspension per pot
T6	1% compost + *Bacillus megaterium* var. *phosphaticum* (wt/wt)	30 g compost and bacteria mixture per pot
T7	2% compost + *Bacillus megaterium* var. *phosphaticum* (wt/wt)	60 g compost and bacteria mixture per pot
T8	4% compost + *Bacillus megaterium* var. *phosphaticum* (wt/wt)	120 g compost and bacteria mixture per pot

wt = weight

The treatments were thoroughly mixed with 3 kg of air-dried 4 mm sieved soil and then carefully potted in the selected pots. All the pots (including controls) were irrigated to the field capacity (34%).

Figure 5. Taking the weight of different doses of compost treatments (left) and application of treatments to the homogenized soil for study (right)

3.6. Development of plants

In this study, “Altındane” wheat variety (*Triticum aestivum* L.) was selected as a test crop, and it was grown from direct seed application to the maturity stage (4 months). Fifteen (15) seeds were manually sown in each pot to a depth of 3 cm from the soil surface. Rainwater was sprayed on the soil as crucial to keep the moisture content at field capacity. The first seedling germination was recorded five days after sowing. Two weeks later, seedlings were thinned to 12 plants per pot. Every week the growth of wheat plants was monitored through manual weeding. Within the growing period, N (urea) was added to the soil twice at a rate of 0.31g urea per pot which was half of the recommended dose (19 kg N per decare).

Figure 6. Seedlings of wheat

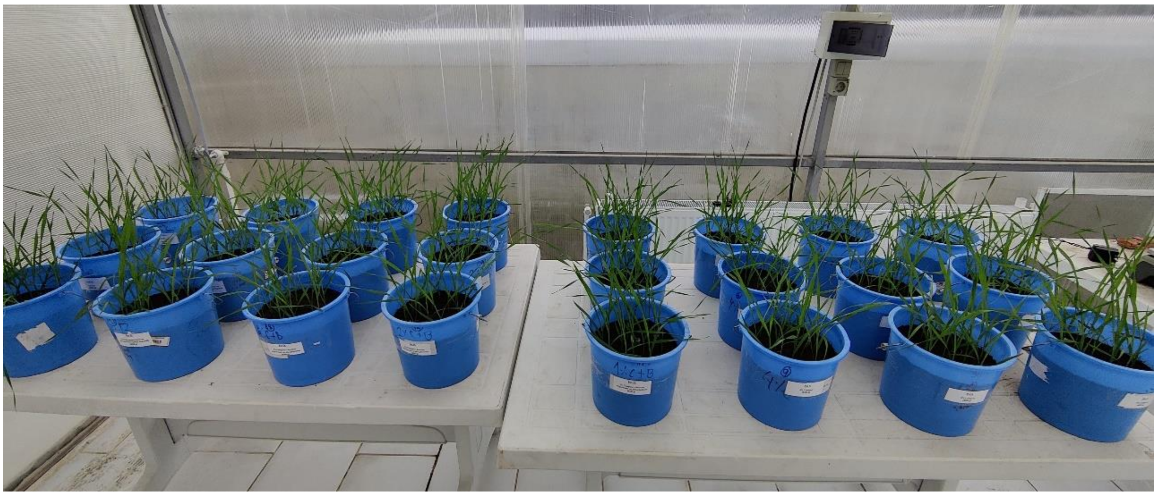

Figure 7. Growing wheat plants in greenhouse

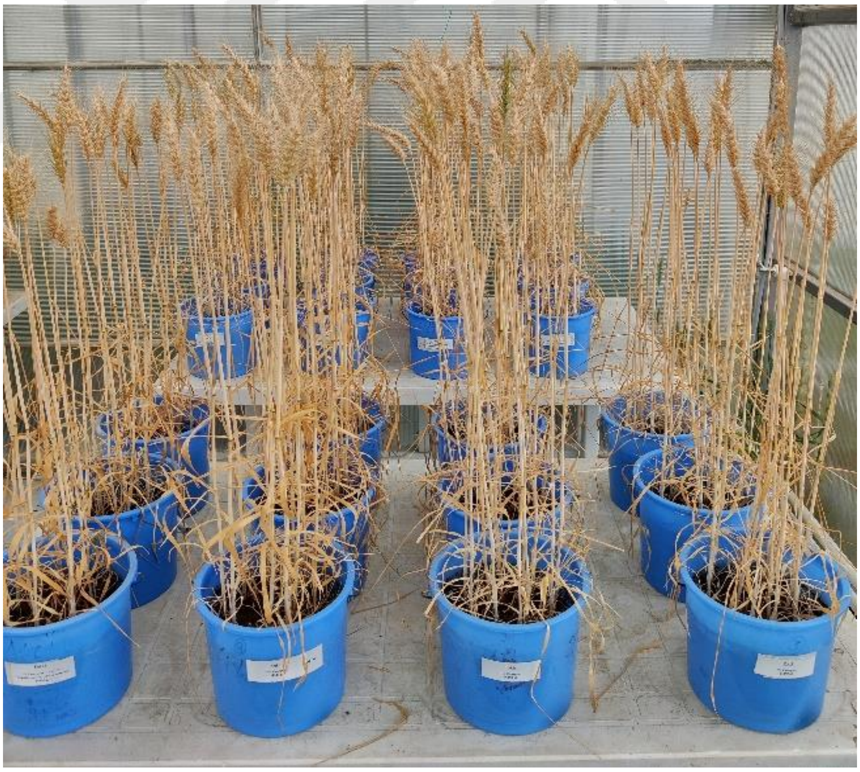

Figure 8. Mature wheat plants in greenhouse

3.7. Laboratory analysis for soil health assessment

After plant growth trials, soil health was assessed by evaluating its different properties. The soil sample was collected from every pot of 5 cm depth from the surface. The fresh soil samples were put in polythene bags and taken into the laboratory for performing further analysis. For soil microbiological analysis, the soil samples were saved in the refrigerator at 4^0C.

3.7.1. Analysis of soil chemical properties

2 mm sieved 20 g air-dried soil was soaked in 20 ml distilled water (1:1 solid/water ratio) and shaken thoroughly for 1 hour in a reciprocal shaker at 150 rpm. The slurry was then measured for pH using a Seven Compact S210 pH meter, and EC (electrical conductivity) was determined using a Five Easy Plus FP30 Conductivity meter (Rowell, 1994). The exchangeable cations (Ca, Mg, Na and K) were determined with the 1N NH_4OAc (Ammonium Acetate) extraction method (Rowell, 1994). The bioavailability of inorganic orthophosphate (PO_4^-) in soil samples were analyzed through the Olsen method, where alkaline (pH 8.5) 0.5M sodium bicarbonate ($NaHCO_3$) solution was used (Olsen et al., 1954).

Figure 9. Measurement of pH (left) and EC (right) of different soil samples

3.7.2. Analysis of soil biological properties

3.7.2.1. Alkaline phosphatase enzyme activity

The phosphatase enzyme is responsible for hydrolyzing the soil organic P compounds into inorganic polyphosphates that are important for P cycling in soil. Phosphatase enzyme activity was estimated according to Tabatabai & Bremner (1969). Toluene (0.25 ml), phosphate buffer (4 ml, pH = 8), and 0.115 M *p*-nitrophenyl phosphate (1 ml) were poured to an Erlenmeyer flask containing 1 g fresh soil sample. All the samples were incubated at 37^0C for one hour. By using a spectrophotometer at 410 nm wavelength, the produced *p*- nitrophenol was examined. The unit was stated as 'µg *p*-NP/ g dry sample'.

3.7.2.2. Basal soil respiration

Carbon dioxide that is released from the decomposition of soil organic matter through the action of diversified soil microorganisms and respiration of plant roots and soil fauna can be estimated by basal soil respiration method (Anderson, 1983).

Fresh soil sample equivalent to 5g oven dried soil and a cuvette containing 2.5ml 0.05N NaOH was placed in a jar and incubated for 24 hours at 25^0C for CO_2 absorption. Then $BaCl_2$ and Phenolphthalein indicator were added with NaOH solution and titrated with the standardized hydrochloric acid.

The result was expressed as 'mg CO_2 / g soil/ day'.

3.7.2.3. Microbial biomass carbon

Carbon is one of the most essential energy supplying factor for soil microorganisms. The substrate induced respiration method was followed to measure the microbial biomass carbon (MBC), suggested by Anderson & Domsch (1978). 1ml Glucose (0.5%) was added to the fresh collected soil sample comparable with 5g oven dried soil and kept in a jar for two hours. Then it was tightly covered and incubated for few hours at 25^0C. The production of CO_2 was determined by following the method prescribed by Anderson (1983). MBC was estimated from the respiratory in terms of mg C/ g soil/ day as 40.04 mg CO_2/ g soil/ day + 0.37. The end result was stated as 'mg MBC/ g soil/ day'.

3.8. Plant measurement

At the maturity stage, plants from every pot were examined to record their specific yield contributing characteristics viz. plant biomass (g), plant height (cm), and length of the spike (cm). Plant height (cm) was measured from the base of the shoot to the top of the plant through a measuring tape. The grain yield of wheat was expressed as ‘g/ pot’.

The percent yield increase over control was calculated by following this formula (Durmuş & Kızılkaya, 2022):

$$\% \text{ Yield increase over control} = \frac{Yt\text{-}Yc}{Yc} \times 100$$

Here, ***Yt*** = Yield of wheat grain in treated pot and ***Yc*** = Yield of wheat grain in control

Water use efficiency (WUE) is a measure of the quantity of biomass produced per unit utilization of water by the plant. WUE is expressed here as ‘g/ mm’. The following formula was used to calculate the WUE (Pascale et al., 2011):

$$\text{WUE} = \frac{\text{Plant biomass}}{\text{Utilized water for biomass production}}$$

3.9. Statistical analysis

Microsoft excel 2016 pro was used to calculate the basic calculation and for preparing graphs. Statistical analysis of the final data was analyzed by using SPSS statistical software, version 25. I performed two-way ANOVA to determine the main effects of factors as well as their interactions. I applied Duncan's Multiple Range Test (DMRT) for post-hoc analysis at the $p \leq 0.05$ level. Correlation was executed to see the relationship among different properties of soil.

4. RESULTS AND DISCUSSION

4.1. Effect of HHC and *Bacillus megaterium* var. *phosphaticum* on yield contributing characteristics, yield and water use efficiency of wheat plant

4.1.1. Plant biomass, plant length and spike length

The HHC and bacteria applications generated significant effects on the yield contributing properties of wheat (Figure 10 to 12). Considering wheat plant biomass (Figure 10), the 4% compost + bacteria amendment showed the highest production of biomass, which was 59.33 g. The second most production was found from 2% compost + bacteria application (56.00 g). The control was the lowest in biomass production (45.67 g), and it was statistically similar with only *B. megaterium* var. *phosphaticum* application (46.67 g).

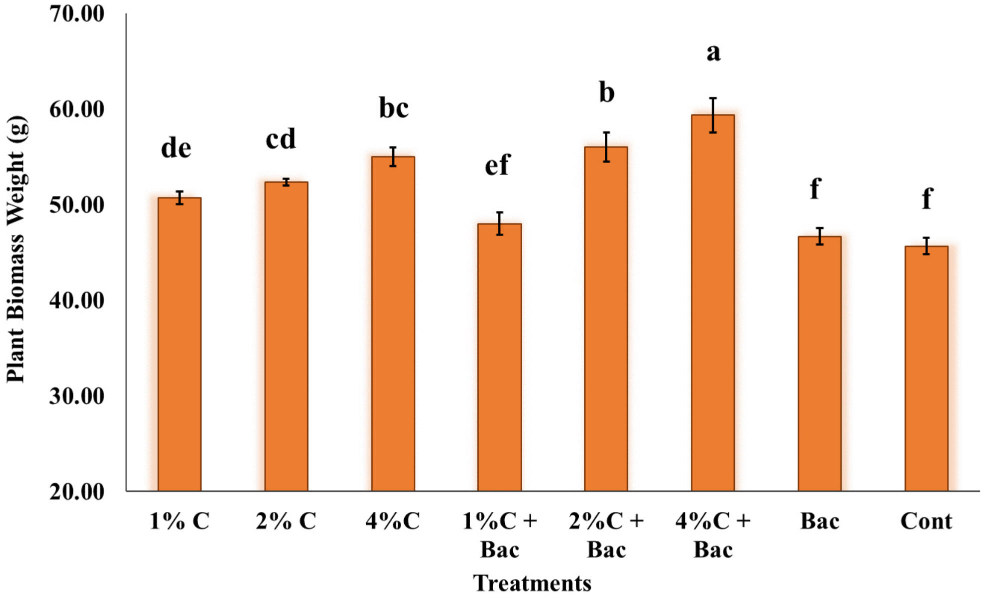

Figure 10. Biomass production of wheat grown in compost (C) and bacteria (Bac) treated soil. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$.

Overall, for biomass production, the integrated applications of compost and bacteria were superior to the individual compost treatments. The findings show a similar direction to Hu & Qi (2013), who conducted a long time research to find out the effectiveness of microbes on the yield and nutrition of wheat with the co-application of compost. They described that the integrated application significantly magnified the wheat biomass. Solís-Domínguez et al. (2011) in their study mentioned that inoculation of microorganisms (arbuscular mycorrhizal fungi) with compost (a combination of green waste and manure) resulted around 44 to 76%

enhance in plant biomass production in acidic metalliferous mine tailings. The combined application of plant growth promoting bacteria and compost was also found quite effective in increasing chickpea biomass, which was about 84% (Shahzad et al., 2008b).

As observed from Figure 11, the combine application of 4% compost and bacteria represented higher plant length (75.54 cm), followed by bacteria (74.14 cm), 2% compost + bacteria (74.08 cm), 2% compost (73.83 cm) and 4% compost (73.29 cm). Control demonstrated the lowest wheat plant height.

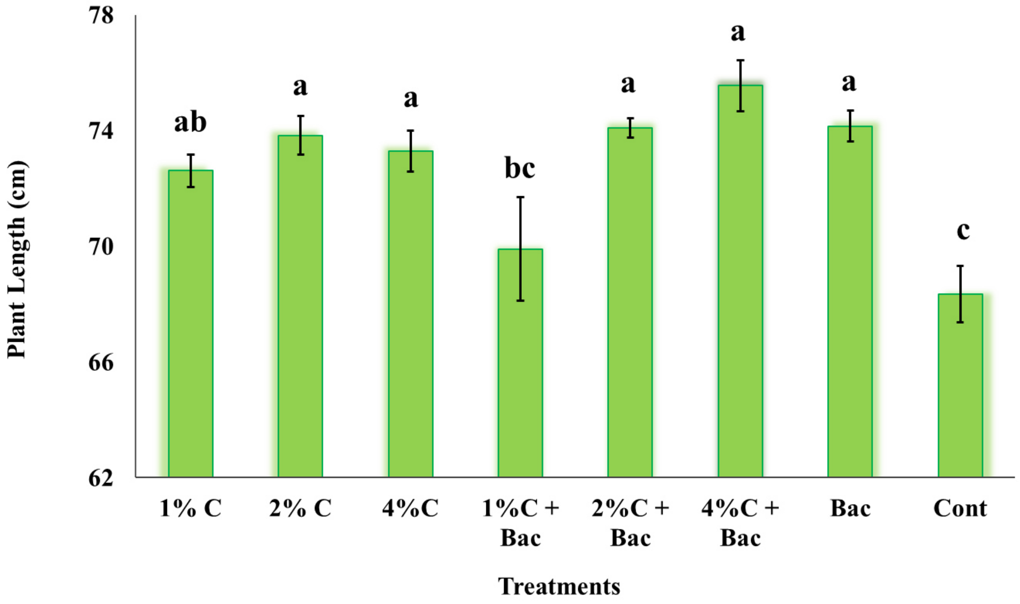

Figure 11. Development in plant length of wheat grown in compost (C) and bacteria (Bac) applied soil. Lowercase letters represent the means separated by applying, DMRT where confidence interval = 95% and $p \leq 0.05$.

The findings resemble Akhtar et al. (2009), who recorded taller wheat plant height with the merged application of compost and PGPR. The application of chick manure compost alone in Cd polluted soil was found to be highly efficient in increasing the wheat plant height (Liu et al., 2009). The highest enlargement in the wheat plant height (16% over control) was also reported by Ibrahim et al. (2008), who used organic manure and compost.

Briefly, 4% compost application represented a greater spike length (10.41 cm), which was statistically similar to 4% compost + bacteria application (10.12 cm). The 2% compost + bacteria, 2% compost, and 1% compost treatmenrts showed statistically similar results in spike length development (9.97 cm, 9.83 cm, and 9.82 cm, respectively) (Figure 12).

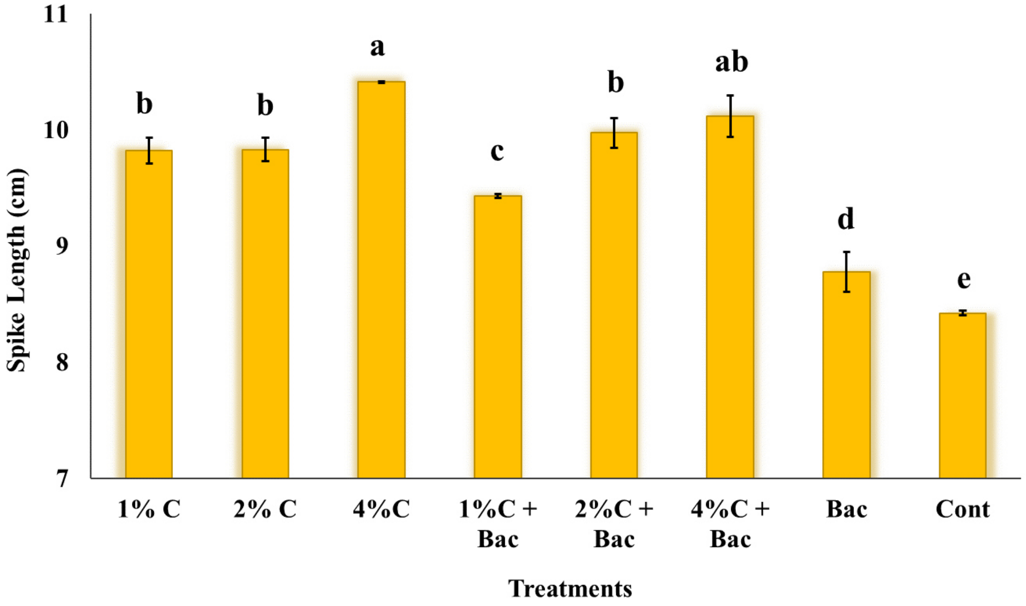

Figure 12. Effect of compost (C) and bacteria (Bac) application on spike length of wheat. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$.

Mohamed et al. (2019), in their research, observed that the addition of biofertilizers (*Azotobacter* and yeast) with sewage sludge compost increased the spike length of wheat plants. In another study, it was recorded that the use of compost during wheat production can increase the spike length, and it was more than manure application (Ibrahim et al., 2008).

4.1.2. Wheat yield

According to the outcomes of the harvested wheat, all the applied treatments demonstrated positive impacts on yield status compared to control (Figure 13). Amending with 4% compost + bacteria was the treatment that performed the highest yield (19.38 g/ pot), which was statistically alike with 2% compost + bacteria (18.60 g/ pot) application. Moreover, they are also statistically similar to the three single applications of compost (1%, 2%, and 4% compost). Here it is notable that these three single compost applications revealed nearly equal wheat grain production (18.29, 18.30 and 18.28 g/ pot in 1%, 2% and 4% compost treated pot, respectively). The lowest wheat grain production was observed in control, 15.87 g/ pot.

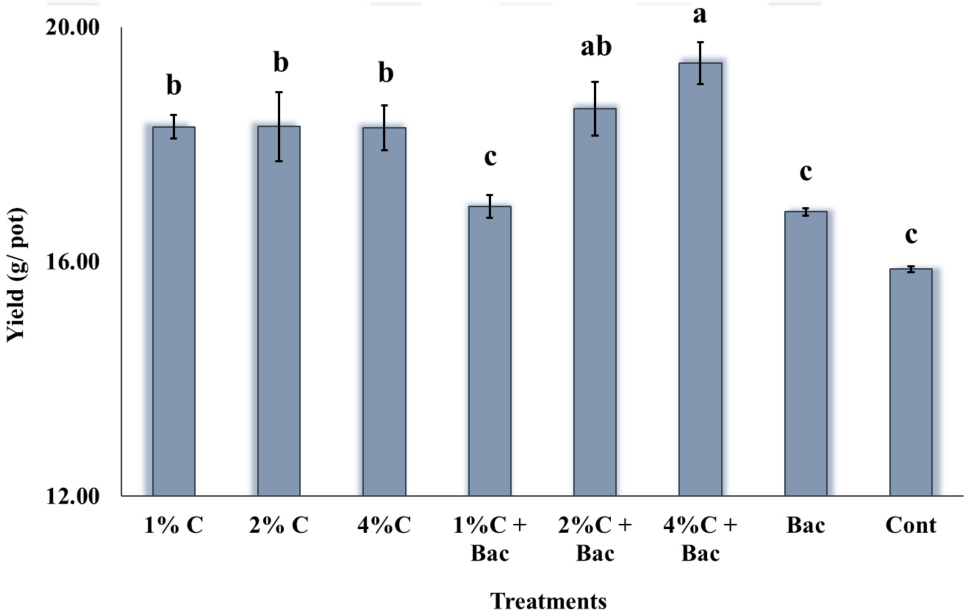

Figure 13. Effect of compost (C) and bacteria (Bac) applications on wheat yield (g/ pot). Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

Synergistic implementation of compost and PGPR in water-stressed salt-affected soil was revealed to increase the wheat production significantly more than their individual treatments (Omara et al., 2022). Andrade et al. (2021) carried out an experiment with organic compost, PGPR, arbuscular mycorrhizal fungus, and *Brachiaria* on wheat and maize. They described that the combination of compost with compost, *Brachiaria*, and fungus improved the overall yield status of both wheat and maize. Billah et al. (2020), from their two-year field study, stated that the rock phosphate-enriched compost, in addition to PGPR (*Pseudomonas* sp. and

Proteus sp.) significantly enhanced the wheat production. Again, Baig et al. (2012), in their research, found that the inoculation of *Bacillus* spp. in P-enriched compost generated a progressive influence on the wheat yield status. They concluded that the existence of two special attributes, P solubilization and ACC deaminase activity in the bacteria, might have created collaborative impacts on wheat growth and yield.

Percent grain yield increase over control was calculated as per the formula mentioned in the materials and methods section and the results are presented in Figure 14. The highest percentage (22.12%) of grain yield increased from the combined use of 4% compost and bacteria. For single compost application, all the three doses exhibited almost similar outcomes in percent yield increase over control.

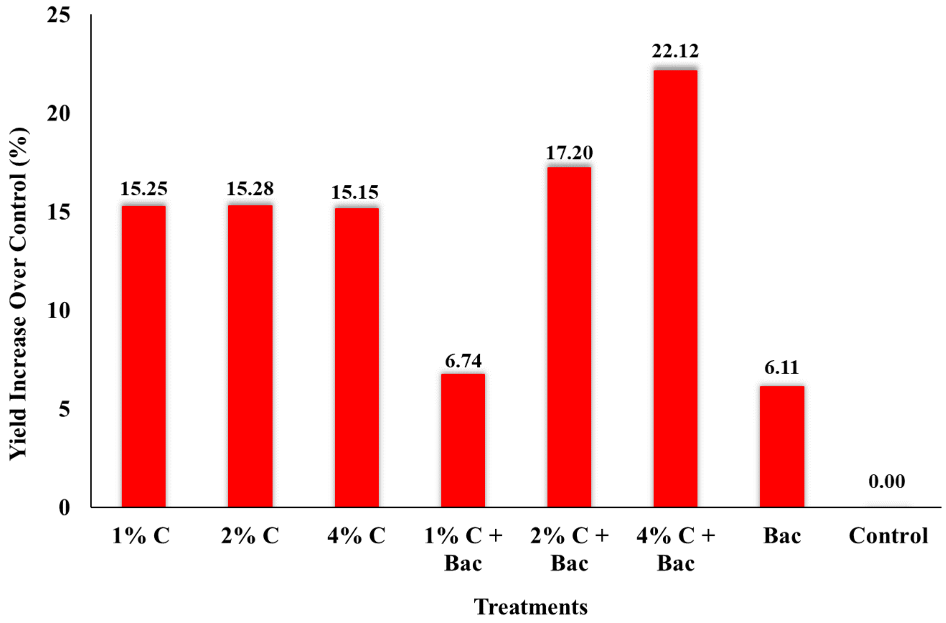

Figure 14. Percent yield increase of wheat plant over control (compost = C and bacteria = Bac)

4.1.3. Water Use Efficiency (WUE)

The treatment applications improved the WUE of the wheat plant as compared to the control (Figure 15). WUE experienced an upward trend with the enhancement of compost doses. The highest WUE value was 0.148 g/ mm in 4% compost + bacteria application. The second maximum was found in 4% compost treated pot (140 g/ mm) representing a very close WUE with 2% compost + bacteria and 2% compost single applied treatments (0.139 and 0.137 g/ mm, respectively). Control was recorded as the lowest one in WUE (0.126 g/mm).

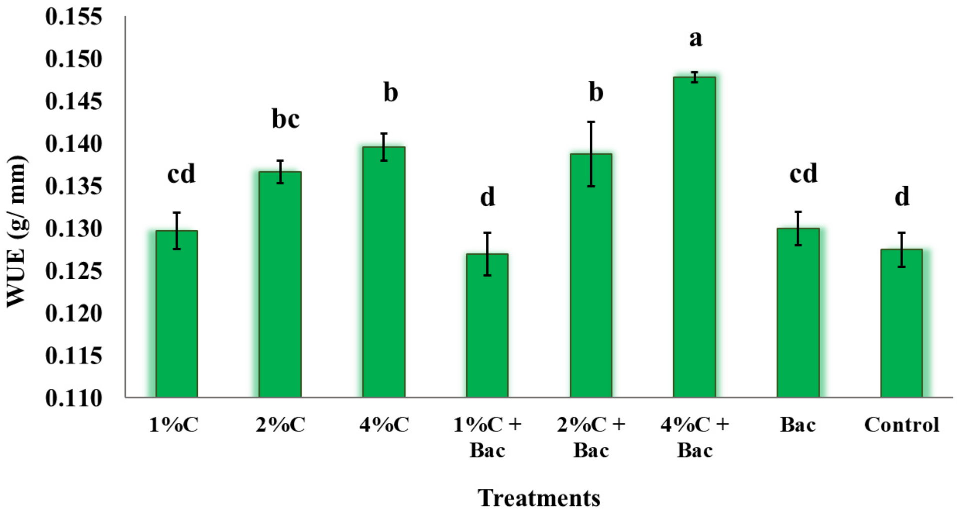

Figure 15. Water use efficiency (WUE) for different doses of compost (C) and bacteria (Bac) application on wheat plant. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

Rivier et al. (2022) reported that the compost application was one of the best amendments in reducing the evaporation loss and enhancing WUE in spring wheat. Another study conducted by Demir & Gülser (2021) stated that in both field and glasshouse conditions, the application of rice husk compost significantly intensified the WUE of tomato plants. Organic compost implementation was recorded to solve the negative consequences of water stress by strengthening WUE and plant water status (Abd El-Mageed et al., 2018). Adamtey et al. (2010) also described the higher WUE of maize plants in N- enriched compost treated pots.

Considering all the plant parameters, it can be explained that bacteria in combination with HHC can bring better outcomes almost in all cases than a single compost application in soil. However, to bring out the significant benefits of Bacillus megaterium var. phosphaticum RK1, it needs to be applied with the aggregation of organic matter like compost. The results also illustrate that to get a higher yield from a single application, 1% HHC is enough, and from the combined application, the greater addition of compost with bacteria is better.

4.2. Effect of HHC and *Bacillus megaterium* var. *phosphaticum* application on soil health properties

4.2.1. On soil pH, EC and organic matter (SOM)

The Figure 16 demonstrates that with the increase of HHC doses, the pH levels were also increasing but the trend was too much slow and they were very close to neutral pH. Only the individual bacteria treated soil was found to have more pH (7.33) but it was also not too far away from neutral. The lowest was observed in control pot (pH = 7.08).

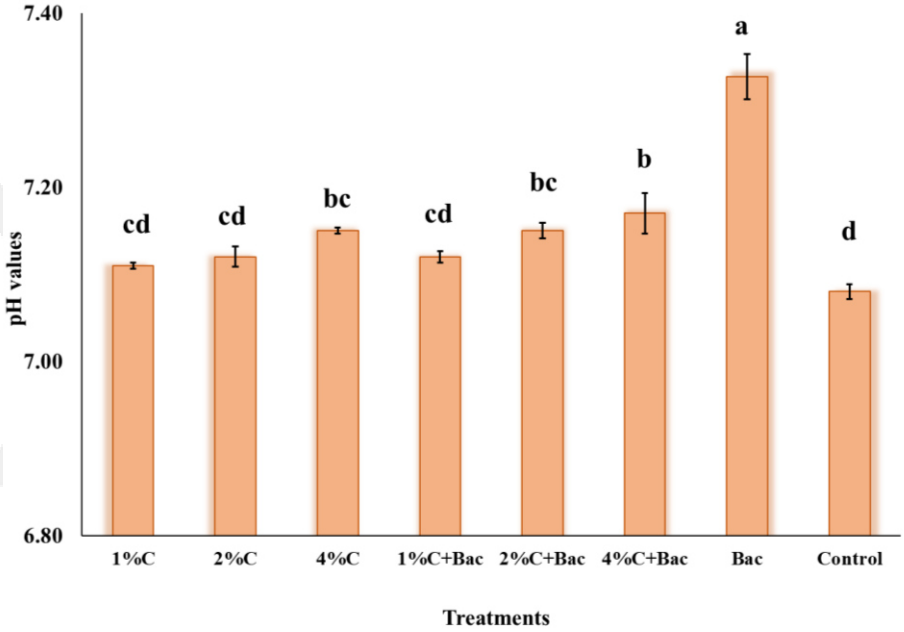

Figure 16. Effect of compost (C) and bacteria (Bac) application on soil pH, lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

Soil pH greatly influences the metal solubility, nutrient uptake from soil and the soil microbial functions by controlling different processes and responses in plants (García-Gil et al., 2004). Compost implementation possesses liming effect because of having more alkaline cations like Ca, Mg and K, which can be free from organic matter by the action of mineralization (Agegnehu et al., 2014; Soheil et al., 2012).

This native strain of *Bacillus megaterium* var. *phosphaticum* was found to be active in increasing the pH. This can be related to other studies, where researchers reported that different *Bacillus* sp. (*Bacillus subtilis* W7, *Bacillus amyloliquefaciens*, *Bacillus velezensis* H-6) were active in increasing the rhizosphere soil pH (Li et al., 2021; Wang et al., 2020; Huang et al., 2019).

Figure 17, which is about EC, demonstrates that in single compost application treatments, the EC rose gradually from 1% to 4% HHC. On the other hand, the soil mixed with the integrated application of HHC and bacteria was showing an opposite pattern, i.e. it was decreasing with more amount of HHC doses. There was no statistically significant difference between the electrical conductivity of bacterial application and control (446 and 441.97 µS/ cm, respectively).

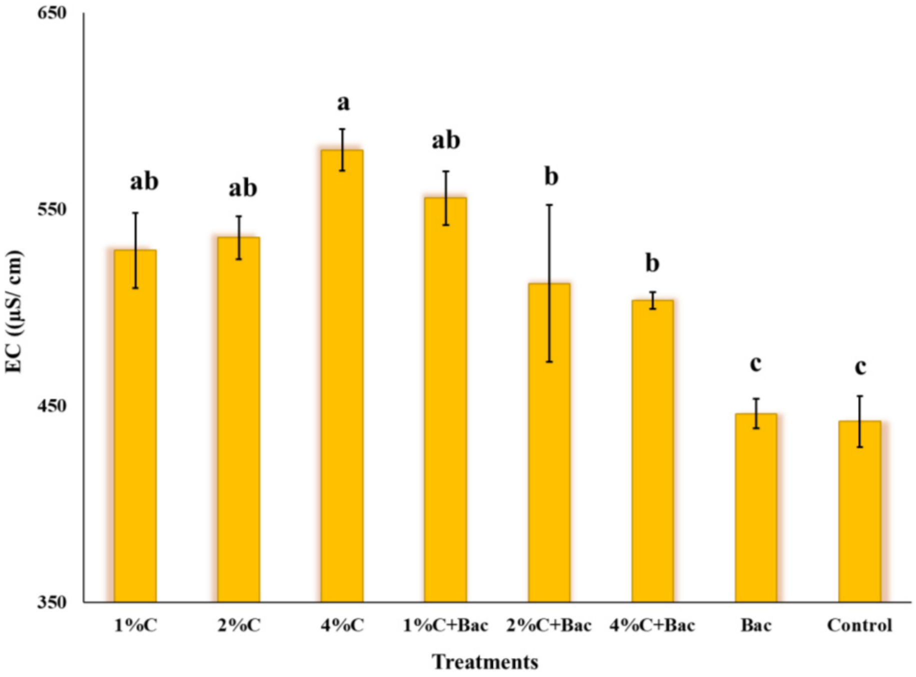

Figure 17. Effect of compost (C) and bacteria (Bac) application on soil electrical conductivity, EC (µS/ cm). Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

In a study, the use of rice husk compost under greenhouse and field was reported to significantly enhance the EC in soil (Demir & Gülser, 2021). Due to the mineralization of organic matter, the EC value was noted to be increasing continuously (Gulser et al., 2010; Eigenberg et al., 2002).

In this study, the co-application of bacteria and HHC reduced the EC because possibly the applied bacteria was much more prone to decompose the organic matter in soil. This action needed energy that the bacteria could get utilizing the available nutrients in soil. This could be why the combined treated soils represented less EC. Ji et al. (2022) described that *Bacillus subtilis* HG-15 strain inoculation in soil remarkably decreased soil EC and promoted wheat growth. Another research by Tahir et al. (2019) in their study evaluated that salt-tolerant *Bacillus* strains viz.

Bacillus sp. SR-2-1, *Bacillus* sp. SR-2-1/1 and their association treatment significantly reduced soil EC with improving the potato production in both normal and saline soils. Furthermore, a negative relationship between soil EC and bacterial community was also reported in another work (Siles & Margesin, 2016).

The organic matter content showed a significant difference in different treatment applications (Figure 18). The most organic matter content was observed in 4%C and 4%C + bacteria application; they were statistically similar in effectivity (3.89% and 3.74% SOM, respectively). The lowest amount, less than 3%, was observed in single bacterial and control application.

Gülser et al. (2015) evaluated that hazelnut husk was more vigorous than compost (manufactured from farmyard manure and wheat straw) in improving soil organic carbon status in hazelnut orchards. The SOM was also indicated to increase significantly with the application of rice husk compost (Demir & Gülser, 2021). In another work, it was reported that the combine utilization of manure compost and bacterial fertilizer enhanced the overall SOM and humus status in soil (Zhen et al., 2014). They got a positive correlation between SOM and microbial biomass.

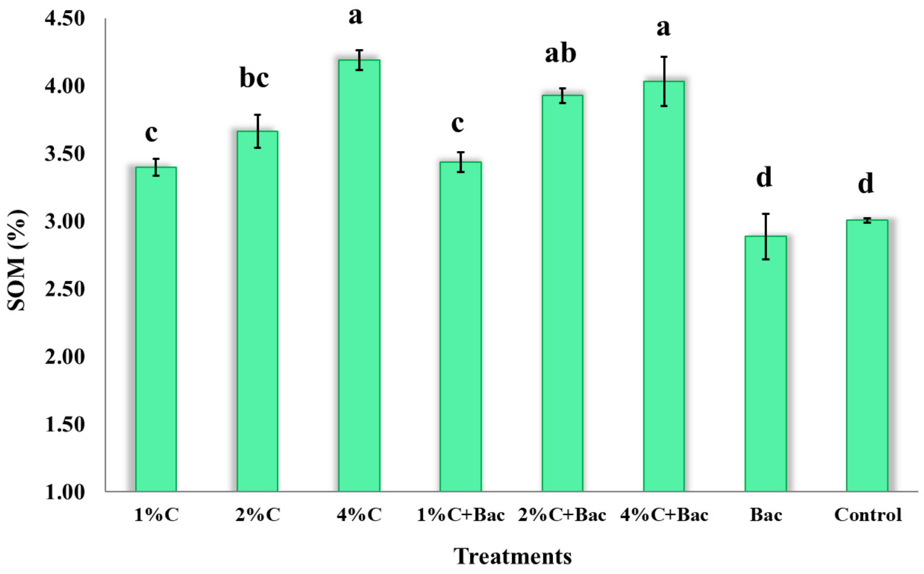

Figure 18. Effect of compost (C) and bacteria (Bac) application on soil organic matter status. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

4.2.2. On the exchangeable cation of soil

The impacts of single and combined implementation of compost and bacteria on the soil exchangeable cation status (Ca^{2+}, Mg^{2+}, and K^+) is illustrated in Table 4. All the treatments showed statistically significant differences in exchangeable cation status in soil.

All the treated soils represented less amount of Ca^{2+} than the control, which was 40.38 meq/ 100g soil. The second highest was observed in single bacterial application 34.28 meq/ 100g soil. The lowest amount was revealed in 2% C application (30.66 meq/ 100g soil).

In the case of Mg^{2+} and K^+, all the applied treatments provided higher concentrations than the control. 1% compost application was revealed as the highest one in intensifying the Mg^{2+} level in soil (18.65 meq/ 100g soil), followed by 2% compost treatment (17.76 meq/ 100g soil). The lowest amount was observed in control treatment, 12.02 meq/ 100g.

K^+ concentration was found maximum in 4% compost amended soil (1.01 meq/ 100g soil), and it was statistically similar to the mix application of 4% compost and bacteria (0.97 meq/ 100g soil). In individual applications, the 4% compost performed better in improving the K^+ level than the 2% and 1% compost applications. For dual combined application, it revealed the same pattern i.e. 4% compost + bacteria > 2% compost + bacteria > 1% compost + bacteria. Moreover, solitary compost treated soil showed a minute increase in potassium concentration than the soils from integrated treatments. Here, bacteria treated soil was evaluated as statistically alike to control.

In clay soil, Ca^{2+} is more potential for adsorbing P. Sharif et al. (2011) reported that after the P application in calcareous soil, only a tiny portion of P could be utilized by plants, while the maximum P gets fixed as calcium phosphate. Several studies also reported the same, that the divalent cation, like Ca^{2+} has high tendency to fix the P in soil (Kalayu, 2019; Sharma et al., 2013; Walpola and Yoon, 2012; Mehrvarz et al., 2008). In our study, compared to control, the HHC application with or without bacteria reduced the Ca^{2+} status in soil. It reflects that maybe the compost application is alleviating the P adsorption by calcium ion. This finding is in agreement with Dede & Ozdemir (2018) that the hazelnut husk growing media showed less calcium level than the other organic treatments (peat and hazelnut husk + sewage sludge). In another experiment, researchers found that *Bacillus megaterium*

SS_3 isolated from calcareous soil reduced the soluble calcium (Dhami et al., 2013).

Table 4. Soil exchangeable Ca^{2+}, Mg^{2+}, and K^+ concentrations (Mean ± Standard Error) (meq/ 100g soil) for different treatments

Treatments	Ca^{2+} (meq/ 100g soil)	Mg^{2+} (meq/ 100g soil)	K^+ (meq/ 100g soil)
1% Compost	31.94 ± 0.21 cd	18.65 ± 1.03 a	0.51 ± 0.01 c
2% Compost	30.66 ± 0.92 d	17.76 ± 0.56 ab	0.67 ± 0.02 b
4% Compost	30.97 ± 0.19 d	16.59 ± 0.48 abc	1.01 ± 0.03 a
1% Compost + Bacteria	30.83 ± 1.00 d	16.47 ± 1.58 abc	0.43 ± 0.03 d
2% Compost + Bacteria	32.41 ± 0.14 bcd	14.24 ± 0.34 cd	0.51 ± 0.02 c
4% Compost + Bacteria	33.33 ± 0.93 bc	15.90 ± 1.00 abc	0.97 ± 0.04 a
Bacteria	34.28 ± 0.50 b	15.55 ± 0.93 bc	0.35 ± 0.01 e
Control	40.38 ± 0.16 a	12.02 ± 0.52 d	0.32 ± 0.02 e
Significance level	**	**	**

'**' $p \leq 0.01$

Same letters within a column indicate no significant differences between treatments ($p \leq 0.05$), by Duncan's Multiple Range Test (DMRT).

Both sole implementation of HHC and merged utilization of HHC and *Bacillus megaterium* var. *phosphaticum* improved the exchangeable magnesium status in soil. Komolafe et al. (2021) in their investigation revealed that the collaborative use of compost and fungus (*Trichoderma asperellum*) was highly competent to raise Mg^{2+} in celosia cultivated soil. The increase of Mg^{2+} uptake by okra was experienced in another study where researchers applied cocoa pod husk compost with neem leaves and poultry manure (Kayode et al., 2018). Likewise, corncob compost application (at a rate of 2%, w/w) in two different types of soil significantly boosted the exchangeable Mg^{2+} availability (Mensah & Frimpong, 2018).

In this study, K^+ significantly increased with the increased doses of compost application. Gülser et al. (2015) reported the significant increase of K^+ in soil with hazelnut husk application, which was even higher than the farmyard manure compost application. A sixteen years of field study also suggested the improvement of K^+ in soil due to long term application of olive mill pomace compost (Roberto et al., 2012). According to a research by Badr (2006), the implementation of K-enriched compost and silicate-dissolving bacteria (SDB): *Bacillus cereus* together represented a remarkable enhancement in K^+ availability and recovery in soil with a better yield of tomato.

4.2.3. On the available P status and alkaline phosphatase activity in soil

Available P in soil increased with individual HHC application. The 4% compost showed higher available P (14.81 ppm) that 2% and 1% compost treated pots (11.71 and 10.13 ppm, respectively). In our findings, we experienced opposite result in combined applications. With the increase of compost doses in combined applications, the P concentration was getting decreased. The 4% compost + bacteria and 1% single compost treated soil was lower in available P (10.36 and 10.13 ppm, respectively). Mix implementation of bacteria with 1% and 2% compost was statistically similar (Figure 19).

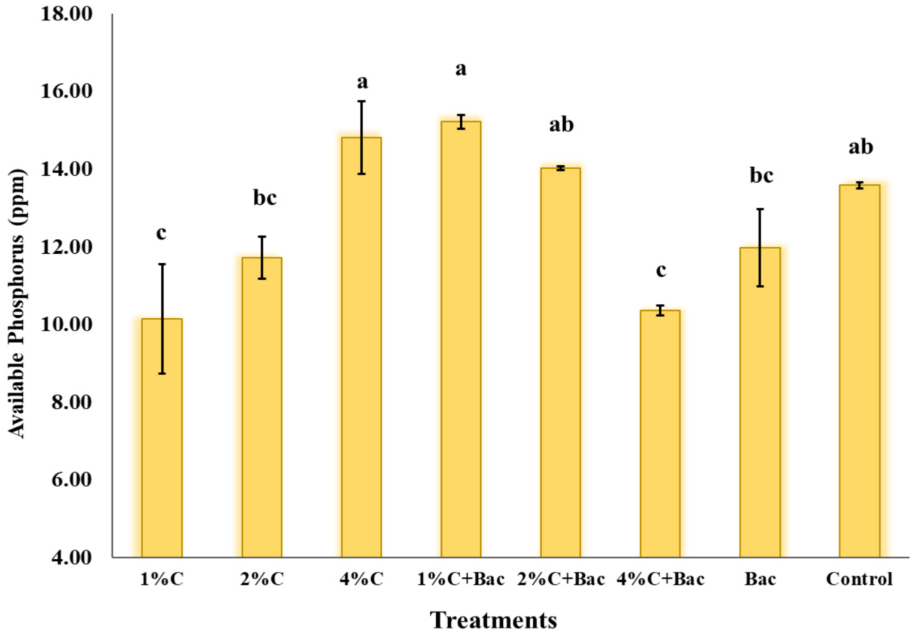

Figure 19. Effect of compost (C) and bacteria (Bac) application on available P in soil, Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

The effect of HHC and *Bacillus megaterium* var. *phosphaticum* amendments on soil alkaline phosphatase enzyme activity is illustrated in Figure 20. Considering sole applications, 1% compost demonstrated higher value (167.62 µg *p*-nitrophenol/ g soil/ hour), followed by 4% (141.44 µg *p*-nitrophenol/ g soil/ hour) and 2% (140.25 µg *p*-nitrophenol/ g soil/ hour) compost. At the same time, the bacteria inoculated compost treated soil exhibited reverse response. 4% compost + bacteria treated soil was the highest in alkaline phosphatase activity (202.60 µg *p*-nitrophenol/ g soil/ hour). The 2% compost and bacteria co-application was the second highest among all (180.69 µg *p*-nitrophenol/ g soil/ hour) and showed statistically identical phosphatase activity with 1% compost + bacteria application. 1% compost and bacteria merged application was the lowest in alkaline phosphatase activity (123.51 µg *p*-nitrophenol/ g soil/ hour).

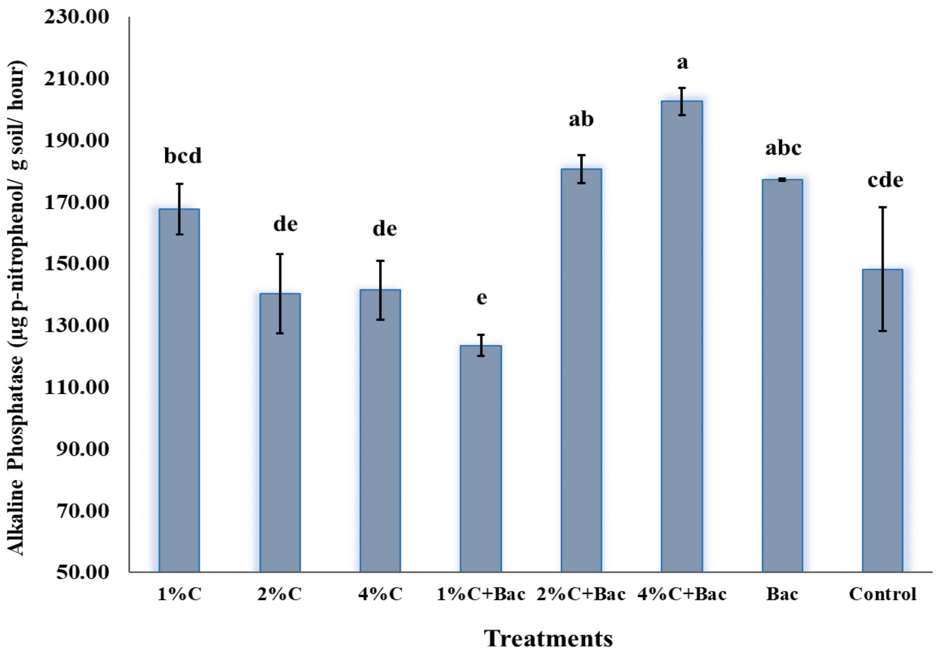

Figure 20. Effect of compost (C) and bacteria (Bac) application on alkaline phosphatase enzyme activity, Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

In several studies, the compost application was reported to increase the plant available P in soil (Dotaniya et al., 2016; Gaind, 2014). The increment of available P in soil was described to improve with the canarium nut compost application (Hannet et al., 2021). They also evaluated that the single compost performed better than the combine application of compost and biochar. Pineapple leaves compost was found effective to improve available P level in high acidic soil (pH 4.56). Özenc & Caliskan (2001) in their field study stated that HHC enhanced P in soil and hazelnut leaves.

The correlation between available P and phosphatase enzyme activity is presented on Table 5. It shows that they have a negative significant relationship with each other ($p \leq 0.05$). It implies that when there is lack of P in soil, the microorganisms tend to release more phosphatase enzyme. From the above mentioned graphs, it is conspicuous that the 4% compost + bacteria applied soil, which is one of the lowest in available P concentration (Figure 19), is the highest one in phosphatase enzyme activity (Figure 20). The same pattern has been observed for the other applications. It also suggests that plants for their growth and development uptake available P from soil. Therefore, when its availability gets low, the *Bacillus* release more phosphatase to make a proper P balance in soil through phosphate solubilization (Kang et al., 2014; Han et al., 2006). This alkaline phosphatase, which is predominantly manufactured by microorganisms, plays an enormous role in regulating the P availability in soil (Garg & Bahl, 2008; Sarapatka, 2003; Oberson et al., 1996). Our findings support diverse studies. Waldrip et al. (2011) mentioned that poultry manure amendment in ryegrass-cultivated soil delivered a negative relationship between initial alkaline phosphatase activity and soil P. A field experiment reported that *Lantana* compost amended soil experienced a strong negative correlation between available P and alkaline phosphatase enzyme (Saha et al., 2008). Another field study reported the similar outcome where they made crop rotation with wheat and maize and did mineral fertilization (Samuel et al., 2010). This is an indicator of inorganic P availability for plants and microbes in soil (Piotrowska-Długosz et al., 2016).

Table 5. Correlation between available P and phosphatase enzyme in soil after compost and bacteria application

	Available P (ppm)	Phosphatase
Available P (ppm)	1	- 0.503*
Phosphatase	- 0.503*	1

* Correlation is significant at the 0.05 level (2-tailed).

4.2.4. On the basal soil respiration and microbial biomass carbon status in soil

The contents of basal soil respiration or carbon dioxide production and microbial biomass carbon of the HHC and *Bacillus megaterium* var. *phosphaticum* treated soils were significantly different between treatments ($p \leq 0.05$) (Figure 21 and 22).

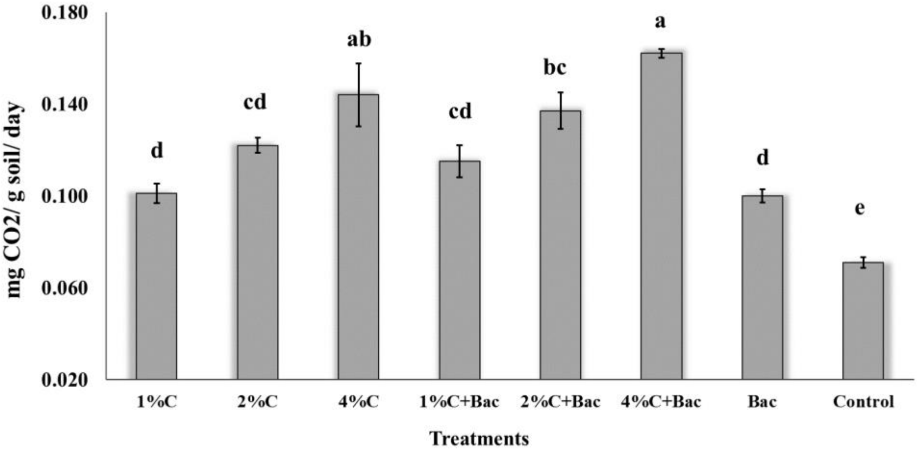

Figure 21. Effect of compost (C) and bacteria (Bac) application on basal soil respiration. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$

In our experiment, we observed that with the addition of higher doses of compost, the carbon dioxide production increased, i.e. 4% > 2% > 1% compost (Figure 21). The trend was common for both single and coupled applications. The highest amount of CO_2 was in 4% compost + bacteria amended soil (0.162 mg CO_2/ g soil/ day), followed by 4% compost treated soil (0.144 mg CO_2/ g soil/ day). They were statistically similar and more than double to control (0.071 mg CO_2/ g soil/ day). A combination of 2% compost and bacteria showed a better outcome than 2% compost application (0.137 and 0.122 mg CO_2/ g soil/ day, respectively). It was noted that the 1% compost + bacteria, single bacteria and 1% compost application were statistically alike in soil carbon dioxide production.

Soil respiration is the production of CO_2 through microbiological activities in soil, and it is considered one of the essential indicators of microbial function (Parkin et al., 1996; Alef & Nannipieri, 1995). Improvement of soil respiration was described by Nair & Ngouajio (2012), who used rye, rye-vetch, rye compost and rye-vetch compost. They mentioned that both composts had a significant positive impact on soil respiration enhancement where there was no difference in the rye and rye-vetch applications. Candemir & Gülser (2010), from their field study, demonstrated that hazelnut husk and tea waste were quite strong in improving the soil respiration in clay and sandy loam soils, respectively. Treonis et al. (2010) investigated the influence of compost application in the tomato-soybean-corn cropping system, where they experienced higher soil respiration than control. Another study reported that microbial soil respiration developed positively with the application of different organic waste compost, where tea waste was the most active one (Kizilkaya & Hepşen, 2007). Moreover, in heavy metal contaminated soils, the inoculation of PGPR was proven to enhance the soil respiration compared to controls (Silva et al., 2021; Sharma et al., 2013).

According to the results of microbial biomass carbon analysis obtained from different amended soils, the higher doses of compost significantly improved the biomass carbon values (Figure 22). The pattern was exactly similar to the basal soil respiration, i.e. 4% > 2% > 1% compost in both individual and combined application cases. Moreover, in biomass carbon production, all the combined treatments performed better than the sole compost and bacteria application. The highest value was noted in 4% compost + bacteria applied to soil (42.61 mg MBC/ g soil/ day). Then the second-highest value was recorded from 2%C + bacteria (41.14 mg MBC/ g soil/ day), and it was statistically similar to the 4% compost and 1% compost + bacteria application (40.07 and 37.52 mg MBC/ g soil/ day). Single bacteria and 1% compost application was statistically similar in biomass carbon yield. The lowest value was observed in the control (29.79 mg MBC/ g soil/ day).

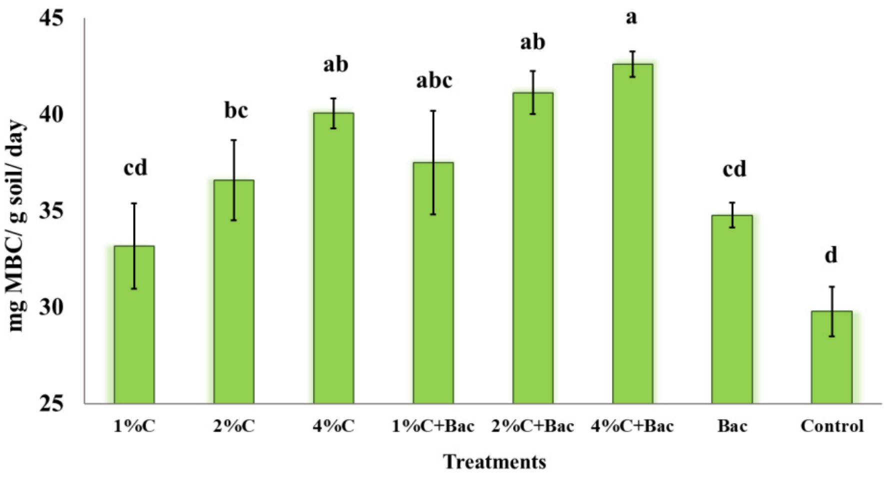

Figure 22. Effect of compost (C) and bacteria (Bac) application on the microbial biomass carbon (MBC) of soil. Lowercase letters represent the means separated by applying DMRT, where confidence interval= 95% and $p \leq 0.05$.

In our research, the compost and bacteria implementation significantly intensified the microbial biomass carbon status. It demonstrates that the HHC compost and *Bacillus megaterium* provided organic carbon to the soil microflora for their higher growth and reproduction, which eventually enhanced the soil microbial enzyme functionalities (Kanchikerimath & Singh, 2001). In a recent study on tomato waste compost, it has been reported that this compost alone and in combination with fertilizer, that is in both cases improved the microbial biomass carbon availability in soil (Durmuş & Kızılkaya, 2022). A field experiment on calendula and marigold conducted by Sharma et al. (2017) recorded that the consortium of efficient microorganisms (EM) and poultry compost significantly increased the biomass carbon status in soil. In another study, researchers found that composted cattle manure was more efficient in magnifying microbial biomass carbon in soil than composted swine manure (Das et al., 2017). Ros et al. (2006), in their crop rotation field study, described that the long-term application of composts could rapidly improve the microbial biomass carbon level. The supplementation of organic materials in soil can improve the soil organic carbon and, at the same time, can enhance the microbial population and their functions (Tejada et al., 2006; Ros et al., 2003).

These results postulated that the addition of HHC and *Bacillus megaterium* var. *phosphaticum* into soil boosts the soil biological properties, as reflected in basal soil respiration and microbial biomass carbon activities.

5. CONCLUSION

The results of this study indicated that the hazelnut husk compost and phosphate solubilizing bacteria (PSB), *Bacillus megaterium var. phosphaticum* contain a significant amount of plant nutrients and have the capability to improve the plant-available P status in soil. The outcome of this research completed the stated hypothesis. Among the single applications (1%, 2%, 4% compost and bacteria), the 1% compost can be considered for better wheat yield as it was statistically significant with 2% and 4%. This can save both cost and time for the crop producers. If it is considered to go for combined application, then 4% compost + bacteria has been proven as the best one for enhancing yield. About 22.12% yield was increased by this treatment compared to control. The water use efficiency was also highest in this application, i.e. this can use the water more potential to increase plant biomass.

HHC and bacteria applications boosted the soil chemical and biological properties. Except for the single bacteria application, the pH was close to the neutral in all the treated pots. The electrical conductivity was also in a position that it was sufficient to make the plant nutrients accessible. Soil organic matter was increased with the accumulation of compost. The exchangeable cations (Ca, Mg and K) were in a suitable condition for plants better growth and development. The available P status in this study demonstrated that with the single application of compost, the P level was enriching with increasing compost doses (4% > 2% > 1%). The combined applications experienced an opposite trend. Interestingly, the correlation between available P and alkaline phosphatase activity revealed a significant negative result. It describes a possible indication that wheat plants are continuously absorbing available P for their vegetative growth and reproduction. When the soil faces a deficiency of P, the *Bacillus megaterium* var. *phosphaticum* is becomes active to make a balance by secreting more phosphatase enzyme. To support the bacteria to maintain this function properly, the compost provides much more energy. That is why we observed higher biomass carbon and respiration status in higher compost treated pots. Therefore, soil amendment with HHC and this PSB may be a promising soil management approach to minimize the use of synthetic fertilizer in commercial agriculture, and in the long run, maintain soil health.

Overall, these results can be implemented in future practices for compost and PSB applications on agricultural lands. Moreover, further researches and long-term field plot experiments are also essential to determine the efficacy of HHC and *Bacillus megaterium* var. *phosphaticum* in proper soil management and increasing plant yield.

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2. Afrad, M.S.I, Galib, A.H., Haque, M.E., Zakaria, M., Sakib, M.H., **Rahman, M.M.** & Shoily, A.A. (2022). Assessing the overall efficiency of two technologies of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. *Journal of Extension Education*. 32(4): 6584-6594. https://doi.org/10.26725/JEE.2020.4.32.6584-6594

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Won Awards, Incentives and Scholarships

1. Erasmus Mundus Joint Master Degree (EMJMD) Scholarship