EVALUATION OF THE USAGE OF PHOSPHATE DEPOSITE IN TURKEY AS A PHOSPHORUS FERTILIZER SOURCES VIA BIOTECHNOLOGY METHODS

by
Sinem Uran

Submitted to Graduate School of Natural and Applied Sciences
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in
Biotechnology

Yeditepe University
2018

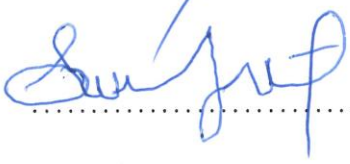
EVALUATION OF THE USAGE OF PHOSPHATE DEPOSITE IN TURKEY AS A PHOSPHORUS FERTILIZER SOURCES VIA BIOTECHNOLOGY METHODS

APPROVED BY:

Prof. Dr. Metin Turan ..... 
(Thesis Supervisor)

Prof. Dr. Fikrettin Şahin ..... 

Prof. Dr. Mehmet Rüştü Karaman ..... 

Assoc. Prof. Dr. Adem Güneş ..... 

Assist. Prof. Dr. Bahar Soğutmaz Özdemir ..... 

DATE OF APPROVAL: ..../..../2018

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Prof. Dr.Metin TURAN for the continuous support of my Ph.D study and related research, for his patience, motivation, and immence knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study.

Similar, profound gratitude goes to Prof. Dr. Fikrettin ŞAHİN, who has been a truly dedicated mentor and for their insightful comments and encouragement, but also fort he hard question which incented me to widen my research from various perspectives.

In addition, Thanks to Leyla TARHAN for assistance during the experiment.

I must thank my unbelievably supportive lovely husband Arcan. He has faith in me and my intellect even when I felt like digging hole and crawling into one because I didn't have faith in myself. These past several years have not been an easy ride, both academically and personally. I truly thank him for sticking by my side, even when I was irritable and depressed. I feel that what we both learned a lot about life and strengthened our commitment and determination to each other and to live life to the fullest.

Last but not the least, I would like to thank my family: my mother, grandmother and to my brother for almost unbelievable support throughout writing this thesis and my life in general. They are the most important people in my world and I dedicate this thesis to them. Many thanks!

Thank you for everything.

ABSTRACT

EVALUATION OF THE USAGE OF PHOSPHATE DEPOSITE IN TURKEY AS A PHOSPHORUS FERTILIZER SOURCES VIA BIOTECHNOLOGY METHODS

As a nonrenewable asset, phosphorus (P) is the second most vital macronutrient for plant development and sustenance. Request of phosphorus application in the farming creation is expanding quick all through the globe. Utilizations of phosphorus-based manures enhance the dirt fruitfulness and agribusiness yield however in the meantime worries over various elements that prompt ecological harm should be tended to appropriately. Appropriate administration of phosphorus alongside its manures is required that may help the most extreme use by plants and least run-off and wastage. The primary target of this survey is to evaluate the part of phosphorus in manures, their take-up alongside different components and motioning amid P starvationThe significance of the impact of phosphate shake relies upon the synthetic type of phosphorus in which this component is consolidated. The study, shows the aftereffects of inorganic and natural types of phosphorus in the phosphate shake from Mazidag-Derik, Semikan store situated at Mardin city at SE Anatolia of Turkey. Add up to phosphorus focus in the stone is a normal 18.5%. The inorganic phosphorus mean % substance were 99.98% for contemplated sedimentary phosphate shake, biotechnological methods for the use of *Bacillus megaterium*(BM), *Pseudomonas aeruginosa*(PA), *Pseudomonas putida*(PP), *Acinetobacter baumannii*(AB), *Micrococcus luteus*(ML), *Burkholderia cepacia*(BC), *Aspergillus niger*(AN) and *Epidermophyton floccosum (EF)* in organic agriculture, which are defined as a result of the preliminary tests of this project and which are characterized by P resolution capacity, are to enrich the P contents of the raw phosphate sources present in our country. According to the results obtained, the effect of fertilizer application on the yield and yield components of wheat, maize, tomato, potato, grape, quince, orange and cabbage plants were investigated as a result of two year field work and the efficient application was found to be more effective than control and the use of rock phosphate alone according to the usage parame

ÖZET

BİYOTEKNOLOJİK YÖNTEMLER YARDIMIYLA ÜLKEMİZDEKİ FOSFAT YATAKLARININ TARIMDA FOSFORLU GÜBRE OLARAK KULLANILABİLİRLİĞİNİN DEĞERLENDİRİLMESİ

Yenilenemez bir kaynak olarak, fosfor (P), bitki büyümesi ve beslenmesi için ikinci en önemli makro besin maddesidir. Tarımsal üretimde fosfor uygulamasının talebi dünya genelinde hızla artıyor. Fosforlu gübrelerin uygulamaları toprak verimliliği ve tarımı verimliliğini artırır, ancak aynı zamanda çevresel zararlara yol açan bir takım faktörlere yönelik endişeler de düzgün bir şekilde ele alınmalıdır. Fosforun gübrelerle birlikte doğru bir şekilde yönetilmesi, bitkiler tarafından azami kullanıma ve minimum dökülmeye ve israfa yardımcı olabilir. Bu gözden geçirmenin temel amacı gübrelerde fosforun rolünü, diğer elementlerle birlikte alımını ve P açlığı sırasında sinyal vermeyi değerlendirmektir.Fosfat kayaçının etkisinin önemi, bu elementin birleştirildiği fosforun kimyasal formuna bağlıdır. Makale, Güneydoğu Anadolu'daki Mardin kentinde bulunan Semikan Mevduatından Mazidag-Derik'teki fosfat kayaçlarındaki inorganik ve organik fosfor formlarının sonuçlarını sunmaktadır. Kayadaki toplam fosfor konsantrasyonu ortalama% 18.5'dir. Çalışılan sedimenter fosfat kayaçları için inorganik fosfor ortalaması% 99.98 idi. Bu çalışmanın amacı, ön denemeler sonucunda tanımlamaları yapılan ve fosfat çözme kapasitesi belirlenen bazı Plant Growth Promoting Bacteria (PGPR) bakterilerin organik tarımda kullanımını konu alan biyoteknolojik yöntemler ile ülkemizde mevcut ham fosfat kaynaklarının fosfat içeriklerinin zenginletilmesi, kullanım amacına göre formülasyon geliştirilmesi ve gübre haline getirilmesidir. Araştırmaya göre gübre olarak kullanımına karar veren formülasyonun iki yıllık tarla çalışması sonucunda buğday, mısır , domates, patates, üzüm, ayva, portakal, ve lahana bitkisinde verim ve verim unsurları üzerine etkileri incelenmiş ve en etkili uygulamanın kontrole göre çok daha etkin olduğu ve kaya fosfatının yalnız başına kullanımına göre birlikte kulanımı aynı zamanada P etkinlik parametresini artırdığı ortaya konmuştur.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

AB	*Acinetobacter baumannii*
AN	*Aspergillus niger*
BC	*Burkholderia cepacia*
BM	*Bacillus megaterium*
CRM	Certified Referene Material
Cu	Copper
DTPA	Diethylenetriaminepentaacetic
EF	*Epidermophyto floccosum*
HPLC	High- Performance Liquiadacid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
K	Potassium
Mg	Magnesium
ML	*Micrococcus luteus*
Mn	Manganese
MPR	Microorganism + Phosphate Rock
N	Nitrogen
Na	Calcium
P	Phosphate
ATP	Adenosine Triphosphate
PA	*Pseudomonas aeruginosa*
PH	Potential of Hydrogen
PP	*Pseudomonas putida*
PR	Phosphate Rock
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
TSP	Triple Super Phosphate
TVC	Total Viable Count
MIS	Microbial Identification System
Zn	Zinc

1. INTRODUCTION

Phosphorus has atomic number 15, atomic weight 30,97 and is located in 5. group of periodic table. Because of its strong interest in Oxygen, it is a lithophile element. For this reason, it never exists in nature independent instead exists in the salt and the ester of phosphoric acid. Besides, since it is an important building element of some living site such as C, H, N, O, it has an importance biologically as well. It acts an active part in evolution of plants[1].

The greatest reserve of phosphorus in nature is phosphorescent rocks on crustal; the second greatest reserve is water supplies. Phosphorus remains in rocks as bound phosphate. The rocks are called “phosphate” or “phosphate rock-rock phosphate”. The term rock phosphate is generally used and it includes minerals with high volume of phosphorus element, phosphate rock and concentrated products.

The phosphorus minerals in nature exist in 3 groups;

i) apatite, ii) phosphorite iii) vivianita.

The Main mineral in these beds are fluorapatite, hydroxylapatite, carbonatite, trankolit and kurskite. The most important one and primary phosphate mineral among all is apatite. The apatite is expressed by the general formula $Ca_5(PO_4)_3F$, Cl, OH, CO_3. In a economic sense, while the term apatite is used in magnetic beds, the term phosphate rock is used in sedimantery beds(Table 1.1) [2].

Table 1.1. Several rock phosphate

Mineral Name	**Chemical Formula**
Fluorine Apatite	$9CaO.3P_2O_5.CaF_2$
Chlorine Apatite	$9CaO.3P_2O_5.CaCl_2$
Hydroxyapatite	$9CaO.3P_2O_5.Ca(OH)_2$
Carbonate Apatite	$9CaO.3P_2O_5.CaCO_3$
Kollofonite	$Ca_3P_2O_8.H_2O$
Francolite	$9CaO.3P_2O_5.Ca(F,Co_3).H_2O$

Phosphate ore can be divided into 3 groups according to included gangue minerals within;

i) Siliceous ores: These are different form of quartz and silica. Some methods like flotation or density difference can be applied to these gang minerals to remove from the ore.

ii) Killin Ores: In this type of phosphate rock, there are clays, hydrated iron oxide, aluminium oxide and silicates as gangue minerals. These impurities can be removed by simple and useful techniques like washing, sifting.

iii) Limy ores: These include impurities of slightly silica and mostly Calcite ($CaCO_3$) and Dolomite ($CaCO_3.MgCO_3$). Carbonate minerals are difficult to remove by flotation or other methods. Because the superficial characteristics of carbonate and phosphate are very close to each other[3, 4].

1.1. NUTRIENT TRANSPORTATION

Plant cells can accumulate nutrients at much higher concentrations than in the soil solution that surrounds them. This allows roots to extract nutrients from the soil solution where they reside in very low concentrations. Movement of nutrients within the plant depends highly upon transportation through cell membranes, which requires energy to oppose the forces of osmosis. Here again, ATP and other high energy P compounds provide the needed energy[5].

1.2. PHOSPHORUS DEFICIENCY

When P is limited, the most striking effects are decrease in leaf expansion and leaf surface area, as well as the number of leaves. Shoot growth is more affected than root growth, which leads to a decrease in dry weight ratio of the shoot root. Nonetheless, root growth is also reduced by P deficiency, leading to less root mass to reach water and nutrients. Generally, inadequate P slows the process of carbohydrate utilization, while carbohydrate production through photosynthesis continues. This results in a build up of carbohydrates and the development of a dark green leaf color. In some plants, P-deficient leaves develop a purple color, for example tomato and corn[6]. Since P is easily mobilized in the plant,

when a deficiency occurs the P is relocated from older tissues to active meristematic tissues, resulting in foliar deficiency symptoms appearing on the older (lower) portion of the plant. However, such symptoms of P deficiency are seldom observed in the field other than loss of yield. Other effects of P deficiency on plant growth include delayed maturity, reduced quality of forage, fruit, vegetable, and grain crops, and decreased disease resistance.

1.3. SOIL- PLANT INTERACTIONS

Phosphorus is taken up from the soil solution by plant roots as orthophosphate ions, principally $H_2PO_4^-$ and to a lesser extent $H_2^-PO_4$. Several factors can influence both the rate and amount of P taken up by the plant and, therefore, can affect the recovery of a single application of P fertilizer. The same factors can also affect the recovery of P reserves accumulated in the soil from past additions of P as a fertilizer or manure. The most important factors controlling the availability of P to plant roots are its concentration in the soil solution and the P-buffer capacity of the soil. The latter controls the rate at which P in the soil solution is replenished, i.e. the rate of desorption of P from the solid phase of the soil, which is faster in soils with a high buffer capacity [7]. Also important are the size of the root system and the extent to which roots grow into the soil, and the efficiency with roots which take up P. When considering a single application of P fertilizer, the efficiency that it is used also depends on how well it was mixed with the volume of soil exploited by roots. Other factors that affect crop yield, and hence the requirement for P, can influence P uptake by the crop and thus the recovery of P and the efficiency that the applied P was used. These factors include soil moisture and the extent to which weeds, pests and diseases have been controlled. Because the effects of these factors vary from year to year, it is essential to estimate average of P recovery over a number of years in order to obtain reliable data.

1.4. AIM OF THE STUDY

The objective of this study is without using chemical fertilizers with the help of microorganisms and to increase the availability of phosphorus by eliminating the negative effects of nitrogen and nitrate.

2. MATERIALS AND METHODS

2.1. BIOTECHNOLOGICAL METHODS IN PHOSPHORUS RESOLUTION

In this study, taken from various P Gubretas phosphate shake prior distinguishing proof made *Acinetobacter baumannii, Pseudomonas aeruginosa, Pseudomonas putida*, *Bacillus megaterium, Micrococcus luteus and Burkholderia cepacia* PGPR bacteria and *Aspergillus niger* and *Epidermophyto floccosum (EF)* fungus assessed individual and relying upon the co-organization effectiveness degree and subjected to ICP-MS (Inductively Coupled Plasma-Mass Spectrometer) instrument with the assistance of improvement tests. Sized piece of ore that used for this purpose, ambient pH, concentration of microorganisms, it tries to optimize the effectiveness assessed in terms of parameters such as time and temperature [8]. Every encapsulation of the pH brings about the arrangement condition, the electrical conductivity of the medium to said phosphorus and Fe (iron), Mn (manganese), Zn (zinc), Cu (copper), Pb (lead), Cd (cadmium), Ni (nickel), Si (silicium), the material staying as hasten notwithstanding overwhelming metals, for example, Al (aliminium), P portions and aggregate Fe, Mn, Zn, Cu, Pb, Cd, Ni, Fe, Si, Al content should be dictated by testing the quality and level of P-insoluble phosphate shake. Furthermore, every application condition because of protein action in arrangement, hormones, amino acids and natural acids tried parameters and instruments that influence the P dissolvability parameters decided[9].

2.1.1. Isolates to be used in the study

All isolates used in the study; colonial structure, colony form, based on criteria such as growth and pigment production of bacteria selected, drawn, purified and isolate MIS, polyphasic taxonomy will be done by comparing according to BIOLOG system and spectrometer[10]. This isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa, Pseudomonas putida, Burkholderia cepacia, Micrococcus luteus* and *Bacillus megaterium* bacteria were kindly provided and Aspergillus niger and *Epidermophyto floccosum (EF)* fungus by Ataturk University has determined that phosphate solvent properties[11].

The Assessment of the Usability of Phosphate Reserves in our Country as a Source of Phosphorus Fertilizer in Agriculture: The provision, isolation and identification of the bacteria to be used in the study were made[12]. As a result of the study made in this section, the selection of the most suitable formulation was made, thus was evaluated as a basic process for the implementation phase in single year and perennial plants grown in different geographies.

In the study, the following CRM strains were provided for use in controls applied to formulation, and environmental isolates, and an attempt was made it determine the biochemical properties of each isolate.

i. *Bacillus megaterium DSM 319*
ii. *Pseudomonas aeruginosa DSM 1117*
iii. *Pseudomonas putida DSMZ 7189*
iv. *Acinetobacter baumannii DSMZ 30008*
v. *Micrococcus luteus DSMZ 1790*
vi. *Burkholderia cepacia DSMZ 7288*
vii. *Aspergillus niger DSM 1988*
viii. *Epidermophyto floccosum (EF)*

Environmental isolates were enriched and thus purified in addition to CRM isolates that were used in the study (Figure 2.1). The aim here is to ensure that strains isolated from nature be used in the organic fertilizer complex once fully defined and biochemically characterized. The enriched species have been identified and several types of activity tests have been assessed, primarily including phosphatase enzyme activities as important criteria in the resolution of rock phosphates and their purity.

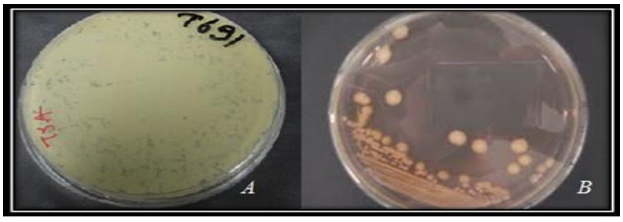


Figure 2.1. A: Enriched cuture, B: Reduced culture

2.1.2. Determination of Morphological Features

The individual morphology of the bacteria due to their small size, but can be determined by microscope. For this purpose, pure preparations prepared from bacteria produced in suitable liquid or solid media were expected to be examined by staining with specific dyes. Under the microscope, the structure of bacteria (coccoid, bacilli, comma, spiral, pleomorphic, etc.), size, sports status (with or without terminal, if any, subterminally, sentral), staining property (Gram negative or positive, etc.). Determining the survival of bacteria growth chart created.

2.2. PATHOGENICITY TESTS OF PHOSPHOROUS FERTILIZER FORMULATION

In this work package, the reciprocal effects of phosphorus fertilizer formulations generated under laboratory conditions with the PGPR bacteria of organisms naturally found in the rhizosphere of annual and perennial plants grown in different geographical areas have been determined. Besides, the antagonistic effects of phosphorus fertilizer formulations applied on *Epidermophyto floccosum (EF)* and *Aspergillus* from the fungus family and on *Bacillus megaterium*, *Micrococcus luteus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* from niger bacteria all of which increase the mechanism of phosphorus fertilizer formulation, which is helpful and effective in breaking down phosphorus and also the plant root growth and increase efficiency of have been determined (Figure 2.2).

Figure 2.2. Vitek system used in biochemical definitions

As a result of the study, it was decided that two formulations having the advantage of being the most effective in terms of fertilizer value in the solution of rock phosphate be used and the antagonistic effect tests of the said formulation were made. With this test, in addition to their individual use, the eventualities of using the organisms together were investigated. Possible formulations determined in line with this are given below.

i. *Bacillus megaterium* (BM)
ii. *Pseudomonas aeruginosa* (PA)
iii. *Pseudomonas putida* (PP)
iv. *Acinetobacter baumannii* (AB)
v. *Micrococcus luteus* (ML)
vi. *Burkholderia cepacia* (BC)
vii. *Aspergillus niger* (AN)
viii. *Epidermophyto floccosum (EF)*

Approximate content and number of microorganism used in the formulations: BM:1.3E+10 cfu/ml, PA:1,6 E+9 cfu/ml, PP:1,2 E+8 cfu/ml, AB:1,0E+8 cfu/ml, ML:2.6E+8 cfu/ml, BC:1,7E+8 cfu/ml, AN:1,3E+7 cfu/ml

In this step, the organisms used in the formulation were compared for their microbial viability and antagonism. In the study, TVC (total viable count) cards and TVC media were used for total live counting parallelly with the help of tempo system, 1 ml of sample was taken from the formulas prepared, and was added to the TVC medium and vortexed at 1000 rpm to add 3 ml of sterile purified water (Figure 2.1). Once the loading process is over after having been revortexed, the cards were taken and left to incubate at 30 ° C for 48 hours (Figure 2.2). The results obtained following the incubation were correlated with the recurrent manual operation concurrently with the tempo system. For this purpose, 1 ml of the formulas was transferred to empty sterile petri plates following which a 20 ml of 45 ° C of PCA was added and was thoroughly mixed. After the congelation, it was kept at incubation at 30 ° C for 48 h, the results evaluated after the incubation. It was observed out of the results of the organisms used as formulations that the number of organisms in the mixture showed a change without any decrease, and in the case of adding rock phosphate into this mixture, there was no decrease in efficiency, but partial increases. After the study, it was determined that the organisms contained in the fertilizer-effective mixture formulation did not show an antagonistic effect on each other (Figure 2.3).

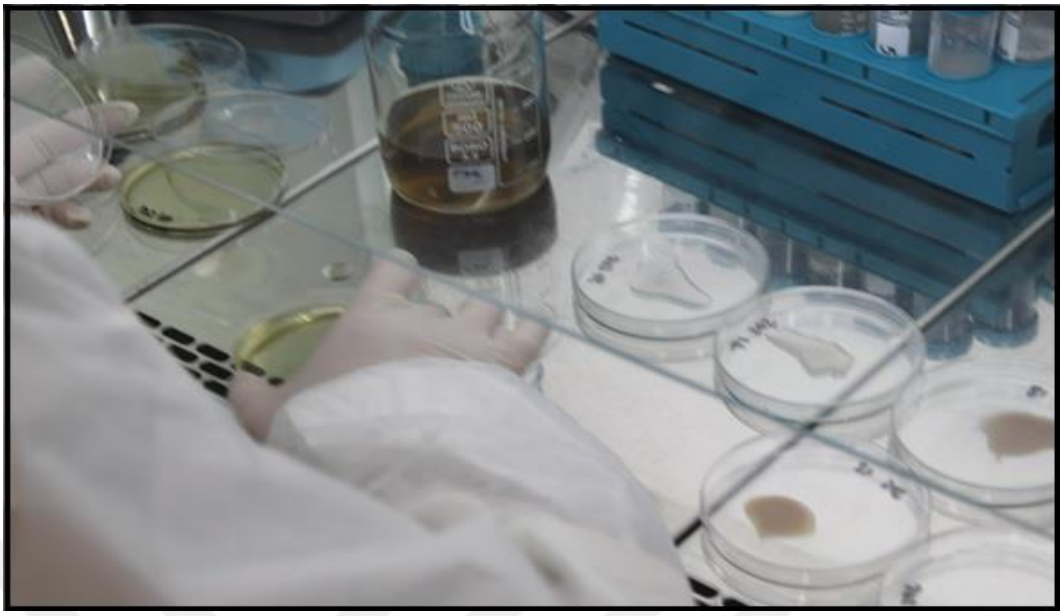


Figure 2.3. Manual Total live work

2.3. CULTURE FEATURES

In solid and liquid media with pure cultures of bacteria colony morphology and cultural properties was determined (Figure 2.4.).

Figure 2.4. Incubator

2.4. IDENTIFICATION OF PHYSIOLOGICAL PROPERTIES

Isolates of maximum, minimum and optimum growth temperature, pH, NaCl needs, and atmospheric oxygen demand detection process was conducted.

2.5. BIOCHEMICAL TESTS

The obtained isolates to determine the biochemical characteristics; Catalase, oxidase, amylase and anaerobic agar tests was done.

Figure 2.5. The class 2 cabin

2.6. MOLECULAR FEATURES

2.6.1. Determining Fatty Acid Profile

Diagnosis will be made of microorganisms in Trypticase with a standard culture medium Soy Agar (TSA) broth produced by incubation for 24-72 hours and purified fatty acids from the cells of the microorganisms have completed the growth gas chromatography

essentially according to running "MIS" (microbial identification system) by reading the fatty acid profile in the device was determined.

2.6.2. Development and preparation of the isolates for trial

The development of isolates to be used in trials were provided in 28 ° C NBA ambient until it reached 108 cfu/ml concentration (24-36hours).

2.6.2.1. Mineral Analysis

2.6.2.1.1 Determination of Microelements (Fe, Mn, Zn, Cu) and Heavy Metals Metal (Pb, Cd, Ni, F, Si, Al)

Sufficient amount of Fe, Mn, Zn and Cu were determined by the ICP-MS spectrophotometry in extracted filters according to DTPA (diethylenetriaminepentaacetic) method (sample is filtered in %2-%5 of nitric acid)[8].

2.6.2.1.2 Sequential Analysis of Phosphorus

Sequential phosphorus fractions were determined according to [10]and [13]taking soil samples which were sifted through the sieve.

2.6.2.2. Determination of the Enzyme in Phosphate Rock

2.6.2.2.1 Acid and Alkaline Phosphatase Enzyme Activity

A certain amount of stock solution of phosphate rock pre-determined events (in two different pH) were put into. Then be released into the p-nitrophenyl phosphate solution is added and incubation. NaOH was added while remaining a shaker our example Whatman (No. 42) was filtered through folded filter paper. The photoelectric calorimeter (Klett-Petersen Summer) performed by measuring the intensity Suzuga yellow. At the same colorimeter with standard solutions (including 0-10-20-30-40-50 mg of p-nitrophenol) was measured by reading a calibration graph to be drawn on the graph and the corresponding p-nitrophenol content filtrate the measured value[6].

2.6.2.3. Hormone Extraction, Purification and Analysis of Operations

2.6.2.3.1 Extraction and Purification Procedures

Extraction and purification of [14] and [15] and were performed according to the methods in triplicate. Deep examples in small parts removed from the freezer and processed into powder in a pestle with liquid nitrogen. Powdered samples on -40 ° C to be added held 80% methanol [16] and Ultra tissue disintegrator (Ultrasonic Processor, Jencons LTD.) Once homogenized, + 4 ° C in the dark for 24 hours to homogenize. It was made. Examples Whatman No. 1 filter paper after the filtered supernatant from the remaining parts were subjected to the same process again, and then combined both the supernatant. The combined supernatants from the re PTFE filter [17] passed through the evaporator then dried using a pump. The dried extracts were subjected to unravel again in $KH2PO_4$ buffer. Located in the soluble extract samples for 1 hour to separate the fatty acids the centrifugal [18][19]. The supernatant was turned into a beaker on the automatic pipette tube. To separate the phenolic compounds and[20, 21], the Polivinilpolipirilido the dissolution of each sample (PVPP, Sigma) was prepared in which the supernatant beaker into released and will be mixed thoroughly [22, 23].

2.6.2.3.2 Preparation of the Polyvinylpolypyrrolidone

Insoluble PVPP dropped off into a beaker placed on suspension filtered in the form of acetic acid after mixing thoroughly. Repeat three times the volume was used after filtration, washed with acetic acid.

PVPP supernatant mixed with Whatman No 1 filter paper will be filtered from leaving the PVPP. The extract was then be taken either immediately or stored at -40 ° C for use [24].

2.6.2.3.3 Conditioning Process

After first methanol cartridges, passed, it was prepared by washed with pure water. The supernatants (after dissolution expected if frozen) to syringes conditioned Sep-Pak C18 cartridge (1 ml / min).

By cartridges, the adsorbed hormones were taken into vials by methanol to dissolve. Samples taken into vials to be used for HPLC analysis [25].

2.6.2.3.4 Analysis of Hormones

High Performance Liquid Chromatography (HPLC) method was used in Hormone analysis.

2.6.2.3.5 Analysis of Operations with High Performance Liquid Chromatography (HPLC)

In our study, HPLC was used for indole acetic acid and abscisic acid measurements [7, 17].

2.6.2.4. Determination of Amino Acid

Amino acid analysis of the phenylisothiocyanat (Pitc) determine the separation columns [23]. Comprising amino acid standards was prepared for the standard solution. Examples of small test tube dissolved in buffer solution. Dried at high pressure and dissolved in the solution again by addition Pitc buffer. At room temperature, the reaction that occurs after time [26]. Pitc second time derivatives are dissolved at high pressure. Amino acid wherein Pitc derivatives such as sodium acetate, sodium acetate and methanol in acetonitrile 9: 1 (v / v) is dissolved in 1 ml of the mixture and analyzed by HPLC in an amount ratio.

2.6.2.5. Determination of Organic Acid

Oxalic acid in analytical measurements of organic acids include tartaric acid, malic acid, succinic acid, malonic acid, L-ascorbic acid, maleic acid, organic acids is mixture comprising citric acid and fumaric acid. Standards prepared and read, with each amino acid mixture will determine the HPLC peaks[27].

2.6.2.6. Ion Analysis

Anions such as CO_3, SO_4 of rock phosphate was determined using ion chromatography.

2.7. ENHANCED USE OF CRUDE PHOSPHATE ROCK IN HERBAL PRODUCTION

After the aforementioned raw phosphate rock process of enrichment by biotechnological methods, usability was tested in plant production of the new product to be obtained [12]. For this purpose, the raw phosphate rock enriched to be obtained by applying different doses of commonly cultured wheat and maize yields and fertilizer use efficiency in our region with output parameters were investigated.

2.8. FIELD STUDIES

With an attempt to determine the effects of the implementation of phosphorus extract obtained from the extraction of phosphate rock by bioextraction method in different regions (Erzincan, Konya, Kayseri, Antalya, Manisa, Mersin, Erzurum and Iğdır) and in different plants (grape, potato, corn, tomato, quince, orange, cabbage and wheat) (pH = 7.9, Total P_2O_5 ratio 29.6%) and at various doses (0, 6, 8 and 12 kg) on the yield, yield parameters and chemical content, P fertilizer usage efficiency of rock pohophate (pH=7.9, Total P_2O_5 ratio %29.6) and *Bacillus megaterium* (BM) + *Pseudomonas aeruginosa* (PA) + *Pseudomonas putida* (PP) + *Acinetobacter baumannii* (AB) + *Micrococcus luteus* (ML) + *Burkholderia cepacia* (BC) +*Aspergillus niger* (AN) formulation, experiments were established and sampled in the fields for 2 years.The P activity test results were determined with the yield, yield parameters and chemical contents obtained from the experiment with macro and micro elements in soil samples[28].

2.9. FIELD ANALYSIS

Soil Reaction (pH): Potentiometrically measured with glass electrode pH meter in 1: 2.5 soil water suspension [29].

Soil lime (CaCO3): It was determined volumetrically by Scheibler calcimetry [29].

Soil Organic Matter: Soil organic matter was determined by the Smith-Weldon [30].

Soluble Phosphorus Determination: The soils were determined after having been extracted with sodium bicarbonate (NaHCO3, adjusted to pH 8.5) with the ICP readable on OES 'spectrophotometer [16].

Interchangeable Cations: The interchangeable cations of the soils were determiend after having been rinsed with Ammonium Acetate and extracted with ICP readable by OES spectrophotometer [31].

Plant Acceptable Micro Element (Fe, Mn, Zn, Cu) Determination: The quantities of suitable Fe, Mn, Zn and Cu were determined by means of ICP in the filters extruded according to the DTPA method being readable on OES spectrophotometer [32].

Chlorophyll Amount: The chlorophyll content of the plant leaves were determined by SPA-502 chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan)

Stoma Permeability: The stoma content of plant samples was determined by measuring with SC-1pormeter.

Measurement of photosynthesis: To determine the physiological processes brought about in the plants during frost stress, photosynthesis measurements were performed aftermath of the frost stress with the Li-COR photosynthesis measurement device.

3. RESULTS AND DISCUSSION

Biochemical and microscopic methods were utilized in the identification process. Environmental isolates and CRM strains were studied comparatively for identification, and enrichment cultures were reduced, and a single colony was used. Gram staining was performed in the first place, and then biochemical pathway selection was made according to type of the gram. At the end of the study, it was determined that especially the phosphatase activities of the biochemical reactions of environmental isolates and CRM strains important for the project were very high and similar .

Biochemical analyzes of the CRM and environmental isolates used in the study were conducted and the efficiency tests of each organism were performed.

3.1. *BACILLUS MEGATERIUM*

Biochemical analysis results of *Bacillus megaterium* strain are given in Table 3.1 and Figures 3.1.A and 3.1.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.1. Biochemical analysis results of *Bacillus megaterium*, CRM and environmental strains

***Bacillus megaterium* DSM 319**		***Bacillus megaterium* environmental isolate**	
L- LYSINE-ARYLAMİDASE LYSA	(POSITIVE)	L- LYSINE-ARYLAMIDASE LYSA	(POSITIVE)
PHENYLALANINE ARILAMIDE PHEA	(POSITIVE)	PHENYLALANINE ARILAMIDE PHEA	(POSITIVE)
L- PROLINE ARYLAMİDASE PROA	(NEGATIVE)	L- PROLINE ARYLAMİDASE PROA	(NEGATIVE)
BETA-GALACTOSIDASE BGAL	(POSITIVE)	BETA- GALACTOSIDASE BGAL	(POSITIVE)
L-PROLIDONIL-ARYLAMİDASE PYRA	(NEGATIVE)	L- PROLIDONIL-ARYLAMİDASE PYRA	(NEGATIVE)
ALPHA - GALACTOSIDASE AGAL	(POSITIVE)	ALPHA-GALACTOSIDASE AGAL	(POSITIVE)

ALANINE ARYLAMIDASE ALAA	(POSITIVE)	ALANINE ARYLAMİDASE ALAA	(POSITIVE)
TYROSINE ARYLAMIDASE TYRA	(POSITIVE)	TYROSINE ARYLAMİDASE TYRA	(POSITIVE)
BETA-N-ACETYL-GLUCOSAMİNİDASE BNAG	(NEGATIVE)	BETA-N-ACETYL-GLUCOSAMİNİDASE BNAG	(NEGATIVE)
ALA-PHE-PRO ARYLAMİDASE APPA	(POSITIVE)	ALA-PHE-PRO ARYLAMİDASE APPA	(POSITIVE)
D- GALACTOSE DGAL	(POSITIVE)	D- GALACTOSE GAL	(NEGATIVE)
GLYCOGEN GLYG	(POSITIVE)	GLYCOGEN GLYG	(POSITIVE)
MYO-INOSITOL INO	(NEGATIVE)	MYO-INOSITOL INO	(NEGATIVE)
MALTOTRIOSE MTE	(POSITIVE)	MALTOTRIOSE MTE	(POSITIVE)
GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)	GLYCINE ARYLAMIDASE GLYA	(POSITIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
ALPHA-GLUCOSIDASE AGLU	(POSITIVE)	ALPHA- GLUCOSIDASE AGLU	(POSITIVE)
D- TAGATOSE DTAG	(NEGATIVE)	D- TAGATOSE DTAG	(NEGATIVE)
D- TREHALOSE DTRE	(NEGATIVE)	D- TREHALOSE DTRE	(NEGATIVE)
INULIN INU	(POSITIVE)	INULIN INU	(POSITIVE)

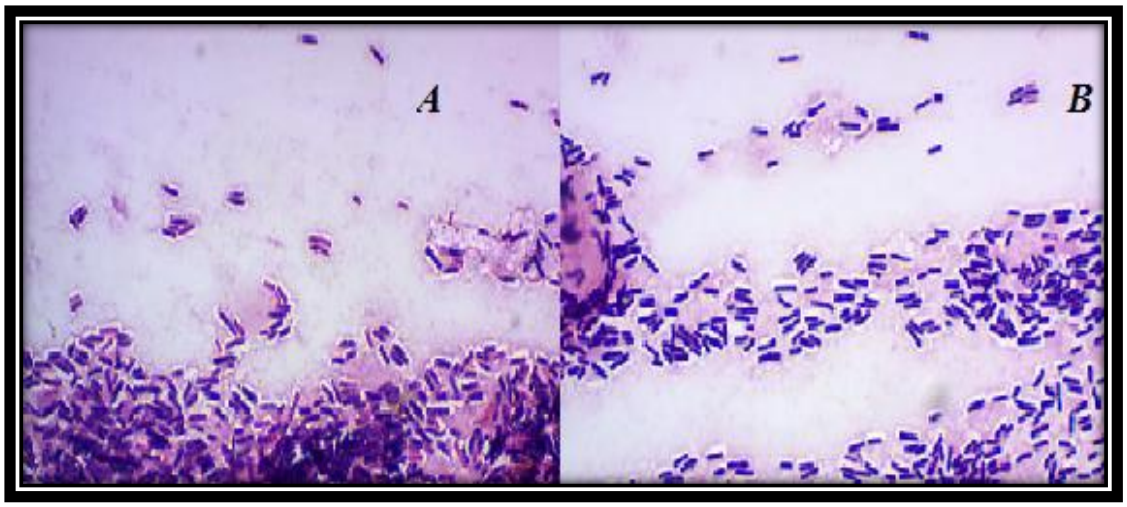


Figure 3.1. A: Environmental isolate, B: CRM strain Gram + 100x *Bacillus megaterium image*

3.2. *PSEUDOMONAS AERUGINOSA*

Biochemical analysis results of *Pseudomonas aeruginosa* strain are given in Table 3.2 and Figures 3.4.A and 3.4.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.2. Biochemical analysis results of *Pseudomonas aeruginosa* CRM and environmental strains results

***Pseudomonas aeruginosa* DSM 1117**		***Pseudomonas aeruginosa* environmental isolate**	
BETA- XYLOSIDASE BXYL	(NEGATIVE)	BETA- XYLOSIDASE BXYL	(NEGATIVE)
L- LYSINE - ARYLAMİDASE LYSA	(NEGATIVE)	L- LYSINE - ARYLAMİDASE LYSA	(NEGATIVE)
L- ASPARTATE ARYLAMİDASE ASPA	(NEGATIVE)	L- ASPARTATE ARYLAMIDASE ASPA	(NEGATIVE)
PHENYLALANINE ARYLAMİDASE PHEA	(NEGATIVE)	PHENYLALANINE ARYLAMİDASE PHEA	(NEGATIVE)
L- PROLİNE ARYLAMİDASE PROA	(POSITIVE)	L- PROLİNE ARYLAMIDASE PROA	(POSITIVE)

BETA-GALACTOSIDASE BGAL	(NEGATIVE)	BETA-GALACTOSIDASE BGAL	(NEGATIVE)
L-PROLİDONİL-ARYLAMİDASE PYRA	(NEGATIVE)	L-PROLIDONIL-ARYLAMİDASE PYRA	(NEGATIVE)
GLYCINE ARYLAMIDASE GLYA	(POSITIVE)	GLYCİNE ARYLAMIDASE GLYA	(POSITIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(NEGATIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N-ASETIL-D-GLUCOSAMİNE NAG	(POSITIVE)	N-ASETİL-D-GLUCOSAMINE NAG	(POSITIVE)
PHOSPHATASE PAZ	(NEGATIVE)	PHOSPHATASE PAZ	(POSITIVE)
OLEANDOMICIN RESİSTANCE OLD	(POSITIVE)	OLEANDOMICIN RESİSTANCE OLD	(POSITIVE)

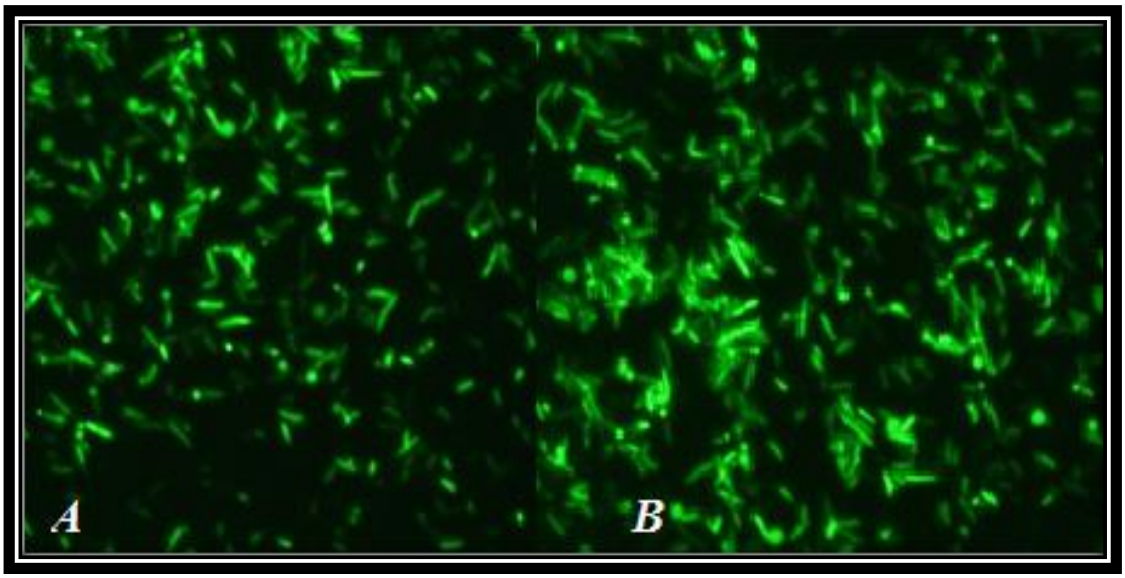


Figure 3.2. A: Environmental isolate, B: CRM strain, cyb green 100x 1M *Pseudomonas aeruginosa* image

3.3. *PSEUDOMONAS PUTIDA*

Biochemical analysis results of *Pseudomonas putida* strain are given in Table 3.3 and Figures 3.5.A and 3.5.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.3. Biochemical analysis results of *Pseudomonas putida* CRM and environmental strains,

Pseudomonas putida DSMZ 7189		***Pseudomonas aeruginosa environmental isolate***	
L-PROLIN ARILAMIDAD PRO	(POSITIVE)	L-PROLIN ARILAMIDAD PRO	(POSITIVE)
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA- GALACTOSIDASE BGAL	(POSITIVE)
L-ASPARTAT ARILAMIDAD ASPA	(NEGATIVE)	L-ASPARTAT ARILAMIDAD ASPA	(NEGATIVE)
FENİLALANIN ARILAMIDAD PHEA	(NEGATIVE)	FENİLALANIN ARILAMIDAD PHEA	(NEGATIVE)
MYO-INOSITOL INO	(POSITIVE)	MYO-INOSITOL INO	(POSITIVE)
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA- GALACTOSIDASE BGAL	(POSITIVE)
L-PROLIDONIL- ARILAMIDAD PYRA	(NEGATIVE)	L-PROLIDONIL- ARILAMIDAD PYRA	(NEGATIVE)
GLISIN ARILAMIDAD GLYA	(NEGATIVE)	GLISIN ARILAMIDAD GLYA	(NEGATIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL -D- GLUCOSAMINE NAG	(NEGATIVE)	N- ACETYL -D- GLUCOSAMINE NAG	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
ESCULIN HYDROLYSIS ESC	(POSITIVE)	ESCULIN HYDROLYSIS ESC	(POSITIVE)

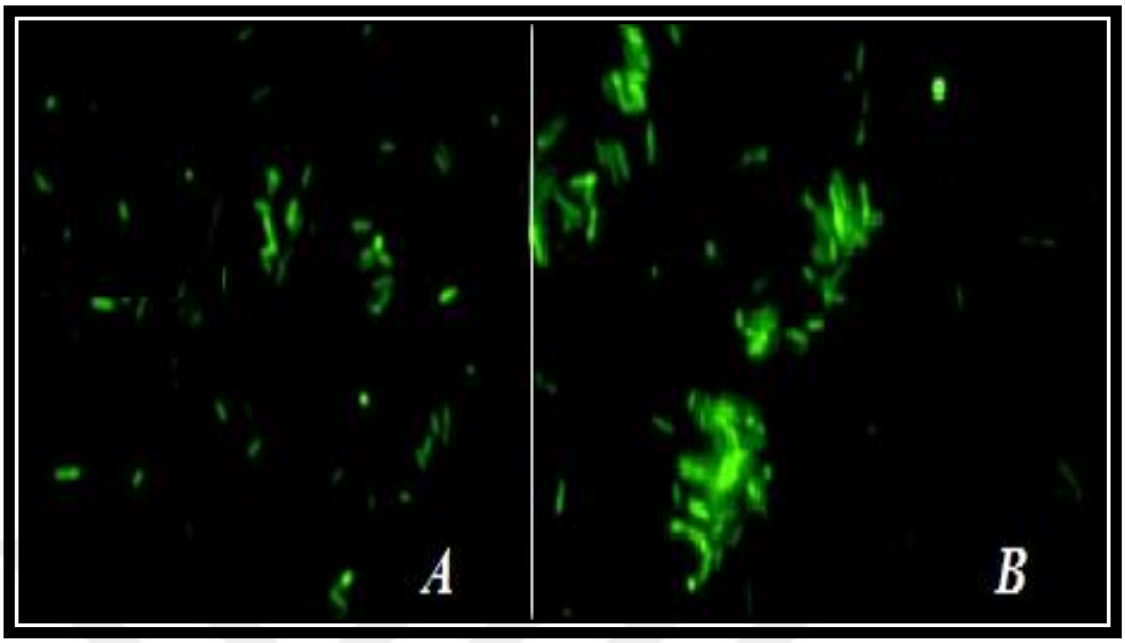


Figure 3.3. A: Environmental isolate, B: CRM strain B green 100x 1M *Pseudomonas putida* image

3.4. *ACINETOBACTER BAUMANNII*

Biochemical analysis results of *Acinetobacter baumannii* strain are given in Table 3.4 and Figures 3.6.A and 3.6.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.4. Biochemical analysis results of *Acinetobacter baumanii* CRM and enviromental strains

Acinetobacter baumannii DSMZ 30008		***Acinetobacter baumannii ENVİRONMENTAL ISOLATE***	
MYO-INOSITOL INO	(POSITIVE)	MYO-INOSITOL INO	(POSITIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)

D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL -D- GLUCOSAMİNE NAG	(NEGATIVE)	N- ACETYL -D- GLUCOSAMINE NAG	(NEGATIVE)
PALATINOSE PLE	(NEGATIVE)	PALATINOSE PLE	(NEGATIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA- GLUCOSIDASE BGLU	(NEGATIVE)	BETA- GLUCOSIDASE BGLU	(NEGATIVE)
BETA- MANNOSIDASE BMAN	(NEGATIVE)	BETA- MANNOSIDASE BMAN	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L- PROLINE ARYLAMIDASE PROA	(POSITIVE)	L- PROLINE ARYLAMIDASE PROA	(POSITIVE)

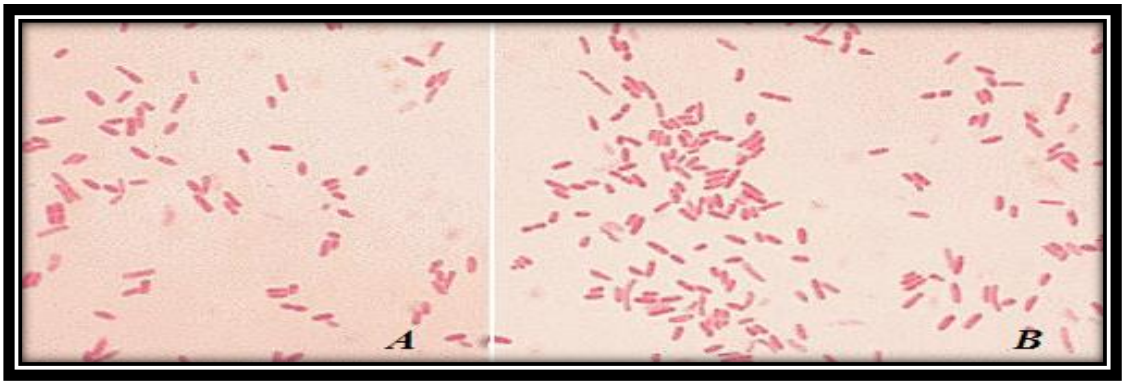


Figure 3.4. A: Environmetal isolate, B: CRM Strain, Gram (-) 100x IM *Acinetobacter baumannii* image

3.5. *MICROCOCCUS LUTEUS*

Biochemical analysis results of *Micrococcus luteus* strain are given in Table 3.5 and Figures 3.7.A and 3.7.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.5. Biochemical analysis results of *Micrococcus luteus* CRM and Environmental strains

Micrococcus luteus DSMZ 1790		***Micrococcus luteus environmental isolate***	
MYO-INOSITOL INO	(POSITIVE)	MYO-INOSITOL INO	(POSITIVE)
METHYL-D-KSILOSID MDX	(NEGATIVE)	METHYL-D-KSILOSID MDX	(NEGATIVE)
ALPHA-MANNOSIDASE AMAN	(NEGATIVE)	ALPHA-MANNOSIDASE AMAN	(POSITIVE)
MALTOTRIOSE MTE	(NEGATIVE)	MALTOTRIOSE MTE	(NEGATIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
PALATINOSE PLE	(POSITIVE)	PALATINOSE PLE	(POSITIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA-GLUCOSIDASE BGLU	(NEGATIVE)	BETA-GLUCOSIDASE BGLU	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L- PROLINE ARYLAMIDASE PROA	(POSITIVE)	L- PROLINE ARYLAMIDASE PROA	(POSITIVE)

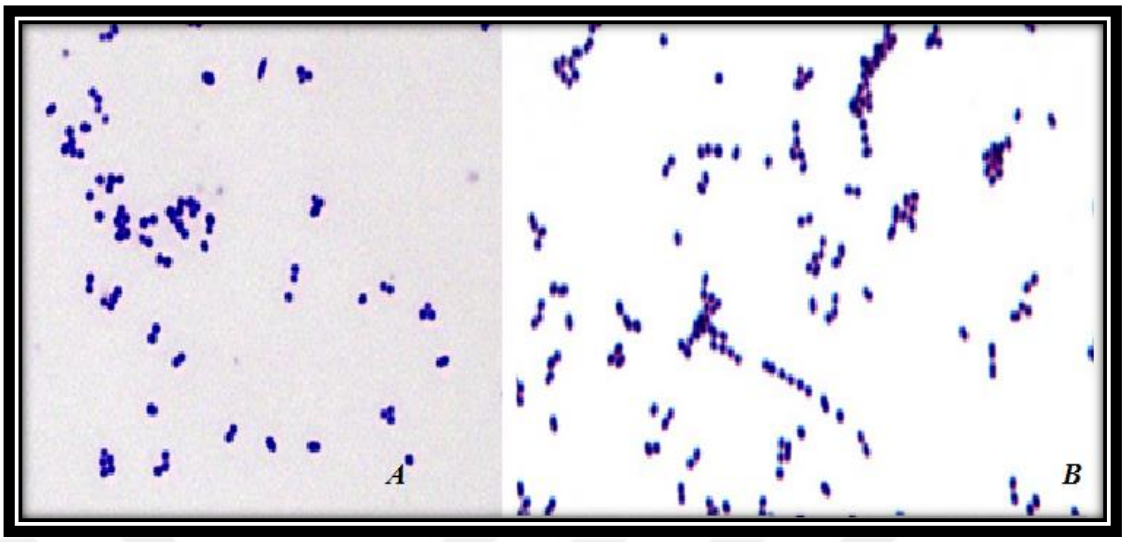


Figure 3.5. A: Environmental Strain B: CRM Strain, Gram (+) 100x IM *Micrococcus luteus i*mage

3.6. *BURKHOLDERIA CEPACIA*

Biochemical analysis results of *Burkholderia cepacia* strain are given in Table 3.6 and Figures 3.8.A and 3.8.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.6. Biochemical analysis results of *Burkholderia cepacia* CRM and Environmental strains

Burkholderia cepacia D MZ 7288		***Burkholderia cepacia environmental isolate***	
MYO-INOSITOL INO	(POSITIVE)	MYO-INOSITOL INO	(POSITIVE)
ALPHA-MANNOSIDASE AMAN	(POSITIVE)	ALPHA-MANNOSIDASE AMAN	(POSITIVE)
MALTOTRIOSE MTE	(NEGATIVE)	MALTOTRIOSE MTE	(NEGATIVE)
GLYCINE ARILAMIDE GLYA	(NEGATIVE)	GLYCINE ARILAMIDE GLYA	(NEGATIVE)

D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL-D-GLUCOSAMİNE NAG	(NEGATIVE)	N- ACETYL-D-GLUCOSAMİNE NAG	(NEGATIVE)
PALATİNOSE PLE	(POSITIVE)	PALATINOSE PLE	(POSITIVE)
L-RHAMNOSUS IR HA	(NEGATIVE)	L-RHAMNOSUS IR HA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA-GLUCOSIDASE BGLU	(NEGATIVE)	BETA-GLUCOSIDASE BGLU	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L-PROLINE ARYLAMİDASE PROA	(POSITIVE)	L-PROLINE ARYLAMİDASE PROA	(NEGATIVE)
D-MANNOSE MNE	(POSITIVE)	D-MANNOSE MNE	(POSITIVE)
D- RIBOSE DRIB	(POSITIVE)	D- RIBOSE DRIB	(POSITIVE)

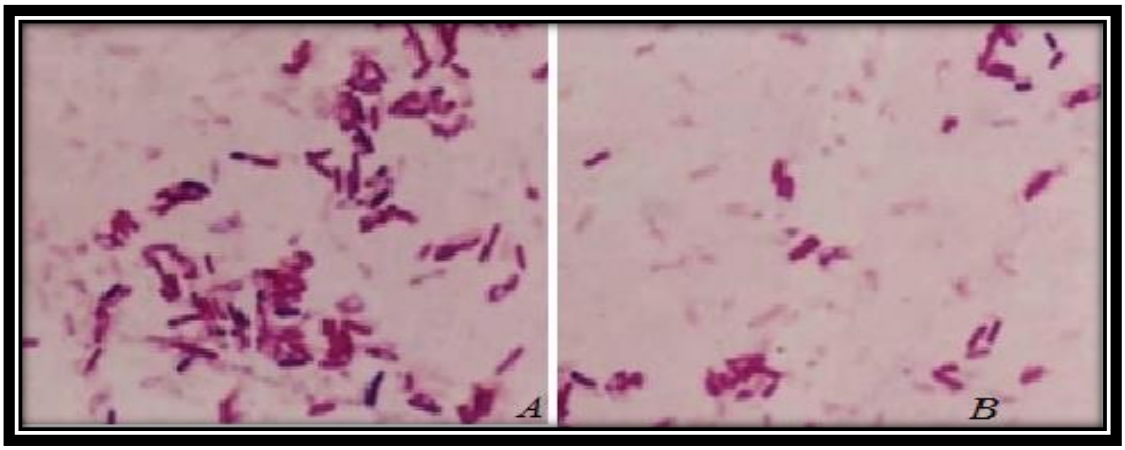


Figure 3.6. A: Environmental isolate, B: CRM strain, Gram (-) 100x IM *Burkholderia cepacia* image

3.7. *ASPERGILLUS NIGER* VE *EPIDERMOPHYTO FLOCCOSUM (EF)*

Biochemical analysis results of *Aspergillus niger* and *Epidermophyto floccosum (EF)* strains are given in Table 3.7 and Figures 3.9.A and 3.9.B. In the results of biochemical tests, microorganisms gave the desired results.

Table 3.7. Biochemical analysis results of *Aspergillus niger and Epidermophyto floccosum (EF)* CRM and environmental strains

Aspergillus niger DSM 1 88, Epidermophyto floccosum (EF)		***Aspergillus niger and Epidermophyto floccosum (EF) environmental isolate***	
ALPHA-MANNOSIDASE AMAN	(POSITIVE)	ALPHA-MANNOSIDASE AMAN	(POSITIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
L- RHAMNOSUS IR	(NEGATIVE)	L- RHAMNOSUS IR	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA	(POSITIVE)	L-PROLINE ARYLAMIDASE PROA	(POSITIVE)
D-RIBOSE DRIB	(POSITIVE)	D-RIBOSE DRIB	(POSITIVE)
D-RIBOSE DRIB 0,06 MG	(POSITIVE)	D-RIBOSE DRIB 0,06 MG	(POSITIVE)
KANAMYCIN BLOOD RESİSTANCE	(NEGATIVE)	KANAMYCIN BLOOD RESİSTANCE	(NEGATIVE)
OLEANDOMYCIN RESISTANCE OLD	(POSITIVE)	OLEANDOMYCIN RESİSTANCE OLD	(POSITIVE)
ESCULIN HYDROLYSIS ESC	(NEGATIVE)	ESCULIN HYDROLYSIS ESC	(NEGATIVE)

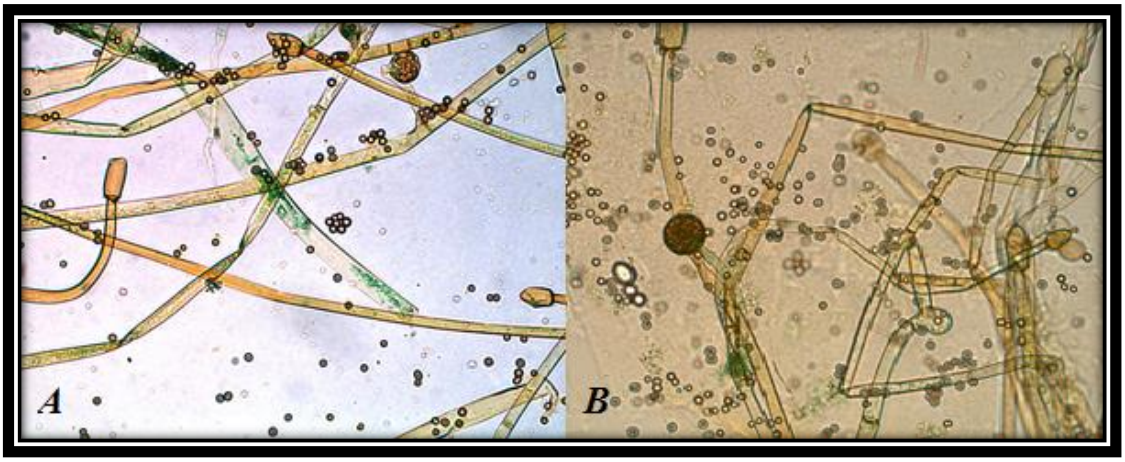


Figure 3.7. A: Environmental isolate, B: CRM strain, Gram (-) 100x IM *Aspergillus niger* image

In order to determine the number of microorganisms in the test content properly, five recurring parallel study was conducted as a result of which was determined that the average live load of the products generated from the strains obtained in both CRM and environmental isolates as well as the individual live load and the strength of each microorganism used in the product was high, thus revealing that formulations to be used in solving rock phosphate are effective (Table 3.8 and 3.9)

Table 3.8. Average live count results of products obtained from CRM strains

Bacillus megaterium			***Pseudomonas aeruginosa***			***Pseudomonas putida***			***Acinetobacter baumannii***		
1st dish	2nd dish	average	1st dish	2nd dish	average	1st dish	2nd dish	average	1st dish	2nd dish	average
6,5E+9	2,5 E+9	4,5E+9	1,2 E+8	2,1E +7	7,1E+7	2,0E+ 8	4,1 E+6	1,0E+8	6,6E+ 7	3,6E +7	5,1E+7
2,5E+8	1,1 E+9	6,8E+8	2,2 E+7	2,6E +8	1,4E+8	3,1E+ 6	1,4 E+7	8,6E+6	5,2E+ 6	2,2E +7	1,4E+7
1,2E+8	2,1 E+9	1,1E+9	1,4 E+6	1,8E +7	9,7E+6	1,8E+ 6	2,3 E+7	1,2E+7	1,0E+ 8	1,9E +9	1,0E+9
2,3E+9	1,4 E+8	1,2E+9	3,2 E+6	1,1E +7	7,1E+6	2,9E+ 5	1,8 E+5	2,4E+5	3,1E+ 8	4,1E +7	1,8E+8
2,7E+8	2,3 E+9	1,3E+9	2,8 E+8	1,3E +6	1,4E+8	5,1E+ 7	2,6 E+6	2,7E+7	1,9E+ 9	1,9E +9	1,1E+9
avg		1,8E+9	avg		7,4E+7	avg		3,0E+7	avg		4,6E+8
Micrococcus luteus			***Burkholderia cepacia***			***Aspergillus niger***			**Average results for CRM Strains**		
1st dish	2nd dish	average	1st dish	2nd dish	average	1st dish	2nd dish	average	***B. megaterium***		1,8E+9
2,3E+1 0	2,9 E+9	1,3E+1 0	6,0 E+8	2,4E +9	1,5E+9	4,2E+ 5	2,5 E+5	3,4E+5	***P. aeruginosa***		7,4E+7

1,4E+7	2,1 E+7	1,8E+7	2,4 E+7	3,7E +7	3,1E+7	1,2E+ 7	1,4 E+6	1,3E+6	***P.putida***	3,0E+7
2,6E+7	2,6 E+6	1,4E+7	1,9 E+7	3,4E +9	1,7E+9	2,3E+ 5	3,4 E+4	1,3E+5	***A. baumannii***	4,6E+8
2,3E+9	4,5 E+6	1,2E+9	3,2 E+9	4,0E +8	1,8E+9	1,8E+ 6	3,5 E+5	1,1E+6	***M. luteus***	2,9E+9
2,8E+8	5,3 E+8	4,1E+8	2,8 E+7	3,7E +8	2,0E+8	2,3E+ 4	4,1 E+4	3,2E+4	***B. cepacia***	1,0E+9
average		2,9E+9	avg		1,0E+9	avg		5,7E+5	***A. niger***	5,7E+5
Total viable microorganisms in the product									6,3E+10	

Table 3.9. Average live count results of products from environmental strains

Bacillus megaterium			***Pseudomonas aeruginosa***			***Pseudomonas put ida***			***Acinetobacter baumannii***		
1st dish	2nd dish	avg	1st dish	2nd dish	avg	1st dish	2nd dish	avg	1st dish	2nd dish	avg
2,3E +9	2,5 E+8	1,3E +9	4,2E +8	2,0 E+8	3,1E +8	3,0E +8	2,8 E+7	1,6E +8	3,2E +6	2,8E+6	3,0E +6
2,3E +8	3,4 E+7	1,3E +8	2,3E +6	3,0 E+7	1,6E +7	1,9E +7	4,5 E+6	1,2E +7	2,4E +6	4,6E+6	3,5E +6
5,2E +6	4,2 E+8	2,1E +8	3,6E +7	6,0 E+7	4,8E +7	1,7E +7	2,6 E+6	9,8E +6	5,1E +6	2,6E+7	1,6E +7
5,1E +8	2,0 E+8	3,6E +8	1,9E +7	3,8 E+7	2,9E +7	3,0E +7	7,0 E+7	5,0E +7	4,0E +7	5,8E+8	3,1E +8
3,8E +8	6,0 E+9	3,2E +9	5,2E +7	2,7 E+8	1,6E +8	1,6E +6	2,7 E+6	2,2E +6	3,1E +8	4,0E+8	3,6E +8
avg		1,0E +9	avg		1,1E +8	avg		4,8E +7	avg		1,4E +8

Micrococcus luteus			***Burkholderia cepacia***			***Aspergillus niger***			**Average results on environmental isolates**	
1st dish	2nd dish	avg	1st dish	2nd dish	avg	1st dish	2nd dish	avg	***B. megaterium***	1,0E +9
3,5E +9	3,1 E+9	3,3E +9	5,0E +9	3,7 E+9	4,4E +9	3,9E +5	4,0 E+5	4,0E +5	***P. aeruginosa***	1,1E +8
1,6E +9	4,0 E+8	1,0E +9	3,4E +7	6,1 E+8	3,2E +8	5,6E +5	5,0 E+5	5,3E +5	***P.putida***	4,8E +7

3,2E +8	3,5 E+7	1,8E +8	2,3E +8	1,0 E+8	1,7E +8	3,8E +4	1,6 E+5	9,9E +4	***A. baumannii***	1,4E +8
3,1E +8	4,5 E+7	1,8E +8	4,1E +7	1,6 E+8	1,8E +8	1,8E +6	1,0 E+5	9,5E +5	***M. luteus***	1,0E +9
2,4E +8	5,0 E+8	3,7E +8	3,0E +7	3,7 E+8	2,0E +8	4,0E +6	4,0 E+5	2,2E +6	***B. cepacia***	1,0E +9
avg		1,0E +9	avg		1,0E +9	avg		8,3E +5	***A. niger***	8,3E +5
Total viable microorganisms in the product									4,3E+10	

After the products having fertilizer values were identified, efficiency tests were carried out by conducting the necessary work to determine the shelf life of the said formulations. Accordingly, the microorganisms produced were kept at 25 ° C and 8 ° C in 100 ml packages after the live counting, and total live countings were monitored monthly. As a result of the studies, no change or deterioration was to be found in the content of the products during the first 4 months of the shelf life studies conducted in the first phase while the numbers of the organisms in the formulations showed no change until the 4th month after the initial live count but took a course of a stable change at a rate of 5-10% decrease after the 4th month (Table 3.9, Figures 3.8, 3.9).

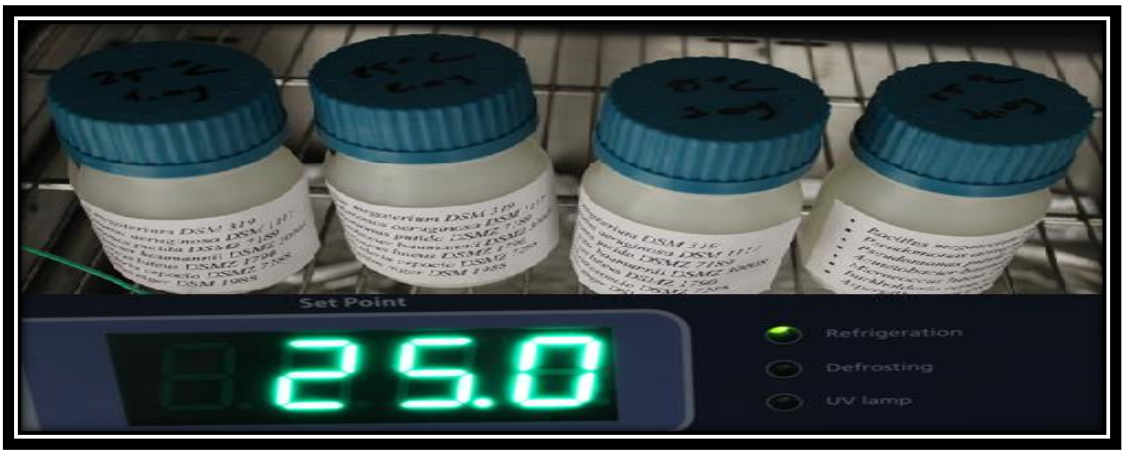


Figure 3.8. Shelf life samples stored at 25 ° C and 60% humidity

Figure 3.9. Shelf life samples held at 8 °C

Table 3.10. Average live count results of products obtained in CRM and environmental strains by months

CRM Product 1st month results			***CRM Product 2nd month results***			***CRM Product 3th month results***			***CRM Product 4th month results***		
1st dish	**2 nd dish**	**average**	**1st dish**	**2 nd dish**	**average**	**1st dish**	**2 nd dish**	**average**	**1st dish**	**2 nd dish**	**average**
4,5E+8	4,1E+	4,3E+8	1,2E+8	2,0E+8	1,6E+8	3,0E+8	3,5E+7	1,7E+8	2,2E+7	2,0E+5	1,1E+7
2,3E+8	1,2E+8	1,8E+8	3,4E+8	3,1E+8	3,3E+8	2,5E+7	4,4E+7	3,5E+7	4,0E+6	4,2E+7	2,3E+7
2,8E+9	3,5E+8	1,6E+9	2,5E+7	6,0E+7	4,3E+7	1,5E+8	2,6E+6	7,6E+7	4,1E+7	3,6E+7	3,9E+7
3,7E+8	7,5E+9	3,9E+9	3,5E+7	4,2E+7	3,9E+7	6,3E+8	7,1E+8	6,7E+8	3,2E+8	6,4E+8	4,8E+8
2,8E+8	2,0E+9	1,1E+9	1,6E+8	3,5E+6	8,2E+7	5,0E+7	5,0E+7	5,0E+7	2,6E+7	5,2E+8	2,7E+8
average		1,5E+9	**average**		1,3E+8	**average**		2,0E+8	**average**		1,7E+8

The formulations which had established shelf life were also tested for pathogenicity to check whether the products were contaminated with other bacteria or pathogens or not, and

was also tested to be free of microorganisms that threaten human and animal health as a result of the study (Table 3.11).

Table 3.11. Results of pathogen analysis performed on the products

Microorganism	**Resut**
Salmonella spp	not detected
Aeromomas spp	not detected
Mycobacterium spp	not detected
E.coli O157	not detected
E.coli	not detected
Listeria spp	not detected
Bacillus cereus	not detected
Bacillus antrachis	not detected
Cl. botulinum	not detected
Cl. tetani	not detected
Cl.perfringens	not detected
Legionella spp	not detected
Vibrio spp	not detected
Shigella spp	not detected
Fusarium spp	not detected
Verticillium spp	not detected
Puccinia spp	not detected

3.8. PATHOGENICITY TESTS OF PHOSPHOROUS FERTILIZER FORMULATION

Table 3.12. Microbial viability results in BM + PA + PP + AB + ML + BC + AN mixture

Microbial viabilite (cfu/ml)						
BM	PA	PP	AB	ML	BC	AN
4,4E+9	5,5E+9	7,6E+7	3,9E+8	4,0E+8	4,4E+6	2,0E+6
7,5E+8	8,0E+8	2,8E+8	1,6E+8	4,0E+6	5,0E+7	2,7E+7
2,2E+8	4,0E+9	3,3E+7	4,2E+7	2,0E+5	5,2E+7	2,6E+7
5,3E+9	5,2E+8	3,7E+7	5,4E+7	1,0E+6	1,0E+6	1,0E+6
2,0E+10	3,7E+9	4,0E+7	7,5E+7	7,4E+8	2,0E+7	3,8E+6

Table 3.13. Microbial viability results in 2. BM + PA + PP + AB + ML + BC + AN + Phosphorus mixture

Microbial viabilite (cfu/ml)						
BM	PA	PP	AB	ML	BC	AN
1,0E+9	1,2E+10	6,7E+9	7,6E+7	4,4E+7	2,0E+8	4,1E+6
4,0E+8	5,0E+8	4,5E+8	6,6E+8	3,4E+8	3,1E+6	1,4E+7
1,0E+9	5,7E+9	3,4E+9	2,5E+8	6,5E+7	1,8E+6	2,3E+7
1,1E+8	4,0E+7	7,5E+7	3,6E+7	3,7E+8	1,2E+6	4,5E+6
3,0E+8	3,0E+9	1,7E+9	1,1E+8	1,0E+7	5,1E+7	6,0E+6

Both phosphorus-free and non-phosphorus formulations were scanned against pathogenic bacterial strains and they were tested for proof to not contain microorganisms that threaten especially human or animal health. In this step, *Salmonella spp, Listeria spp, E. coli O157* analyzes were performed by using Vidas blue detector device, 25 ml samples from the formulations were added to 100 ml Tripton Water and incubated at 25 ° C for 24 h, following the incubation, LMX, SALM, O157 ICE kits were loaded with 500ul and added to the device. After 80 minutes when the results were observed (Figures 4.7 and 4.8). no pathogen was detected. (Tables 3.12 and 3.13). The other pathogenic microorganisms were identified by Vitek 2 compact device and manually sowed and incubated in TSA medium for 36 hours at 30 ° C. After the incubation, 0,65-2,20 MacFarland bacteria were prepared in 3 ml of physiological saline, GP, GN, YST , BCL, ANC and NH cards, and the results were determined after about 16 hours (Tables 3.14 and 3.15).

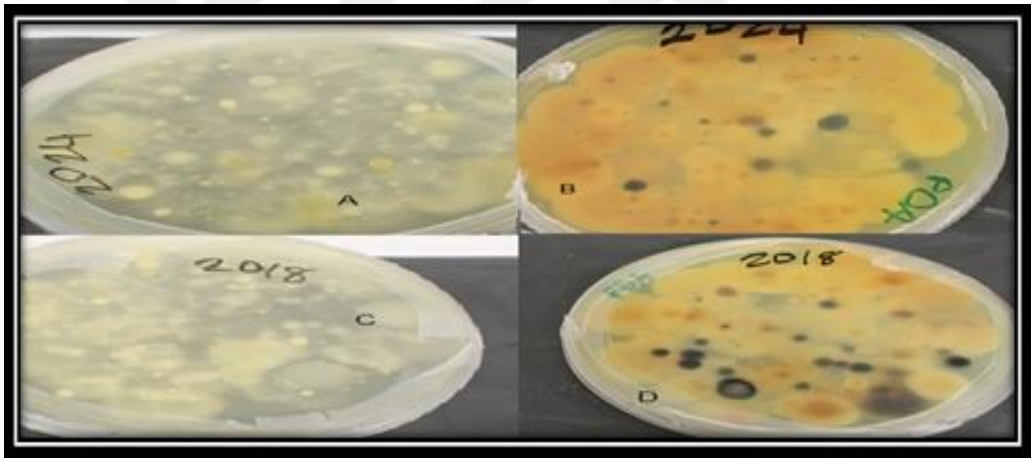


Figure 3.10. A and C; Microbial viability petri images of the mixture (BM + PA + PP + AB + ML + BC + AN)

Table 3.14. Screening tests results against pathogenic bacteria

BM+PA+PP+AB+ML+BC+AN mixture	
Microorganism	**Result**
Salmonella spp	not detected
Aeromomas spp	not detected

Mycobacterium spp	not detected
E.coli O157	not detected
E.coli	not detected
Listeria spp	not detected
Bacillus cereus	not detected
Bacillus antrachis	not detected
Cl. botulinum	not detected
Cl. tetani	not detected
Cl.perfringens	not detected
Legionella spp	not detected
Vibrio spp	not detected
Shigella spp	not detected
Fusarium spp	not detected
Verticillium spp	not detected
Puccinia spp	not detected

Table 3.15. Screening tests results against pathogenic bacteria

BM+PA+PP+AB+ML+BC+AN+ P mixture	
Microorganism	**Result**
Salmonella spp	not detected
Aeromomas spp	not detected

Mycobacterium spp	not detected
E.coli O157	not detected
E.coli	not detected
Listeria spp	not detected
Bacillus cereus	not detected
Bacillus antrachis	not detected
Cl. botulinum	not detected
Cl. tetani	not detected
Cl.perfringens	not detected
Legionella spp	not detected
Vibrio spp	not detected
Shigella spp	not detected
Fusarium spp	not detected
Verticillium spp	not detected
Puccinia spp	not detected

After the patogenity test in the formulations (BM + PA + PP + AB + ML + BC + AN) and (BM + PA + PP + AB + ML + BC + 2.10 and 2.11) both of which used as mixture, studies were conducted for the shelf life of the products (Figures 3.9 and 3.10) the microorganisms produced for this purpose were re-counted every month after a live count was made after having been kept in 100-1000 ml packages, at 25 ° C and 8 ° C. As a result of the studies, it was determined that the initial live count and the 9th live count are close values, without any change or deterioration in the product content and the shelf life studies conducted for 9 months in the first phase (Table 3.16).

Figure 3.11.Microorganism formula shelf life samples stored at 25 ° C temperature and 60% humidity (BM+PA+PP+AB+ML+BC+AN)

Figure 3.12. Samples of microorganism + phosphorus phosphorus (BM + PA + PP + AB + ML + BC + AN + Phosphorus) fertilizer kept at 25 ° C temperature and 60% humidity

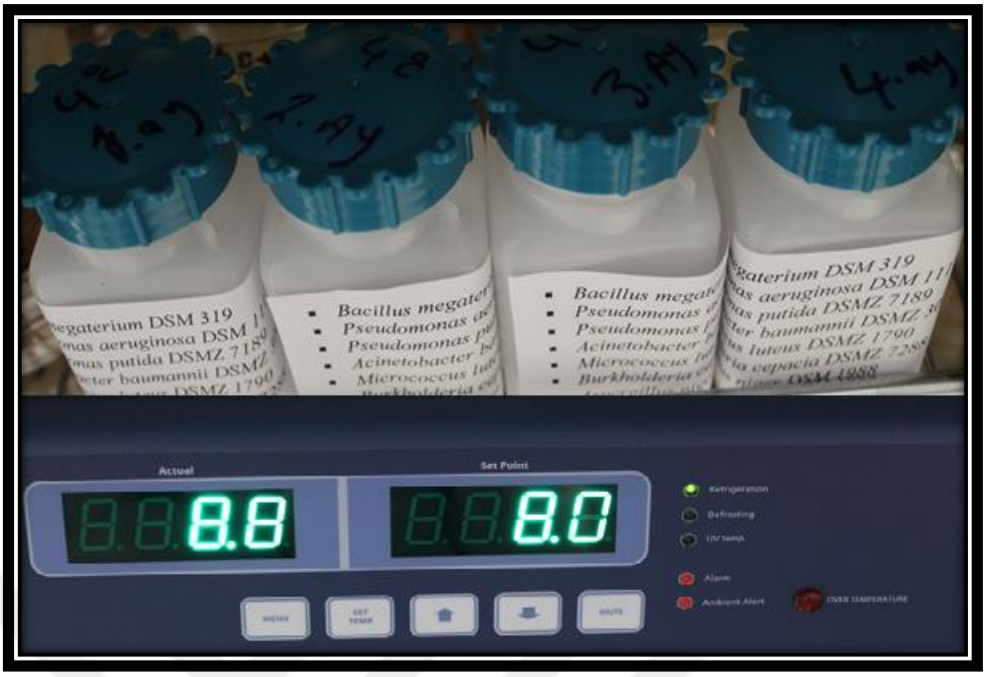


Figure 3.13. Shelf life samples held at 8 ° C temperature and 35% humidity

Table 3.16. Viability results of products shelf life studies

BM+PA+PP+AB+ML+BC+AN mixture (cfu/ml)									
Months	**1st month**	**2nd months**	**3th months**	**4th months**	**5th months**	**6th months**	**7th months**	**8th months**	**9th months**
Results	4,3E+08	1,6E+08	1,7E+08	1,1E+08	2E+08	3,2E+08	4E+07	1,1E+08	1,3E+08
	1,8E+08	3,3E+08	3,5E+07	2,3E+07	3E+07	1,3E+07	4E+07	1,3E+08	5,1E+08
	1,6E+09	4,3E+0	7,6E+07	3,9E 07	2E+08	5,1E+07	6E+07	7,1E+08	2,1E+08
	3,9E+09	3,9E+07	6,7E+08	4,8E+08	8E+07	3,6E+07	5E+08	4,6E+07	1,6E+08
	1,1E+09	8,2E+07	5,0E+07	2,7E+08	1E+07	1,1E+08	3E+07	5,0E+08	3,0E+08

BM+PA+PP+AB+ML+BC+AN + P mixture (cfu/ml)				
Months	**1st months**	**2nd months**	**3th months**	**4th months**
Results	2,5E+09	6,6E+09	1,7E+08	1,1E+08
	6,2E+09	2,5E+09	3,5E+07	2,3E+07
	5,6E+09	1,4E+08	7,6E+07	8,5E+08
	3,2E+10	8,5E+09	6,1E+08	3,0E+08
	3,3E+09	1,8E+08	3,5E+08	7,1E+09

The change was monitored by conducting repeated biochemical analyzes of each organism in the formulation decided to be used as a mixture,. Thus, was used in Vitek 2 Compact system. Microorganisms enriched on TSA were suspended in tryptic strain broth at 0.55 to 2.80 MacFarland. For this purpose, 0.55-0.65 MacFarland and approx. 2.20-2.80 MacFarland of microorganism suspensions were prepared for bacteria and mold-yeast respectively and placed in the Vitek 2 Compact device. Biochemical results were obtained after a work of approximately 16 hours. The mixtures of both phosphorus-containing and non-phosphorus microorganisms were investigated in the studies,. As a result of the studies no antagonistic effect or biochemical structure change was observed (Table 3.17, 3.18, 3.19, 3.20, 3.21, 3.22 and 3.23).

Table 3.17. Biochemical analysis results of *Bacillus megaterium*

BM+PA+PP+AB+ML+BC+AN through the mixture *Bacillus megaterium*		**BM+PA+PP+AB+ML+BC+AN+Phosphorus through the mixture *Bacillus megaterium***	
L- LYSINE - ARYLAMIDASE LYSA	(POSITIVE)	L- LYSINE - ARYLAMIDASE LYSA	(POSITIVE)
PHENYLALANINE ARYLAMIDASE HEA	(POSITIVE)	PHENYLALANINE ARYLAMIDASE HEA	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA		L-PROLINE ARYLAMIDASE PROA	
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA- GALACTOSIDASE BGAL	(POSITIVE)
L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)	L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)
ALFA- GALACTOSIDASE	(POSITIVE)	ALFA- GALACTOSIDASE	(POSITIVE)

AGAL		AGAL	
ALANINE ARYLAMIDASE ALAA	(POSITIVE)	ALANINE ARYLAMIDASE ALAA	(POSITIVE)
TYROSİNE ARYLAMIDASE TYRA	(POSITIVE)	TYROSİNE ARYLAMIDASE TYRA	(POSITIVE)
BETA-N-ACETYL-GLUCOSAMINIDASE BNAG	(POSITIVE	BETA-N-ACETYL-GLUCOSAMINIDASE BNAG	(POSITIVE
ALA-PHE-PRO ARYLAMIDASE APPA	(POSITIVE)	ALA-PHE-PRO ARYLAMIDASE APPA	(POSITIVE)
D- GALACTOSE DGAL	(POSITIVE)	D- GALACTOSE DGAL	(NEGATIVE)
GLYCOGEN GLYG	(POSITIVE)	GLYCOGEN GLYG	(POSITIVE)
MYO-İNOSİTOL INO	(NEGATIVE)	MYO-İNOSİTOL INO	(NEGATIVE)
MALTOTRIOSE MTE	(POSITIVE)	MALTOTRIOSE MTE	(POSITIVE)
GLYCINE ARYLAMIDASE GLYA	(POSITIVE	GLYCINE ARYLAMIDASE GLYA	(POSITIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
ALPHA- GLUCOSIDASE AGLU	(POSITIVE)	ALPHA- GLUCOSIDASE AGLU	(POSITIVE)
D- TAGATOSE DTAG	(NEGATIVE)	D- TAGATOSE DTAG	(NEGATIVE)
D- TREHALOSE DTRE	(NEGATIVE)	D- TREHALOSE DTRE	(NEGATIVE)
INULIN INU	(POSITIVE)	INULIN INU	(POSITIVE)

Table 3.18. Biochemical analysis results of *Pseudomonas aeruginosa*

BM+PA+PP+AB+ML+BC+ AN through the mixture *Pseudomonas aeruginosa*		***BM+PA+PP+AB+ML+BC +AN+P through the mixture Pseudomonas aeruginosa***	
BETA- XYLOSIDASE BXYL	(NEGATIVE)	BETA- XYLOSIDASE BXYL	(NEGATIVE)
L- LYSINE - ARYLAMIDASE LYSA	(NEGATIVE)	L- LYSINE - ARYLAMIDASE LYSA	(NEGATIVE)
L- ASPARTATE ARYLAMIDASE ASPA	(NEGATIVE)	L- ASPARTATE ARYLAMIDASE ASPA	(NEGATIVE)
PHENYLALANINE ARYLAMIDASE PHEA	(NEGATIVE)	PHENYLALANINE ARYLAMIDASE PHEA	(NEGATIVE)
L-PROLINE ARYLAMIDASE PROA	(NEGATIVE)	L-PROLINE ARYLAMIDASE PROA	(NEGATIVE)
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA- GALACTOSIDASE BGAL	(POSITIVE)
L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)	L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)
GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)	GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL -D-GLUCOSAMINE NAG	(NEGATIVE)	N- ACETYL -D-GLUCOSAMINE NAG	(POSITIVE)

PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
OLEANDOMYCIN RESISTANCE OLD	(NEGATIVE)	OLEANDOMYCIN RESISTANCE OLD	(POSITIVE)
ESCULIN HYDROLYSIS ESC	(POSITIVE)	ESCULIN HYDROLYSIS ESC	(POSITIVE)

Table 3.19. Biochemical analysis results of *Pseudomonas putida*

BM+PA+PP+AB+ML+BC+AN through the mixture *Pseudomonas putida*		***BM+PA+PP+AB+ML+BC+AN+P through the mixture Pseudomonas putida***	
L-PROLINE ARYLAMIDASE PRO	(POSITIVE)	L-PROLINE ARYLAMIDASE PRO	(POSITIVE)
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA-GALACTOSIDASE BGAL	(POSITIVE)
L- ASPARTATE ARYLAMIDASE ASPA	(NEGATIVE)	L- ASPARTATE ARYLAMIDASE ASPA	(NEGATIVE)
PHENYLALANINE ARYLAMIDASE PHEA	(NEGATIVE)	PHENYLALANINE ARYLAMIDASE PHEA	(NEGATIVE)
MYO-İNOSİTOL INO	(POSITIVE)	MYO-İNOSİTOL INO	(POSITIVE)
BETA- GALACTOSIDASE BGAL	(POSITIVE)	BETA-GALACTOSIDASE BGAL	(POSITIVE)
L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)	L-PROLIDONIL-ARYLAMIDASE PYRA	(NEGATIVE)
GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)	GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL -D-GLUCOSAMINE NAG	(NEGATIVE)	N- ACETYL -D-GLUCOSAMINE NAG	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
ESCULIN HYDROLYSIS ESC	(POSITIVE)	ESCULIN HYDROLYSIS ESC	(POSITIVE)

Table 3.20. Biochemical analysis results of *Acinetobacter baumannii*

BM+PA+PP+AB+ML+BC+AN through the mixture *Acinetobacter baumannii*		**BM+PA+PP+AB+ML+BC+AN+P through the mixture *Acinetobacter baumannii***	
MYO-INOSITOL INO	(POSITIVE)	MYO-INOSITOL INO	(POSITIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N-ASETIL-D-GLUCOSAMINE NAG	(NEGATIVE)	N-ASETIL-D-GLUCOSAMINE NAG	(NEGATIVE)
PALATINOSE PLE	(NEGATIVE)	PALATINOSE PLE	(NEGATIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA-GLUCOSIDASE BGLU	(NEGATIVE)	BETA- GLUCOSIDASE BGLU	(NEGATIVE)
BETA-MANNOSIDASE BMAN	(NEGATIVE)	BETA- MANNOSIDASE BMAN	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA	(POSITIVE)	L-PROLINE ARYLAMIDASE PROA	(POSITIVE)

Table 3.21. Results of *Micrococcus luteus* biochemical analysis

BM+PA+PP+AB+ML+BC+AN through the mixture Micrococcus luteus		***BM+PA+PP+AB+ML+BC+AN+P through the mixture Micrococcus luteus***	
MYO-İNOSİTOL INO	(POSITIVE)	MYO-İNOSİTOL INO	(POSITIVE)
METHYL -D- KSİLOSİD MDX	(NEGATIVE)	METHYL -D-KSİLOSİD MDX	(NEGATIVE)
ALPHA - MANNOSIDASE AMAN	(NEGATIVE)	ALPHA- MANNOSIDASE AMAN	(POSITIVE)

MALTOTRIOSE MTE	(NEGATIVE)	MALTOTRIOSE MTE	(NEGATIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
PALATINOSE PLE	(POSITIVE)	PALATINOSE PLE	(POSITIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA- GLUCOSIDASE BGLU	(NEGATIVE)	BETA- GLUCOSIDASE BGLU	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PİRUVAT PVATE	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA	(POSITIVE)	L-PROLINE ARYLAMIDASE PROA	(POSITIVE)

Table 3.22. Results of *Burkholderia cepacia* biochemical analysis

BM+PA+PP+AB+ML+BC+AN through the mixture Burkholderia cepacia		***BM+PA+PP+AB+ML+BC+AN+P through the mixture Burkholderia cepacia***	
MYO-İNOSITOL INO	(POSITIVE)	MYO-İNOSİTOL NO	(POSITIVE)
ALPHA- MANNOSIDASE AMAN	(POSITIVE)	ALPHA- MANNOSIDASE AMAN	(POSITIVE)
MALTOTRIOSE MTE	(NEGATIVE)	MALTOTRIOSE MTE	(NEGATIVE)
GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)	GLYCINE ARYLAMIDASE GLYA	(NEGATIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
N- ACETYL -D- GLUCOSAMINE NAG	(NEGATIVE)	N- ACETYL -D- GLUCOSAMINE NAG	(NEGATIVE)
PALATINOSE PLE	(POSITIVE)	PALATINOSE PLE	(POSITIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
BETA- GLUCOSIDASE BGLU	(NEGATIVE)	BETA- GLUCOSIDASE BGLU	(NEGATIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA	(POSITIVE)	L-PROLINE ARYLAMIDASE PROA	(NEGATIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- RIBOSE RIB	(POSITIVE)	D- RIBOSE DRIB	(POSITIVE)

Table 3.23. Biochemical analysis results of *Aspergillus niger and Epidermophyto floccosum (EF)*

BM+PA+PP+AB+ML+BC+AN + EF through the mixture Aspergillus niger		***BM+PA+PP+AB+ML+BC+AN+ EF+P through the mixture Aspergillus niger***	
ALPHA- MANNOSIDASE AMAN	(POSITIVE)	ALPHA- MANNOSIDASE AMAN	(POSITIVE)
D- MANNITOL DMAN	(POSITIVE)	D- MANNITOL DMAN	(POSITIVE)
D- MANNOSE DMNE	(POSITIVE)	D- MANNOSE DMNE	(POSITIVE)
D- MELEZITOSE DMAL	(POSITIVE)	D- MELEZITOSE DMAL	(POSITIVE)
L- RHAMNOSUS IRHA	(NEGATIVE)	L- RHAMNOSUS IRHA	(NEGATIVE)
PHOSPHATASE PAZ	(POSITIVE)	PHOSPHATASE PAZ	(POSITIVE)
PYRUVATE PVATE	(POSITIVE)	PYRUVATE PVATE	(POSITIVE)
L-PROLINE ARYLAMIDASE PROA	(POSITIVE)	L-PROLINE ARYLAMIDASE PROA	(POSITIVE)
D-RİBOSE DRIB	(POSITIVE)	D-RİBOSE DRIB	(POSITIVE)
D-RİBOSE DRIB 0,06 MG	(POSITIVE)	D-RİBOSE DRIB 0,0 MG	(POSITIVE)
BLOOD BINARY RESISTANCE	(NEGATIVE)	BLOOD BINARY RESISTANCE	(NEGATIVE)
OLEANDOMYCIN RESISTANCE OLD	(POSITIVE)	OLEANDOMYCIN RESISTANCE OLD	(POSITIVE)
ESCULIN HYDROLYSIS ESC	(NEGATIVE)	ESCULIN HYDROLYSIS ESC	(NEGATIVE)

3.9. DETERMINATION OF EFFECTIVE BACTERIAL FORMULATION

As a result of preliminary studies conducted, an attempt was made to determine the strength of microorganism formulations to dissolve the phosphate rock. For this purpose, 5 g of phosphate rock was taken and different microorganism formulations were applied within 100 ml of medium. Phosphorus analysis was performed by performing indivudal sampling at different times such as in 0, 2, 4, 6, 24 hours and 1 week (Table 3.22.). As a result of the study, it was determined that the effects of different microorganism formulations on phosphorus solubility are different and vary depending on the time.

In view of efficieny time, it was determined that the *B. megaterium x Epidermophyto floccosum (EF) x A. niger* formulation affects the phosphorus solubility in a shorter period of time (Table 3.23.). It was found out that 70% of the total phosphorus content of the phosphate rock conveyed to solvent environment and converted into a beneficial form within 24 hours.It was also determined that with the implementation of *A. baumannii x B. megaterium* formulation in 1 week period, 84% of phosphorus content of phosphate rock conveyed to solvent environment and was converted into useful form. Considering the duration of the activity out of these results, experiments were carried out by adding *B. megaterium* x *Epidermophyto floccosum (EF)* x *A. niger* formulation to the phosphate rock for the field studies..

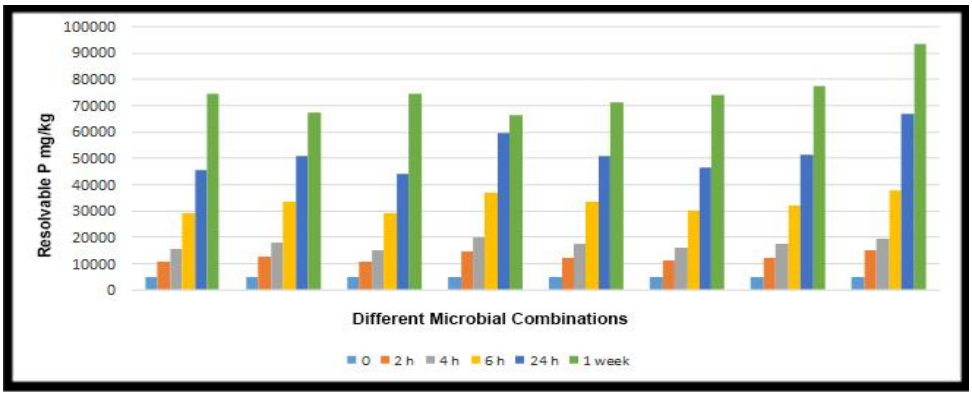


Figure 3.14. Time-dependent phosphorylstion activities of microorganism formulation at 22 ° C

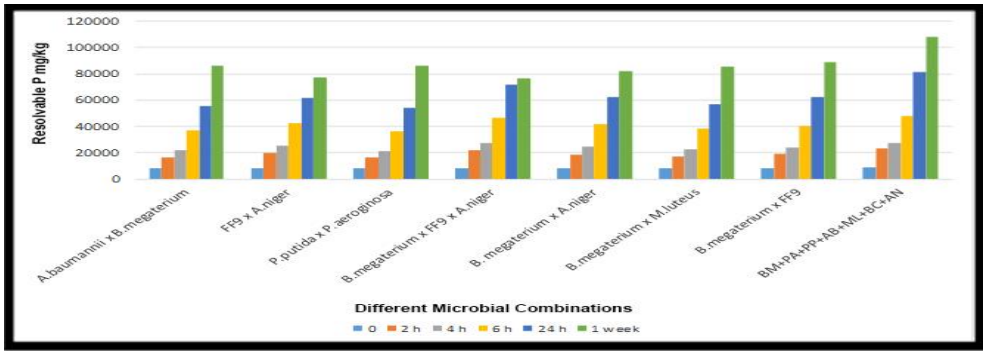


Figure 3.15. Time dependent phosphorylation activities of microorganism formulations at 32 ° C

3.9.1. WHEAT EXPERIMENT

According to the aleatoric trial design of summer wheat kind (Cracked) grown in the field conditions, seed sowing was done in rows of 17 fertilizer with combined grain drill, each row range being 10 cm and 34 (17x2) row design, 180 kg of seed, 120 kg of N/ha, 80 kg of P_2O_5/ha for each hectare, with the parcel length being 7 m while the parcel width is 4 (10cm x 34= 340 cm) at an area of 28 m^2 parcels and 2 meters of distance were kept between each parcel. The experiment was made in 6 parcels in total with 3 replications such as phosphate rock (FK) and microorganism + phosphate rock (MFK).

3.9.1.1. Some Properties of Wheat-Grown Soils

Samples were taken from the soils of each parcel from a 0-30 cm depth and chemical analyzes were carried out at the harvest time in order to determine the change in the soil and the amount of plant nutrients in both years throughout the development period of the wheat plant (Tables 3.24 and 3.25).

Table 3.24. Analysis results of the soil where the 2016 wheat plant was grown (n=3)

Application	Dose	pH	$CaCO_3$	Organic Matter	N	P	K
		(1:2,5)	(%)		kg/da		
Pre-trial		7,54	1,87	1.22	1,1	2,45	78,76
	Control	7,57	1,92	1,23	3,24	3,12	79,12
Bio+FK	6	7,58	1,89	1,23	3,88	4,55	80,23
	12	7,52	1,77	1.24	4,55	5,14	81,24
	18	7,56	1,72	1,24	4,12	6,11	81,12
TSP	6	7,62	1,95	1,18	3,24	3,24	70,23
	12	7,64	1,94	1.20	3,56	3,88	72,33
	18	7,66	1,92	1,17	3,59	4,56	72,11

Table 3.25. Analysis results of the soil where the 2016 wheat plant was grown (n=3)

Application	Dose	Ca	Mg	Na	Fe	Cu	Mn	Zn
		me/100 gr			mg/kg			
Pre-trial		16,57	2,33	0,56	3,24	5,44	4,12	3,44
	Control	16,12	2,30	0,51	2,88	5,67	4,24	3,12
Bio+FK	6	15,88	2,32	0,54	3,12	5,87	4,15	3,40
	12	15,78	2,35	0,48	3,54	5,76	4,02	3,55
	18	16,11	2,38	0,43	3,78	5,57	3,88	3,48
TSP	6	15,98	2,24	0,53	2,98	5,76	3,96	3,15
	12	16,77	2,28	0,52	2,95	4,67	3,88	3,21
	18	16,23	2,31	0,55	2,81	4,45	3,76	2,78

Table 3.26. Analysis results of 2017 wheat plant cultivated soil (n=3)

Application	Dose	pH	$CaCO_3$	Organic Matter	N	P	K
		(1:2,5)	(%)		kg/da		
Pre-trial		7,98	4,51	1.12	2,1	2,45	78,70
	Control	7,87	4,22	1,16	3,10	3,12	78,20
Bio+FK	6	7,85	2,89	1,48	4,8	5,30	80,10
	12	7,82	2,97	1.54	4,85	6,20	81,24
	18	7,86	2,82	1,62	4,82	6,50	81,12
TSP	6	7,92	3,95	1,10	3,64	4,24	70,23
	12	7,94	4,34	1.12	3,76	4,88	72,33
	18	7,96	4,32	1,15	3,99	4,56	72,11

Table 3.27. Analysis results of 2017 wheat cultivated soil

Application	Dose	Ca	Mg	Na	Fe	Cu	Mn	Zn
		mg/100 gr			mg/kg			
Pre-trial		15,20	2,30	0,46	1,24	0,44	4,10	1,25
	Control	15,10	2,30	0,51	1,88	0,67	4,20	1,20
Bio+FK	6	15,45	2,22	0,55	1,12	0,87	4,10	1,40
	12	15,24	2,25	0,48	1,54	0,76	4,02	1,50
	18	16,10	2,27	0,42	1,78	0,57	3,78	1,48
TSP	6	15,74	2,50	0,50	1,98	0,76	3,96	1,15
	12	16,45	2,66	0,55	1,95	0,67	3,18	1,20
	18	16,10	2,45	0,58	1,81	0,45	3,70	1,72

3.9.1.2. Yield and Yield Parameters of Wheat Plant

The measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the efflux within the development period of wheat plant (Tables 3.27, 3.28). During the harvest period, the plants were harvested and the yields were calculated by separating the granules. As for the plant samples were taken and dried in

drying-oven and were made ready for analysis. For the physiological analyzes, the plants were placed in the freezer set at -800C, kept frozen until the analysis process and then analyzed

Table 3.28. Yield and yield parameters of 2016 wheat plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
		kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		265c	42c	14,24c	0,27c
Bio+FK	6	288b	44b	15,32b	0,31b
	12	332a	48a	16,54a	0,35a
	18	327a	47a	15,78b	0,35a
TSP	6	275c	44b	14,65c	0,26c
	12	288b	45b	15,12b	0,28c
	18	302ab	47a	15,47b	0,31b

Table 3.29. Yield and yield parameters of the 2017 wheat plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
		kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		244d	48c	13,10c	0,27c
Bio+FK	6	278c	69b	16,32b	0,30b
	12	310b	66b	19,24a	0,36a
	18	355a	77a	20,15a	0,38a
TSP	6	260c	50c	14,10c	0,29b
	12	290b	51c	15,10b	0,29b
	18	287b	51c	15,20b	0,30b

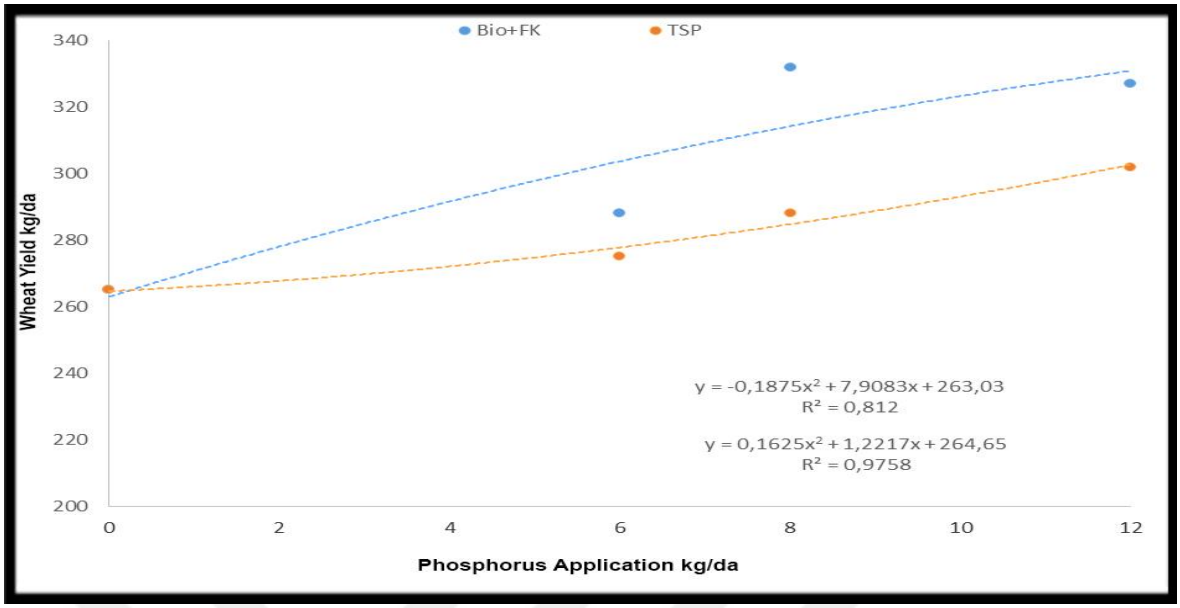


Figure 3.16. Effect of applications on yield of 2016 wheat plant

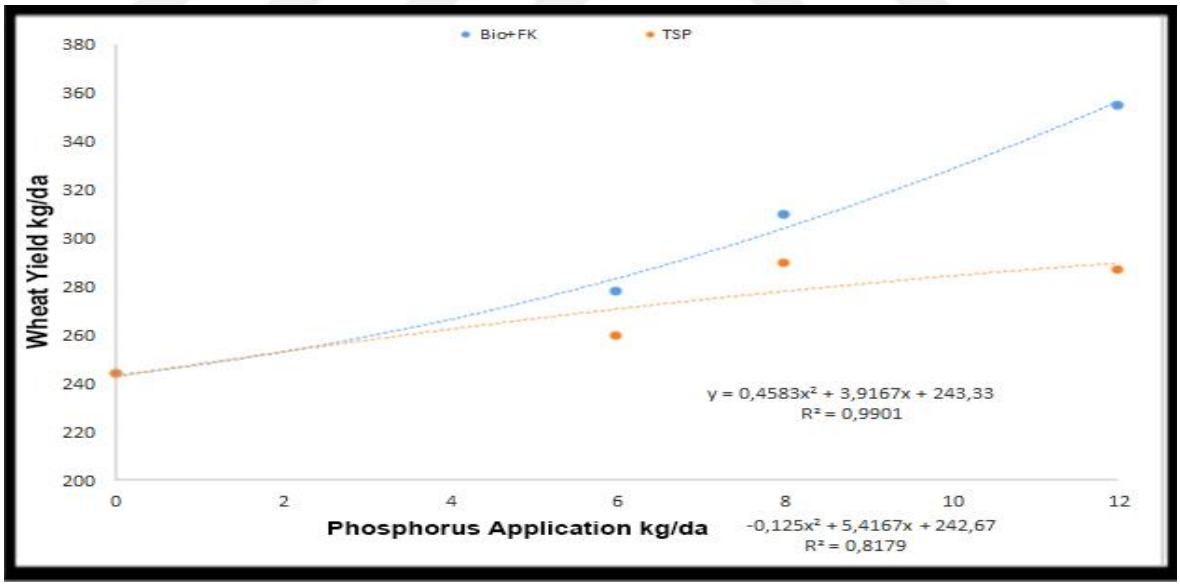


Figure 3.17. The effect of applications on yield of 2017 wheat plant

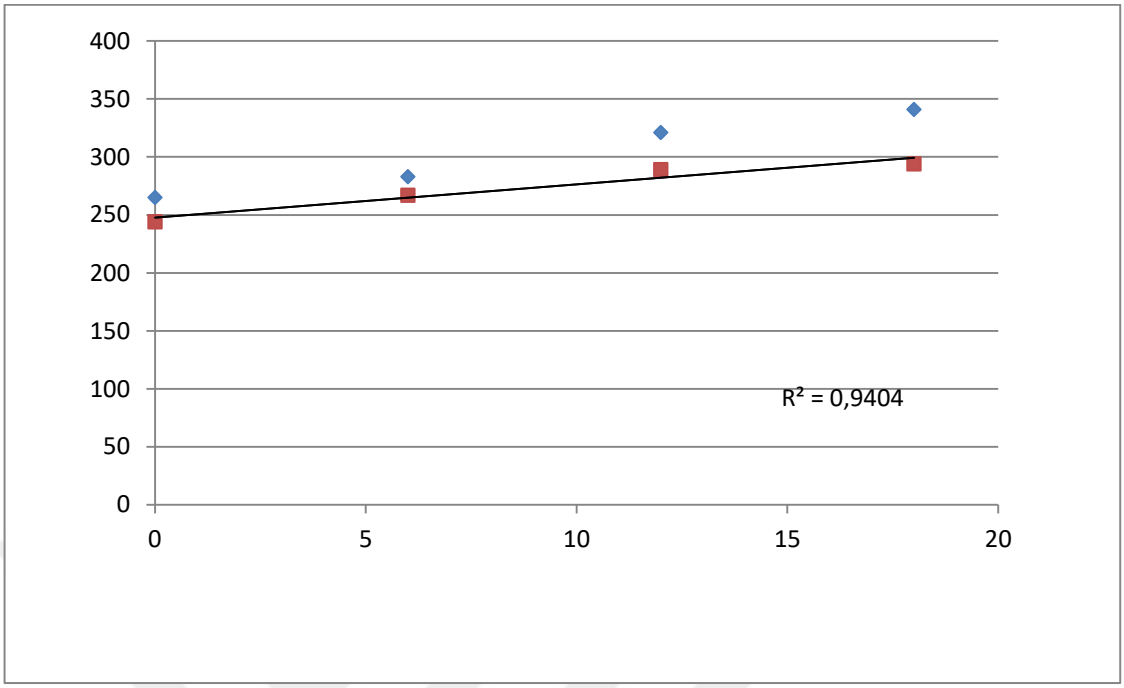


Figure 3.18. 2016-2018 years average

Figure 3.19. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in wheat plant

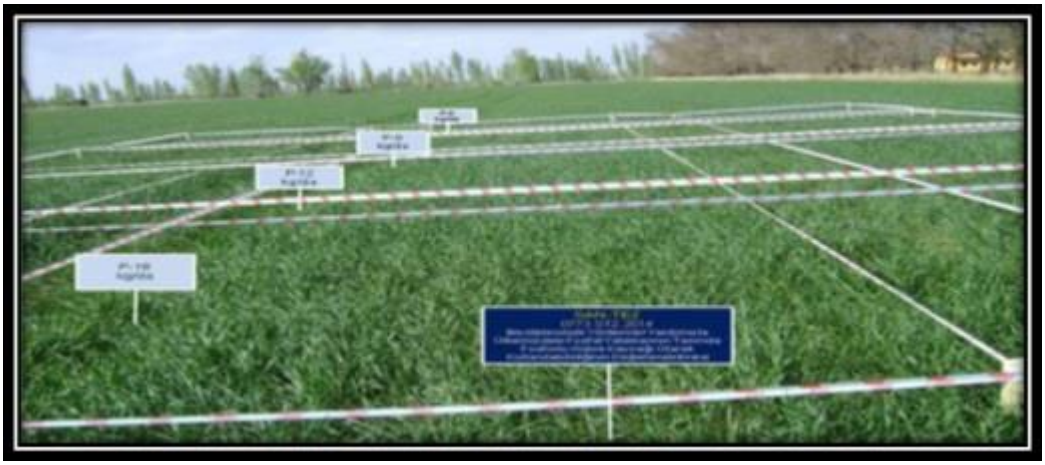


Figure 3.20. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in wheat plant

3.9.2. CORN EXPERIMENT

3.9.2.1. Some Properties of Corn-Grown Soils

In order to determine the amount of plant nutrients as well as the change that takes place in the soil in both years during the development period of the corn plant, chemical analyzes were performed by taking samples from a 0-30 cm depth of the soil of each parcel at harvest time (Tables 3.29, 3.30 and 3.31.)

Table 3.30. Analysis results of the soil of the year 2016 corn planted (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
		(1:2,5)	(%)		kg/da		
Pre-trial		7,48	2,24	1.67	1,88	2,11	65,4
	Control	7,51	2,27	1,57	2,88	3,12	66,45
Bio+FK	6	7,53	2,20	1,66	3,10	3,88	67,13
	12	7,57	2,11	1.79	3,89	4,23	69,12

	18	7,59	2,02	1,81	3,92	5,26	70,21
TSP	6	7,50	2,25	1,58	2,88	3,22	63,44
	12	7,52	2,35	1.55	2,93	3,42	63,66
	18	7,47	2,27	1,52	2,96	3,88	65,46

Table 3.31. Analysis results of the soil of the year 2016 corn planted

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
		mg/100 gr			mg/kg			
Pre-trial		17,55	2,11	0,50	4,23	4,35	4,56	3,11
	Control	18,12	2,18	0,54	4,36	4,15	3,89	2,98
Bio+FK	6	18,76	2,19	0,49	4,53	4,25	4,10	3,11
	12	18,99	2,23	0,44	4,76	4,29	4,44	3,24
	18	19,12	2,27	0,40	4,79	4,26	4,35	3,16
TSP	6	17,68	2,06	0,50	3,39	3,82	3,99	2,94
	12	18,11	2,05	0,52	3,36	3,78	3,96	2,87
	18	18,88	2,11	0,54	3,21	3,71	3,88	2,76

Table 3.32. Analysis results of the soil in which the maize plant was grown (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
		(1:2,5)	(%)		kg/da		
Pre-trial		7,80	3,94	1.27	1,88	2,10	55,40
	Control	7,77	3,92	1,27	1,88	2,40	56,45
Bio+FK	6	7,53	3,20	1,26	1,90	3,90	57,10
	12	7,57	3,31	1.29	2,09	4,50	59,10
	18	7,59	3,12	1,31	2,12	5,90	60,20
TSP	6	7,70	3,89	1,28	2,00	2,32	63,40
	12	7,68	3,90	1.25	1,93	3,00	73,60
	18	7,77	3,92	1,22	1,95	3,10	75,40

3.9.2.2. Yield and Yield Parameters of Corn Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the corn plant. During the harvest period, the plants were harvested and the yields were calculated by separating the granules. (Tables 3.32, 3.33). As for macro and mictro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis . For the physiological analyzes, the plants were placed in the freezer set at -800 ° C and would be kept frozen until the analysis process.

Table 3.33. Yield and yield parameters of 2016 maize (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		5766b	52c	14,35b	0,37b
Bio+FK	6	5987b	57b	16,24a	0,38b
	12	6443a	61a	17,65a	0,41a
	18	6233a	60a	16,24a	0,39a
TSP	6	5877b	54b	14,99b	0,34b
	12	6023a	56b	15,44b	0,36b
	18	6135a	57b	16,23a	0,38b

Table 3.34. Yield and yield parameters of 2017 maize (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		5350c	58d	14,20d	0,37d
Bio+FK	6	6200 b	66c	18,10c	0,42c
	12	6920a	74b	20,05b	0,49b
	18	6687a	80a	24,60a	0,55a

TSP	6	5520c	64c	14,80d	0,38c
	12	6250a	66c	18,14c	0,39c
	18	6300a	67c	18,20c	0,39c

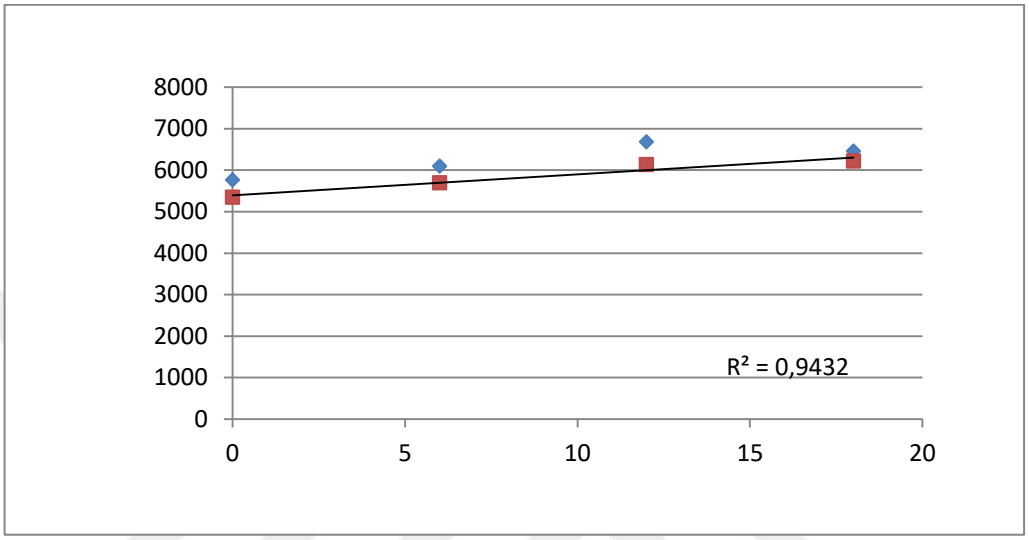


Figure 3.21. 2016-2017 years average

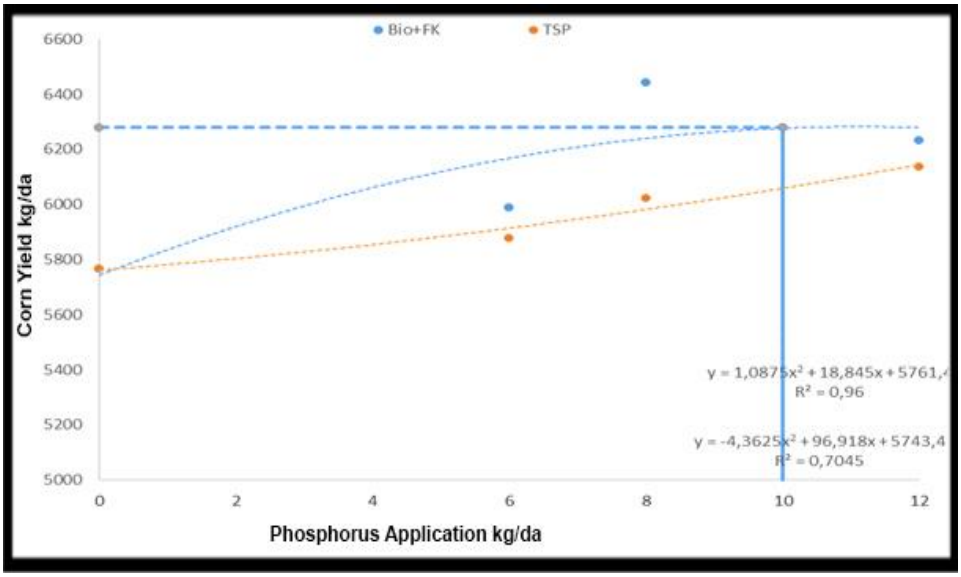


Figure 3.22. Effect of aplications on yield of 2016 wheat plant

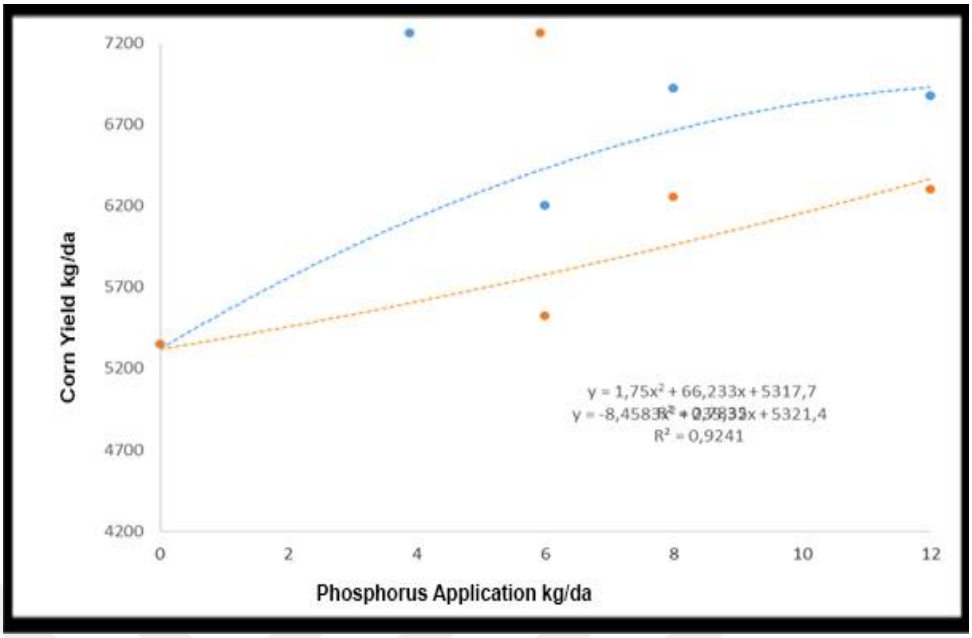


Figure 3.23. Effect of applications on yield of 2017 wheat plant

Figure 3.24. Effect of applications on the yield of corn plant in 2017

Figure 3.25. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in corn plant

3.10. TOMATO EXPERIMENT

3.10.1. Some Properties of Tomato-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the tomato plant, chemical analyzes were performed by taking samples from 0-30 cm depth of the soil of each parcel at harvest time (Tables 3.34, 3.35, 3.36 and 3.37).

Table 3.35. Analysis results of the soil in which the tomato plant was grown in 2016 (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,67	3,22	2,34	2,11	4,23	68,78
	Control	7,61	3,19	2,30	2,78	4,87	67,56

Bio+FK	6	7,58	3,20	2,33	3,12	5,21	68,12
	12	7,55	3,18	2,39	3,45	6,33	67,88
	18	7,54	3,15	2,37	3,58	7,13	68,55
TSP	6	7,69	3,22	2,31	2,89	5,03	66,57
	12	7,76	3,19	2,30	2,99	5,88	67,15
	18	7,71	3,20	2,27	3,08	6,23	68,33

Table 3.36. Analysis results of the soil in which the tomato plant was grown in 2016 (n=3)

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		18,12	2,34	0,67	3,98	5,46	5,44	3,78
	Control	17,68	2,25	0,58	3,20	4,23	4,98	3,65
Bio+FK	6	17,81	2,21	0,55	4,11	5,11	5,11	3,88
	12	17,98	2,11	0,50	4,55	6,11	5,23	4,14
	18	17,45	2,10	0,49	4,43	5,46	5,20	4,01
TSP	6	17,68	2,24	0,60	3,18	4,98	5,16	3,73
	12	18,66	2,28	0,65	3,11	4,78	5,11	3,52
	18	18,92	2,14	0,71	3,01	4,12	4,87	3,25

Table 3.37. Analysis results of the soil in which the year 2017 tomato plant was grown (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,85	4,88	1,34	2,10	4,23	58,70
	Control	7,81	4,69	1,30	2,70	4,87	57,56
Bio+FK	6	7,65	3,80	1,38	3,10	6,20	58,10

	12	7,50	3,60	1,89	3,40	6,80	57,88
	18	7,50	3,45	1,97	3,50	7,80	58,50
TSP	6	7,78	4,20	1,31	2,80	5,03	56,57
	12	7,76	4,40	1,30	2,90	5,88	57,15
	18	7,76	4,48	1,27	2,88	5,90	58,30

Table 3.38. Analysis results of the soil in which the year 2017 tomato plant was grown (n=3)

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		18,12	2,14	0,60	1,98	5,46	5,44	1,78
	Control	17,60	2,20	0,58	1,20	4,23	4,98	1,65
Bio+FK	6	15,80	2,30	0,56	2,01	5,50	6,10	2,88
	12	15,90	2,10	0,52	2,35	6,18	6,28	2,84
	18	14,40	2,30	0,55	2,13	6,96	6,20	2,81
TSP	6	17,60	2,24	0,60	1,18	4,98	5,16	1,73
	12	18,60	2,20	0,66	1,11	4,78	5,10	1,52
	18	18,90	2,10	0,68	1,01	4,12	4,80	1,25

3.10.2. Yield and Yield Parameters of Tomato Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the tomato plant. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calcualted (Tables 3.38 and 3.39 Figures 3.26, 3.27, 3.28 and 3.29) As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis. For the physiological analyzes, the plants were placed in the freezer set at -80C and would be kept frozen until the analysis process.

Table 3.39. Yield and yield parameters of tomato plant n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		7988d	58b	16,54b	0,38b
Bio+FK	6	8412c	61a	17,45b	0,42a
	12	9853a	63a	18,79a	0,45a
	18	9233b	62a	18,11a	0,42b
TSP	6	8233c	53b	17,11b	0,40b
	12	8855c	55b	17,56b	0,38b
	18	8997c	58b	16,44b	0,38b

Table 3.40. Yield and yield parameters of the 2017 tomato plant (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		8250c	55c	15,20c	0,38c
Bio+FK	6	8840c	58c	18,45b	0,44b
	12	10250 a	83b	20,90a	0,50a
	18	9680b	88a	22,10a	0,52a
TSP	6	8320c	58c	17,10c	0,42b
	12	9075b	60c	17,50c	0,44b
	18	9220b	68c	17,20c	0,45b

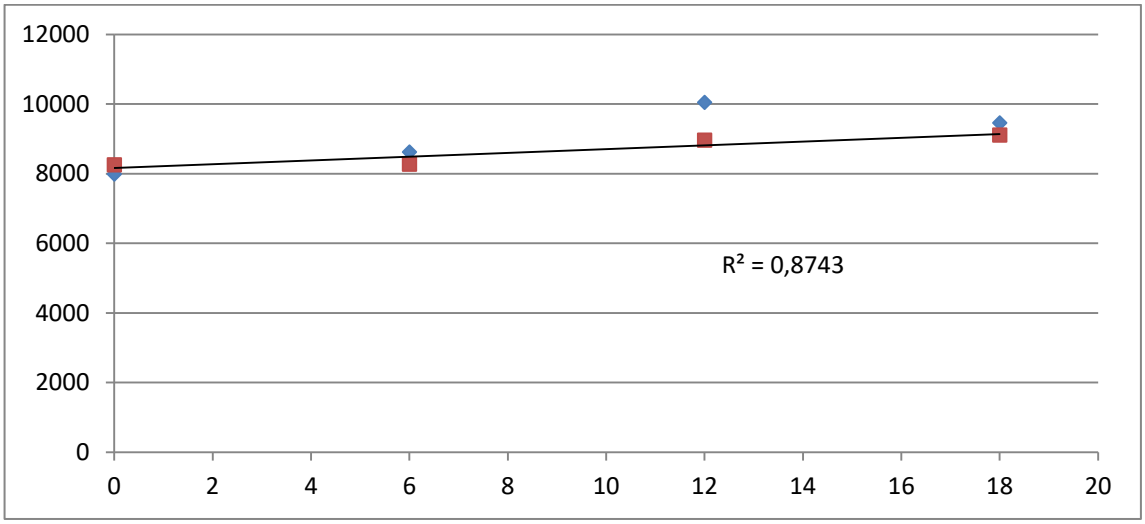


Figure 3.26. 2016-2017 years average

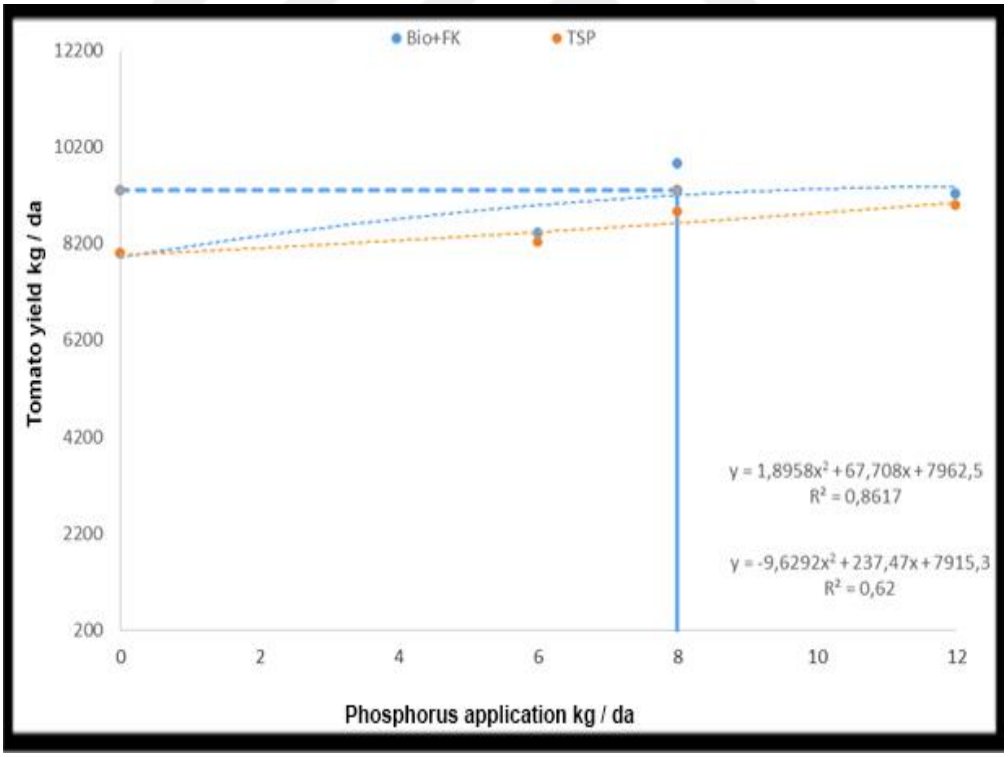


Figure 3.27. Effect of applications on yield of 2016 tomto plant

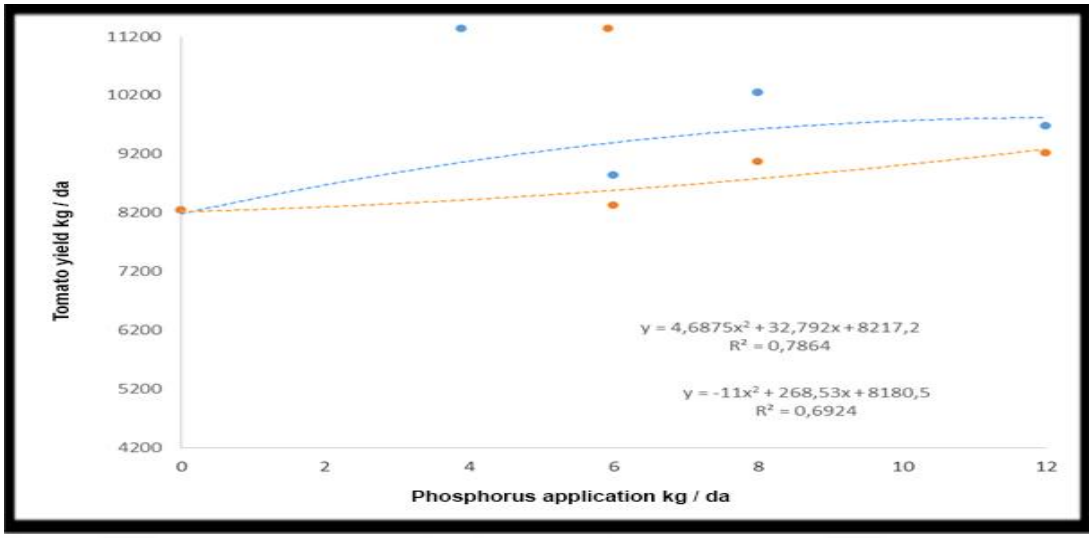


Figure 3.28. Effect of applications on yield of tomato plant of 2017

Figure 3.29. Field experiment where the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in tomato plant

3.11. POTATO EXPERIMENT

3.11.1. Some Properties of Patato-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the potato plant, chemical analyzes were performed by taking samples at 0-30 cm depth from the soil of each parcel at harvest time (Tables 3.40, 3.41 and 3.42).

Table 3.41. Results of the analysis of the soil where the potato plant was grown in 2016 (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,33	0,76	1,98	1,88	3,45	55,46
	Control	7,36	0,73	2,01	1,96	3,87	55,46
Bio+FK	6	7,33	0,72	2,12	2,03	4,65	56,12
	12	7,30	0,70	2,21	2,11	5,25	56,44
	18	7,29	0,67	2,22	2,13	6,11	57,43
TSP	6	7,37	0,75	2,01	1,98	4,01	55,55
	12	7,42	0,79	2,03	2,01	4,52	55,31
	18	7,45	0,78	2,05	2,04	5,11	54,32

Table 3.42. Results of the analysis of the soil where the potato plant was grown in 2016 (n=3)

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		15,44	2,11	0,42	4,35	6,11	4,88	3,12
	Control	16,11	2,03	0,40	4,44	6,23	4,76	3,24
Bio+FK	6	16,78	2,13	0,38	4,65	6,35	4,87	3,38
	12	16,23	2,18	0,35	4,98	6,45	4,93	3,66

	18	16,55	2,15	0,34	4,90	6,40	4,88	3,45
TSP	6	15,44	2,07	0,39	4,51	5,99	4,67	2,98
	12	15,78	2,10	0,41	4,43	5,89	4,34	2,45
	18	15,12	2,11	0,44	4,11	5,77	4,21	2,14

Table 3.43. Analysis results of the soil in which the potato plnat was grown in 2017 (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,50	0,96	1,98	1,80	3,45	58,46
	Control	7,50	0,93	2,01	1,84	3,87	58,46
Bio+FK	6	7,30	0,72	2,12	2,23	5,60	60,12
	12	7,30	0,70	2,21	2,21	5,90	62,44
	18	7,25	0,60	2,22	2,33	6,10	67,40
TSP	6	7,40	0,87	2,01	1,98	4,30	59,55
	12	7,42	0,89	2,03	2,21	4,80	58,30
	18	7,44	0,88	2,05	2,24	5,50	54,32

3.11.2. Yield and Yield Parameters of Potato Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the potato plant. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calculated (Tables 3.43 and 3.44 Figures 3.30, 3.31, 3.32 and 3.33). As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis. For the physiological analyzes, the plants were placed in the freezer set at -80 ° C and would be kept frozen until the analysis process.

Table 3.44. Yield and yield parameters of potato plant 2016 (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		2876d	48b	14,33c	0,26b
Bio+FK	6	3343c	51a	15,46b	0,28b
	12	3766a	53a	17,56a	0,33a
	18	3654b	54a	16,57b	0,32a
TSP	6	3056c	46b	14,88c	0,27b
	12	3231c	45b	15,43b	0,28b
	18	3431c	47b	15,94b	0,30a

Table 3.45. Yield and yield parameters of potato plant 2017 (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		3120c	40c	14,20c	0,22c
Bio+FK	6	3980c	48b	15,80b	0,26b
	12	4896a	56a	17,75a	0,34a
	18	4250b	58a	17,90a	0,36a
TSP	6	3470c	42c	14,80c	0,23b
	12	4120b	44b	15,10b	0,24b
	18	4070b	45b	15,20b	0,26b

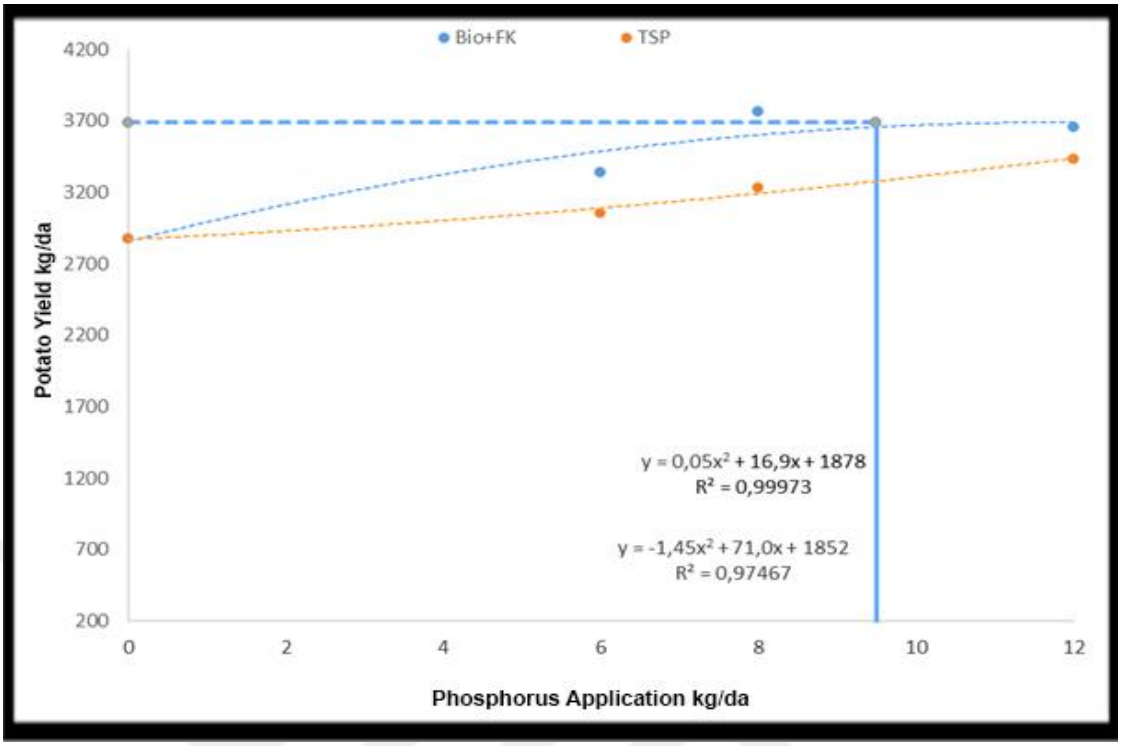


Figure 3.30. Effect of application of potato plant of 2016 on yield

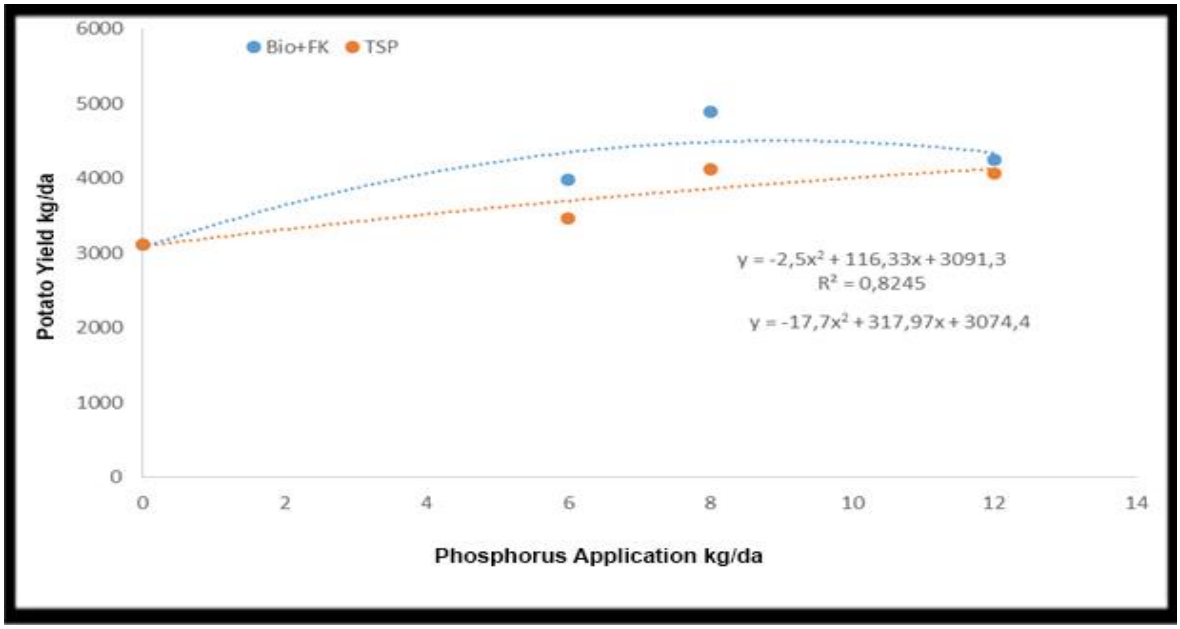


Figure 3.31. Effect of application of potato plant of 2017 on yield

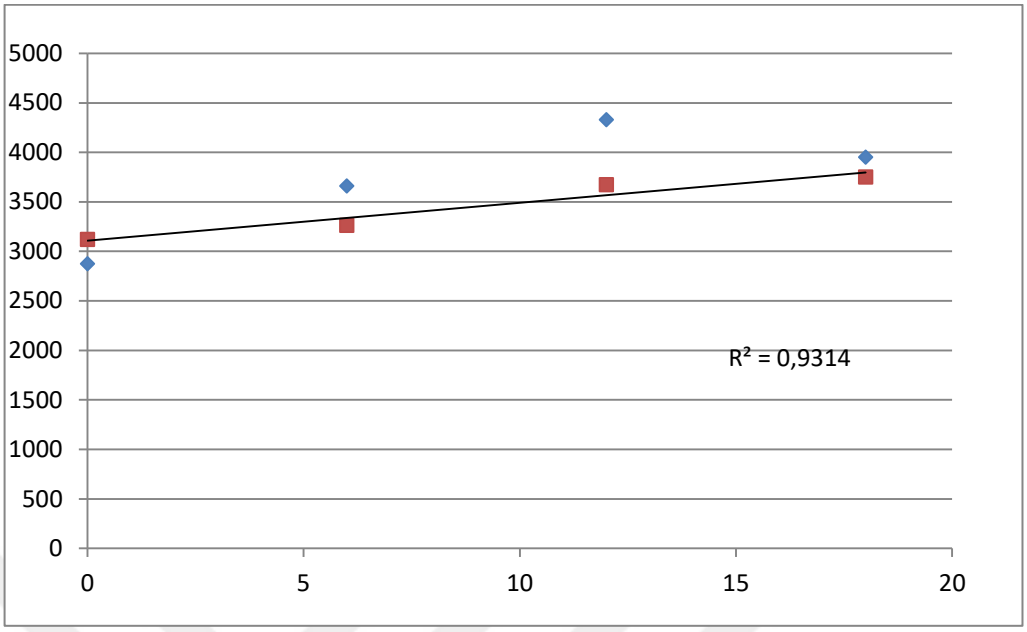


Figure 3.32. 2016-2017 years average

Figure 3.33. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in potato

3.12. GRAPE EXPERIMENT

3.12.1. Some Properties of Grape-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the potato plant, chemical analyzes were performed by taking samples at 0-30 cm depth from the soil of each parcel at harvest time (Tables 3.45, 3.46 and 3.47).

Table 3.46. Analysis results of the soil in which the grape plant of 2016 was grown (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,75	1,22	1,56	1,54	5,44	67,88
	Control	7,72	1,19	1,62	1,88	5,78	68,66
Bio+FK	6	7,70	1,1	1,65	2,11	6,23	68,14
	12	7,68	1,17	1,72	2,44	6,98	68,79
	18	7,69	1,16	1,70	2,38	7,86	68,77
TSP	6	7,74	1,22	1,63	1,96	5,98	69,76
	12	7,79	1,23	1,62	1,98	6,23	70,12
	18	7,81	1,20	1,64	2,04	6,98	68,77

Table 3.47. Table 3.48. Analysis results of the soil in which the grape plant of 2016 was grown (n=3)

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		16,57	2,34	0,66	3,88	5,75	4,24	3,88
	Control	16,88	2,18	0,62	3,44	5,34	3,88	3,42
Bio+FK	6	17,10	2,21	0,58	3,98	5,98	3,96	3,65

	12	17,13	2,27	0,55	4,43	6,11	4,13	3,75
	18	17,34	2,29	0,49	4,12	6,02	4,20	3,77
TSP	6	16,93	2,25	0,63	3,76	5,31	3,76	3,32
	12	17,01	2,28	0,67	3,50	5,33	3,72	3,10
	18	16,57	2,23	0,72	3,34	5,24	3,45	3,05

Table 3.49. Analysis results of the soil where the grape plant of 2017 was grown (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,80	1,20	1,36	1,04	5,10	60,50
	Control	7,76	1,18	1,32	1,88	5,20	62,60
Bio+FK	6	7,74	1,14	1,30	2,10	6,20	65,10
	12	7,65	1,17	1,64	2,64	7,90	68,70
	18	7,55	1,16	1,40	2,38	8,80	68,50
TSP	6	7,74	1,20	1,30	1,90	5,20	69,76
	12	7,74	1,20	1,24	1,70	6,23	70,30
	18	7,80	1,20	1,25	1.30	6,50	68,40

3.12.2. Yield and Yield Parameters of Grape Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the grape fern. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calculated. As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis (Tables 3.48 and 3.49, Figures 3.34, 3.35, 3.36 and 3.37). For the physiological analyzes, the plants were placed in the freezer set at -80C and would be kept frozen until the analysis process.

Table 3.50. Yield and yield parameters of the 2016 grape plant (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		6454c	44c	17,68c	0,31c
Bio+FK	6	6980b	46b	19,67b	0,36b
	12	7453a	47a	21,30a	0,39a
	18	7231a	48a	20,33a	0,37b
TSP	6	6675b	45c	18,11b	0,32c
	12	6877b	47a	18,77b	0,33c
	18	6934b	46b	19,23b	0,35b

Table 3.51. Yield and yield parameters of grape plant (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		6980c	40d	17,10c	0,30d
Bio+FK	6	7200b	44c	19,20b	0,38c
	12	7900a	52b	21,50a	0,42b
	18	7670b	56a	22,75a	0,48a
TSP	6	7100b	42c	18,40b	0,33d
	12	7750a	42c	18,88b	0,35c
	18	7380b	44c	19,50b	0,38c

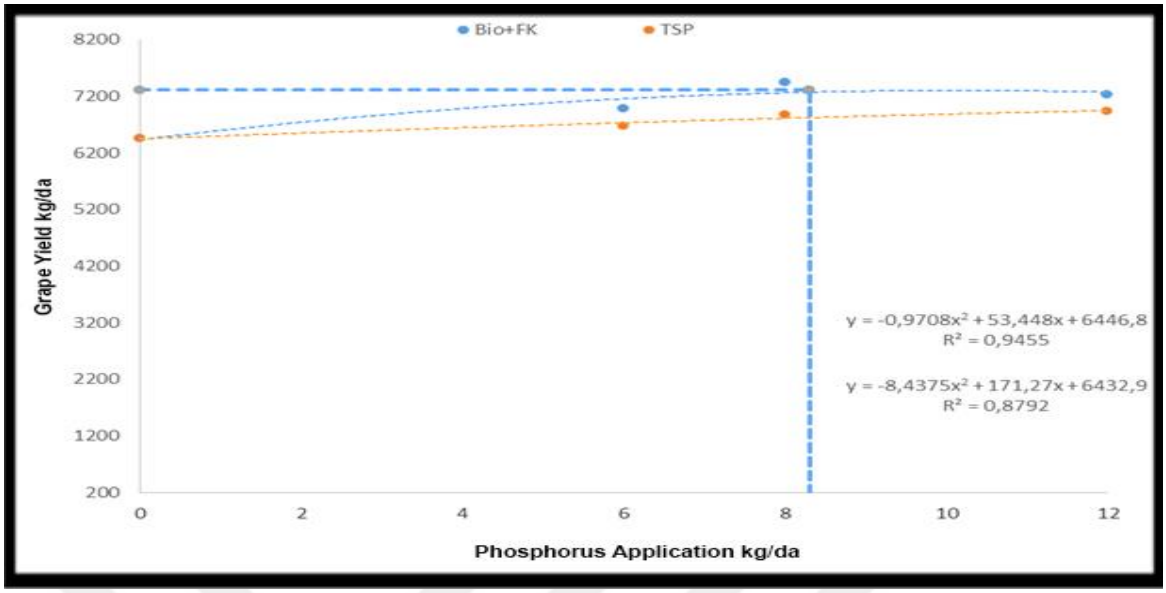


Figure 3.34. Effect of applications on yield of 2016 grape plant

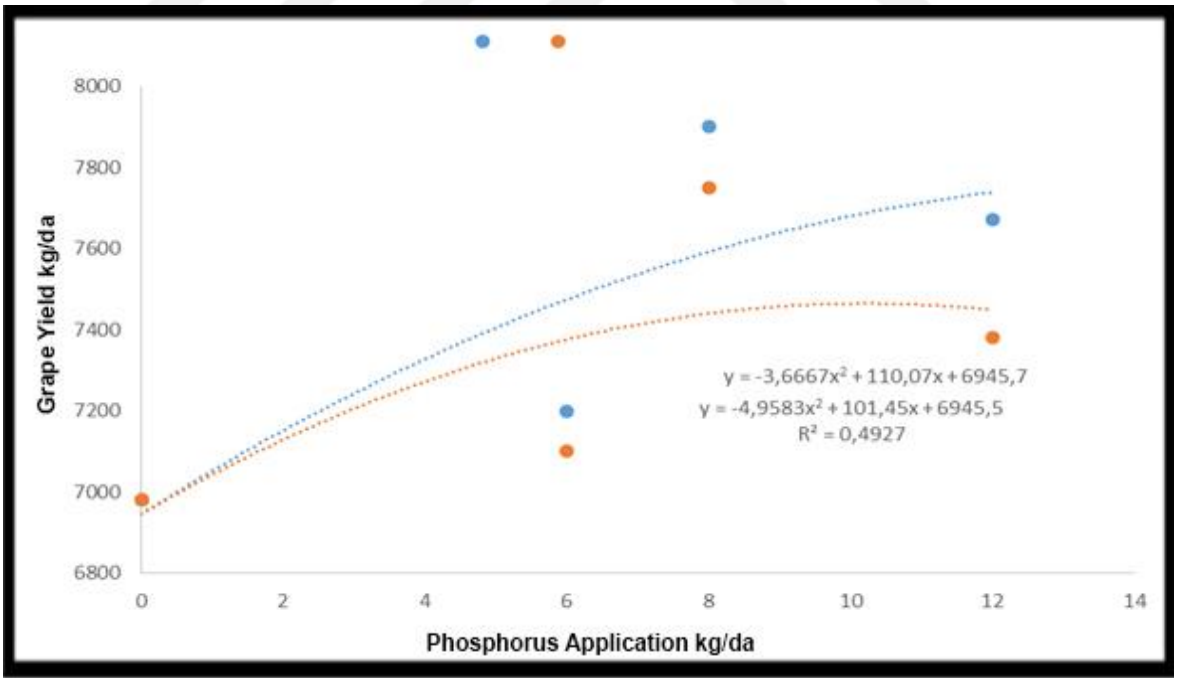


Figure 3.35. Effect of applications on yield of 2017 grape plant

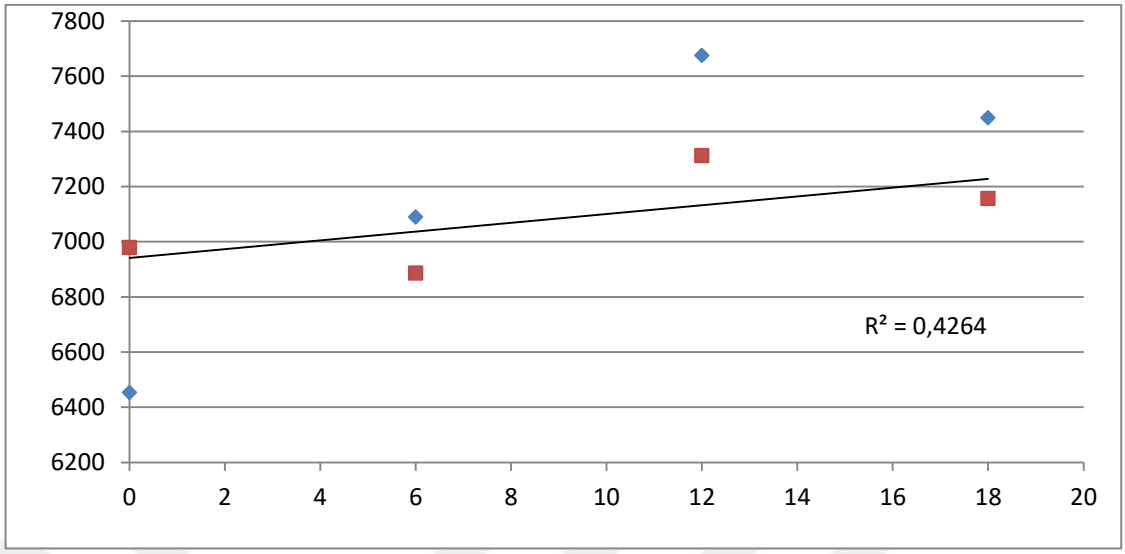


Figure 3.36. 2016-2017 years average

Figure 3.37. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in grape fern

3.13. QUINCE EXPERIMENT

3.13.1. Some Properties of Quince-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the quince plant, chemical analyzes

were performed by taking samples at 0-30 cm depth from the soil of each parcel at harvest time (Tables 3.51, 3.52, 3.53 and 3.54).

Table 3.52. Analysis results of the soil in which the year 2016 quince plant was grown (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,45	1,75	1,92	1,83	4,33	61,12
	Control	7,44	1,73	1,95	2,11	5,46	62,11
Bio+FK	6	7,43	1,7	1,98	2,35	6,45	63,24
	12	7,38	1,71	2,01	2,88	7,66	62,77
	18	7,37	1,74	2,02	3,11	8,97	62,33
TSP	6	7,47	1,76	1,90	2,23	4,89	61,23
	12	7,53	1,78	1,88	2,33	6,23	61,15
	18	7,55	1,76	1,85	2,16	6,98	62,34

Table 3.53. Analysis results of the soil in which the year 2016 quince plant was grown (n=3)

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	mg/100 gr			mg/kg			
Pre- Trial		17,24	2,54	0,51	4,13	4,98	3,96	3,14
	Control	17,45	2,44	0,55	3,78	4,56	3,76	3,01
Bio+FK	6	17,86	2,49	0,47	4,23	4,67	3,88	3,15
	12	18,11	2,55	0,45	4,34	4,78	3,92	3,24
	18	18,45	2,51	0,46	4,21	4,55	3,94	3,17
TSP	6	17,33	2,41	0,58	3,66	4,34	3,54	2,89
	12	17,43	2,37	0,61	3,59	4,23	3,44	2,83
	18	17,56	2,29	0,62	3,57	4,20	3,51	2,82

Table 3.54. Analysis results of the soil where the year 2017 quince plant was grown (n=3)

Application	Dose	pH	$CaCO_3$	Organic Matter	N	P	K
	kg/da	(1:2,5)	(%)		kg/da		
Pre- Trial		7,80	4,25	1,72	1,70	4,30	61,12
	Control	7,79	4,23	1,65	2,15	5,42	62,11
Bio+FK	6	7,40	4,62	1,68	2,30	6,40	65,24
	12	7,30	4,71	2,00	2,88	8,60	68,70
	18	7,30	4,750	2,00	3,10	9,90	68,83
TSP	6	7,76	4,36	1,70	2,20	4,89	61,53
	12	7,73	4,78	1,78	2,30	6,23	61,25
	18	7,69	4,70	1,80	2,10	6,90	62,30

Table 3.55. Analysis results of the soil where the year 2017 quince plant was grown (n=3)

Application	Dose	Ca	Mg	Na	Fe	Cu	Mn	Zn
	kg/da	me/100 gr			mg/kg			
Pre-Trial		17,00	2,54	0,50	1,20	4,10	3,16	1,14
	Control	17,10	2,44	0,55	1,70	4,26	3,16	1,10
Bio+FK	6	17,86	3,40	0,40	1,20	4,37	3,18	2,45
	12	19,11	3,82	0,45	1,30	4,55	3,12	2,54
	18	19,45	3,60	0,49	1,20	4,50	3,14	2,30
TSP	6	17,30	2,40	0,65	1,60	4,30	3,14	1,75
	12	17,40	2,30	0,68	1,50	4,42	3,14	1,65
	18	17,50	2,40	0,70	1,50	4,33	3,11	1,60

3.13.2. Yield and Yield Parameters of Quince Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the quince plant. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calculated. As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis (Tables 3.55 and 3.56, Figures 3.38, 3.39, 3.40 and 3.41.). For the physiological analyzes, the plants were placed in the freezer set at -80 ° C and would be kept frozen until the analysis process.

Table 3.56. Yied and yield parameters of the year 2016 quince plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		1132c	47ab	15,44c	0,38c
Bio+FK	6	1244b	45b	16,34b	0,39b
	12	1305a	49a	17,24a	0,42a
	18	1278b	48a	16,57b	0,43a
TSP	6	1178b	45b	16,11b	0,38b
	12	1211b	45b	16,45b	0,40ab
	18	1272b	46b	16,52b	0,41a

Table 3.57. Yield and yield parameters of the year 2017 quince plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		1250d	52c	15,20b	0,38c
Bio+FK	6	1370c	55c	16,60a	0,44b

	12	1590a	60b	17,80a	0,48a
	18	1510a	78a	16,90b	0,50a
TSP	6	1208d	52c	15,10b	0,39b
	12	1410b	56c	15,75b	0,40b
	18	1400b	55c	15,82b	0,41b

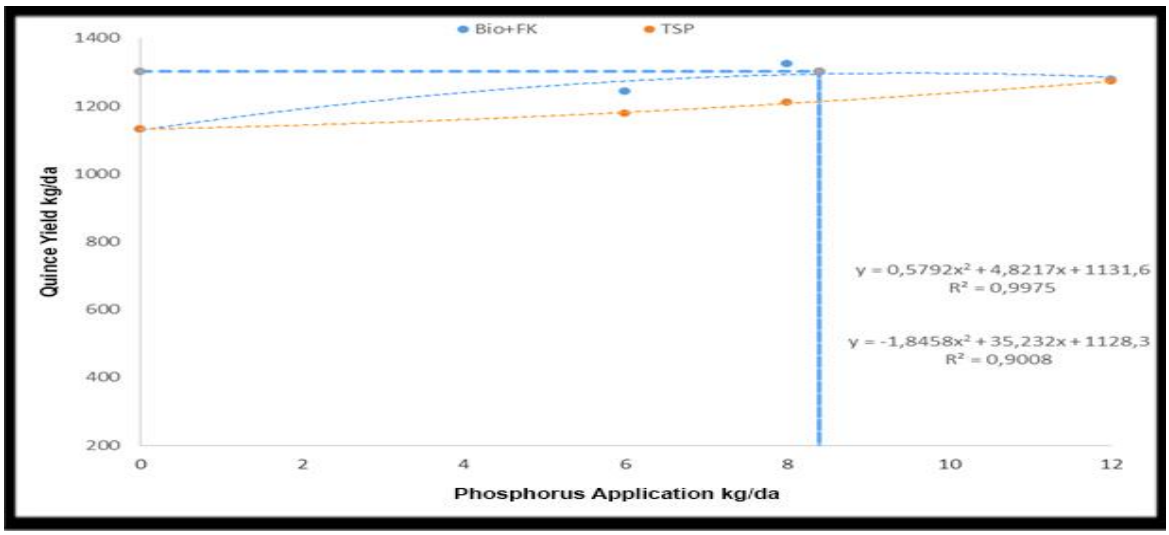


Figure 3.38. The effect of applications on yield of 2016 quince plant

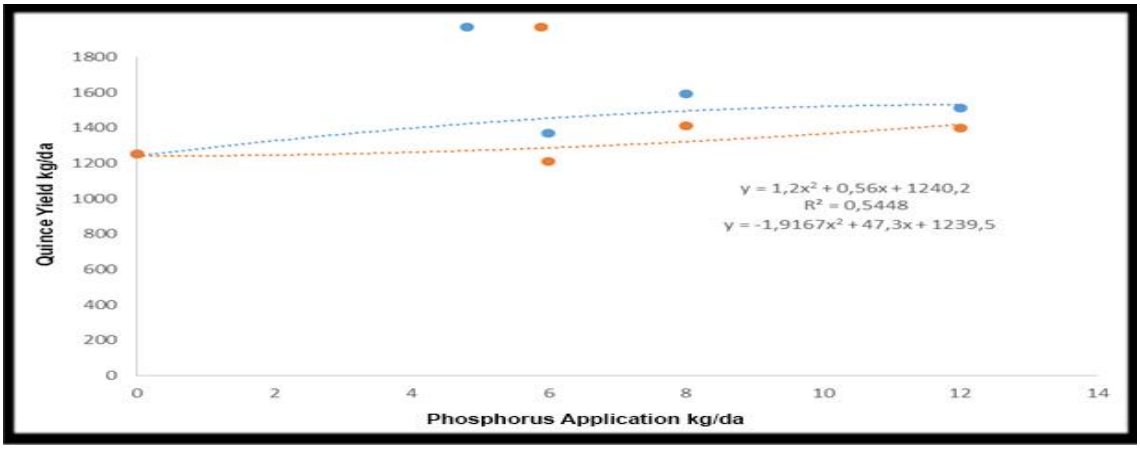


Figure 3.39. The effect of applications on yield of 2017 quince plant

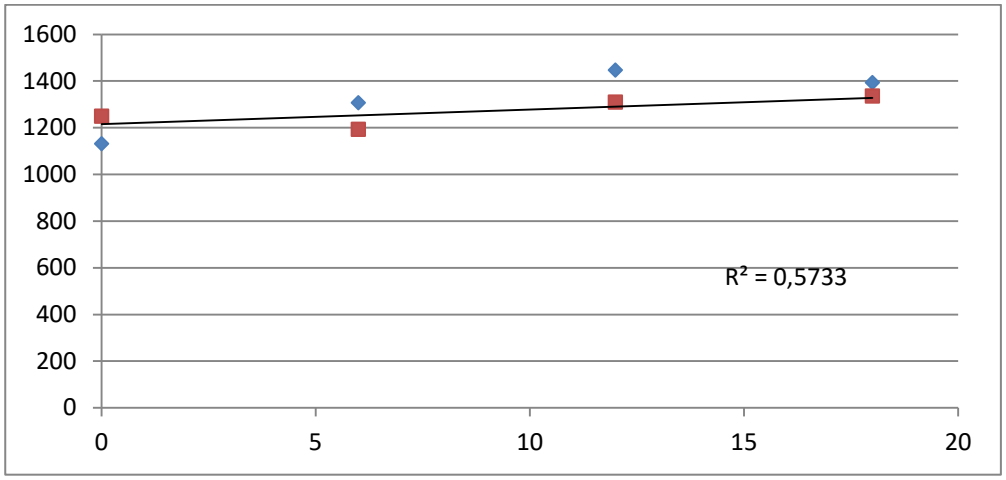


Figure 3.40. 2016- 2017 years average

Figure 3.41. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in quince plant

3.14. ORANGE EXPERIMENT

3.14.1. Some Properties of Orange-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the quince plant, chemical analyzes were performed by taking samples at 0-30 cm depth from the soil of each parcel at harvest time (Tables 3.57, 3.58, 3.59 and 3.60.).

Table 3.58. Results of the analysis of the soil in which the orange vegetable was grown in 2016 (n=3)

Application	Dose	pH	$CaCO_3$	Organic Matter	N	P	K
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		6,45	0,34	2,36	2,13	4,11	78,45
	Control	6,54	0,32	2,28	2,56	4,45	79 1 2
Bio+FK	6	6,52	0,31	2,39	2,59	4,67	78,77
	12	6,49	0,33	2,43	2,63	5,38	78,98
	18	6,52	0,32	2,44	2,54	6,24	79,14
TSP	6	6,61	0,34	2,29	2,22	4,56	78,56
	12	6,63	0,35	2,33	2,30	5,02	79,88
	18	6,64	0,35	2,31	2,19	5,48	79,23

Table 3.59. Results of the analysis of the soil in which the orange vegetable was grown in 2016 (n=3)

Application	Dose	Ca	Mg	Na	Fe	Cu	Mn	Zn
	kg/da	mg/100 gr			mg/kg			
Pre-Trial		15,22	1,45	0,34	5,66	6,23	4,90	4,14
	Control	15,65	1,35	0,38	5,78	5,88	4,77	4,01
Bio+FK	6	15,46	1,49	0,32	5,83	6,10	4,88	4,13
	12	15,23	1,52	0,30	5,94	6,14	5,10	4,24
	18	16,34	1,46	0,28	5,90	6,11	5,01	4,18
TSP	6	14,56	1,38	0,43	5,45	5,65	4,65	3,96
	12	14,77	1,33	0,45	5,12	5,58	4,58	3,91
	18	15,11	1,31	0,51	5,04	5,55	4,50	3,85

Table 3.60. Analysis results of the soil in which the orange plant of the year 2017 is grown (n=3)

Application	Dose	pH	$CaCO_3$	Organic Matter	N	P	K
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		6,40	0,24	2,10	2,13	3,90	72,15
	Control	6,30	0,22	2,20	2,10	4,20	74,10
Bio+FK	6	5,40	0,21	2,60	2,80	5,67	79,70
	12	5,30	0,23	2,65	2,90	7,38	78,98
	18	5,50	0,22	2,80	2,98	9,20	82,14
TSP	6	6,10	0,24	2,20	2,20	4,56	77,6
	12	6,20	0,25	2,30	2,28	5,40	76,28
	18	6,30	0,25	2,25	2,26	5,60	76,20

Table 3.61. Analysis results of the soil in which the orange plant of the year 2017 is grown (n=3)

Application	Dose	Ca	Mg	Na	Fe	Cu	Mn	Zn
	kg/da	me/100 gr			mg/kg			
Pre-Trial		14,20	1,40	0,37	5,60	6,23	4,50	1,14
	Control	14,45	1,60	0,38	5,50	5,48	4,47	1,01
Bio+FK	6	13,40	1,88	0,37	5,0	6,15	4,80	2,18
	12	13,20	1,95	0,35	5,44	6,14	5,10	2,28
	18	13,30	1,98	0,48	5,60	6,11	5,01	2,46
TSP	6	14,20	1,48	0,43	5,43	5,70	4,69	1,90
	12	14,40	1,58	0,48	5,12	5,60	4,67	1,90
	18	15,10	1,61	0,51	5,20	5,58	4,58	1,80

3.14.2. Yield and Yield Parameters of Orange Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the orange plant. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calculated (Tables 3.61 and 3.62, Figures 3.42, 3.42, 3.44 and 3.45). As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis.For the physiological analyzes, the plants were placed in the freezer set at -80 ° C and would be kept frozen until the analysis process.

Table 3.62. Yield and yield parameters of the 2016 orange plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		544b	42b	17,56b	0,45b
Bio+FK	6	576b	44b	18,15a	0,47b
	12	655a	45a	18,97a	0,49a
	18	634a	46a	19,21a	0,48a
TSP	6	564b	43b	17,98b	0,46b
	12	598b	42b	18,34b	0,46b
	18	603a	44b	18,11b	0,48a

Table 3.63. Yield and yield parameteres of 2017 orange plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SP D	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		600c	46b	17,20c	0,44c
Bio+FK	6	690c	48b	18,90b	0,49c
	12	850a	56b	19,25a	0,59b

	18	800a	60a	19,96a	0,62a
TSP	6	630b	48b	17,10c	0,46c
	12	790a	52b	17,44c	0,48c
	18	800a	55b	17,68c	0,50b

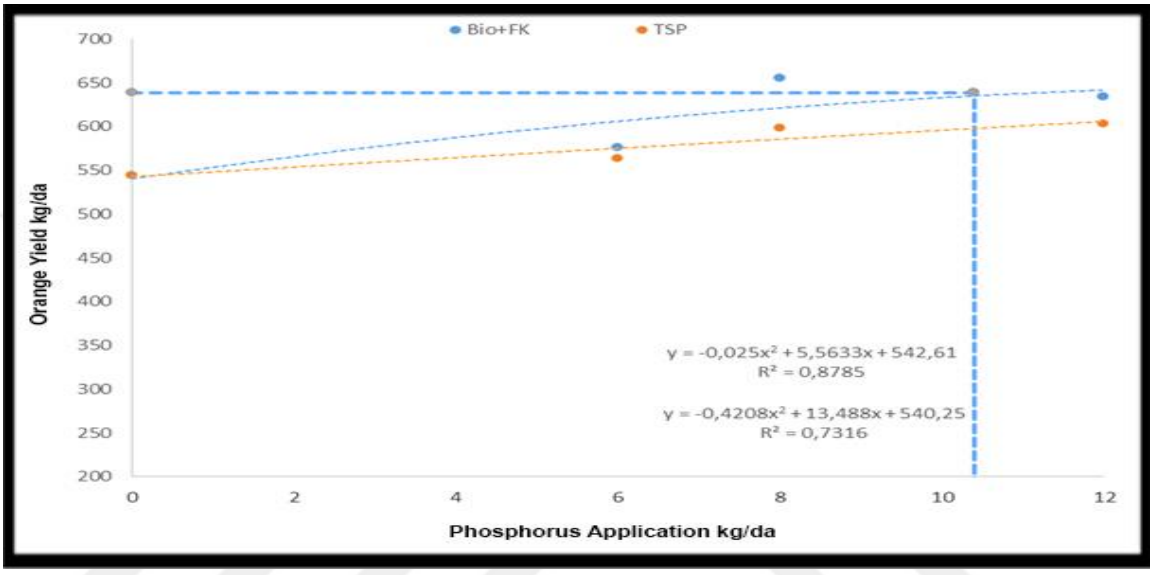


Figure 3.42. Effect of applications on yield of orange fruit of 2016

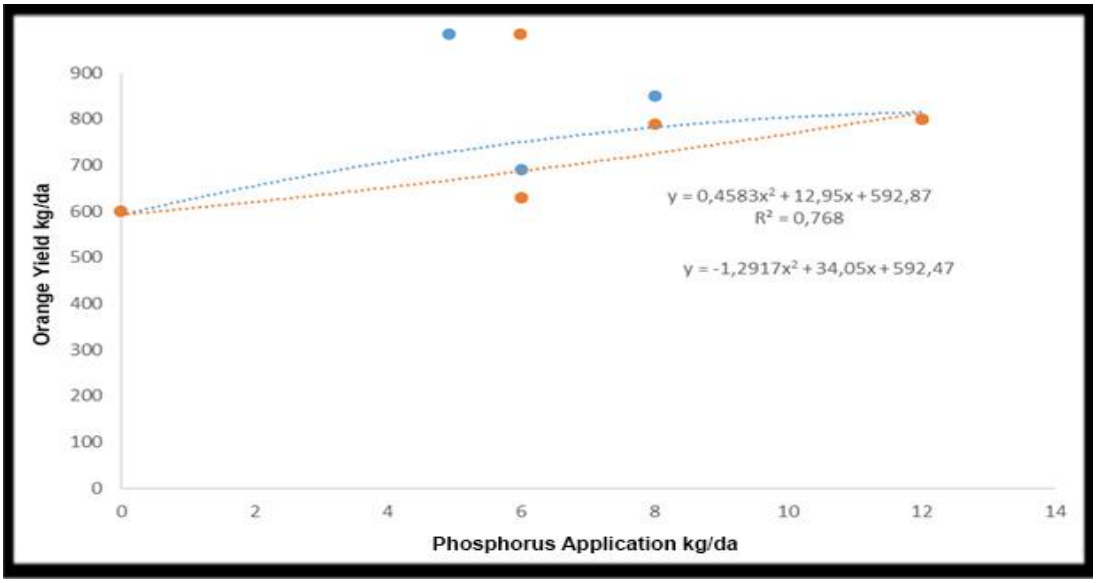


Figure 3.43. Effect of applications on yield of 2017 orange plant

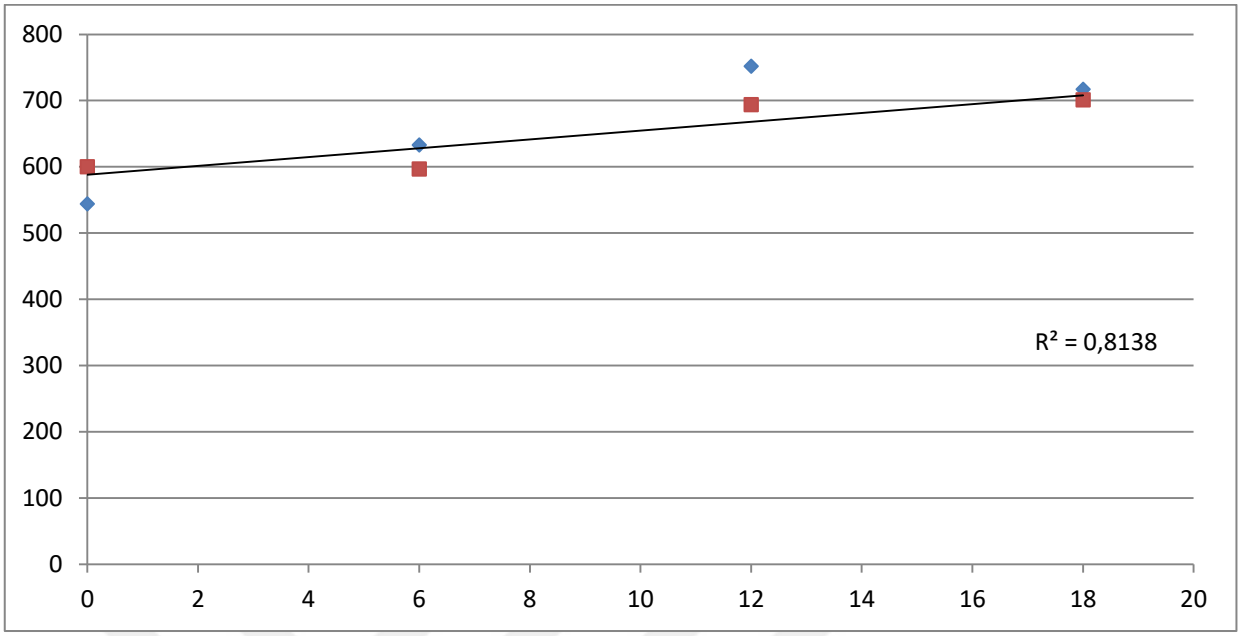


Figure 3.44. 2016-2017 years average

Figure 3.45. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in orange plant

3.15. CABBAGE EXPERIMENT

3.15.1. Some Properties of Cabbage-Grown Soils

In order to determine the amount of plant nutrients and the change that takes place in the soil in both years during the development period of the cabbage plant, chemical analyzes were performed by taking samples at 0-30 cm depth from the soil of each parcel at harvest time (Tables 3.63, 3.64, 3.65 and 3.66.).

Table 3.64. Analysis results of the soil in which the cabbage plant was grown in 2016 (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,63	0,67	1,35	1,11	2,75	54,32
	Control	7,67	0,66	1,30	1,45	3,11	55,45
Bio+FK	6	7,58	0,72	1,33	1,54	3,45	55,65
	12	7,59	0,70	1,39	1,63	4,13	56,12
	18	7,55	0,65	1,35	1,72	5,33	56,46
TSP	6	7,68	0,68	1,30	1,47	3,23	55,34
	12	7,73	0,67	1,27	1,51	3,41	55,12
	18	7,70	0,65	1,28	1,56	4,10	56,45

Table 3.65. Analysis results of the soil of the year 2016 cabbage growing

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		15,67	1,96	0,72	4,11	5,11	4,23	3,54
	Control	15,44	1,87	0,77	3,99	5,34	4,12	3,44

Bio+FK	6	15,87	1,99	0,60	4,14	5,43	4,34	3,76
	12	15,98	2,03	0,55	4,21	5,51	4,25	3,81
	18	15,77	2,01	0,57	4,15	5,58	4,41	3,84
TSP	6	15,23	1,88	0,78	4,03	5,30	4,12	3,56
	12	15,78	1,90	0,82	3,88	5,22	3,90	3,41
	18	15,92	1,86	0,86	3,77	5,11	3,98	3,33

Table 3.66. Analysis results of the soil of the year 2017 cabbage growing (n=3)

Application	**Dose**	**pH**	**$CaCO_3$**	**Organic Matter**	**N**	**P**	**K**
	kg/da	(1:2,5)	(%)		kg/da		
Pre-Trial		7,80	1,60	1,35	1,10	2,70	50,20
	Control	7,74	1,60	1,30	1,12	3,10	52,40
Bio+FK	6	7,50	1,76	1,38	1,86	5,49	56,65
	12	7,40	1,75	1,86	2,10	6,15	58,16
	18	7,35	1,74	1,88	2,22	8,30	60,49
TSP	6	7,69	1,70	1,46	1,40	3,42	55,34
	12	7,74	1,66	1,50	1,34	3,6	55,10
	18	7,76	1,60	1,55	1,38	4,4	56,40

Table 3.67. Analysis result of the soil of the year 2017 cabbage grown

Application	**Dose**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
	kg/da	me/100 gr			mg/kg			
Pre-Trial		15,67b	1,96b	0,70a	4,10a	4,00c	4,23a	1,50b
	Control	15,40b	1,87b	0,77a	3,90c	4,30b	4,12a	1,40b
Bio+FK	6	18,87a	1,99b	0,65b	4,20b	4,30b	4,34a	2,74a
	12	18,98a	2,40a	0,53b	4,30a	4,60a	4,25a	2,79a

	18	18,77a	2,34a	0,57b	4,60a	4,58a	4,41a	2,80a
TSP	6	15,20b	1,80b	0,78a	4,30b	4,45b	4,12a	1,58b
	12	15,60b	1,84b	0,80a	3,80c	4,25b	3,90b	1,40b
	18	15,50b	1,88b	0,82a	3,70c	4,10c	3,98b	1,30b

3.15.2. Yield and Yield Parameters of Cabbage Plant

Measurements of photosynthesis, chlorophyll and stoma permeability were carried out within two years after the ending of development period of the cabbage plant. During the harvest period, the plants were harvested and the yields were weighed in both years and overall yield values were calculated (Tables 3.67 and 3.68, Figures 3.46, 3.47, 3.48 and 3.49). As for macro and micro nutritient elements and other physiological analyzes, samples were taken from the leaves and stems of the plants, dried in drying-oven and were made ready for analysis. For the physiological analyzes, the plants were placed in the freezer set at -80 ° C and would be kept frozen until the analysis process.

Table 3.68. Yield and yield parameters of the 2016 cabbage plant (n=10)

Application	**Dose P_2O_5**	**Yield**	**Chlorophyll**	**Photosynthesis**	**Stoma Permeability**
	kg/da	kg/da	SPAD	μmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		1711c	52b	18,45b	0,48c
Bio+FK	6	1810b	54a	18,98b	0,51b
	12	1945a	55a	19,12a	0,51b
	18	1866b	55a	19,23a	0,53a
TSP	6	1788c	51b	18,57b	0,49b
	12	1835b	52b	18,77b	0,49bc
	18	1889b	52b	18,11b	0,48c

Table 3.69. Yield and yield parameters of the 2017 cabbage plant (n=10)

Application	Dose P_2O_5	Yield	Chlorophyll	Photosynthesis	Stoma Permeability
	kg/da	kg/da	PAD	µmol CO_2 $m^{-2}s^{-1}$	mol H_2O $m^{-2}s^{-1}$
Control		1680d	48c	16,20d	0,40e
Bio+FK	6	1900c	50c	18,40c	0,54c
	12	2425a	59b	22,65b	0,58b
	18	2200b	66a	24,50a	0,64a
TSP	6	1890c	52c	18,30c	0,44d
	12	2200b	55bc	18,40c	0,46d
	18	2120b	54bc	18,30c	0,46d

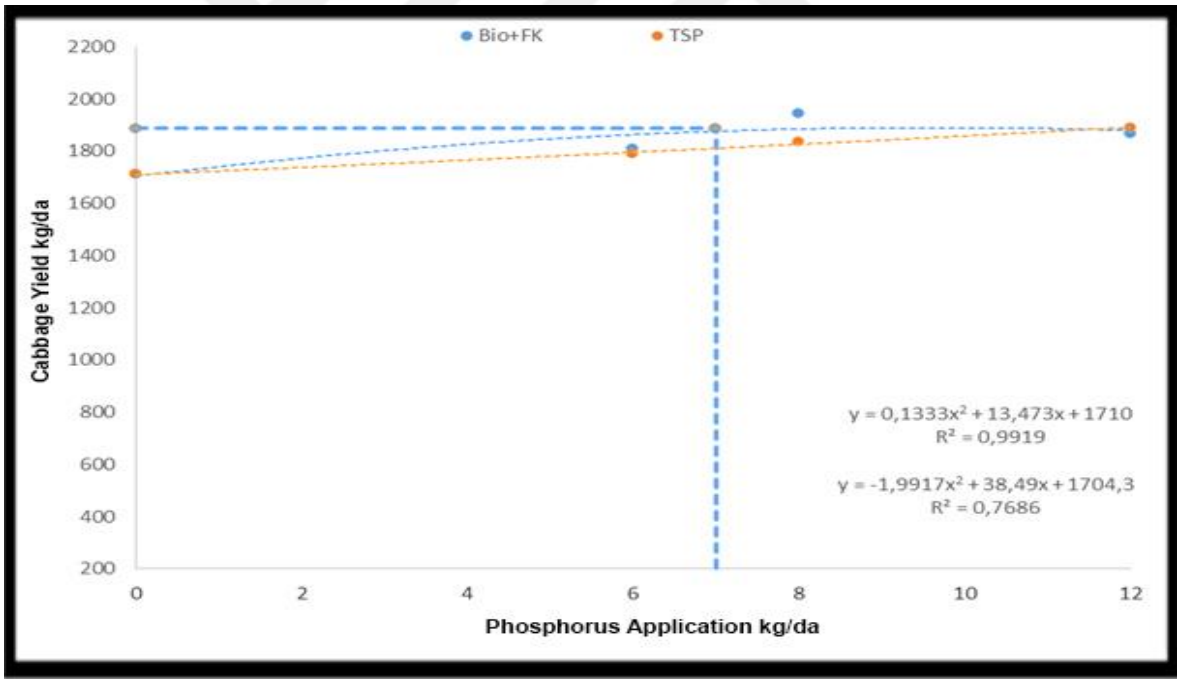


Figure 3.46. Effect of applications on yield of cabbage plant of 2016

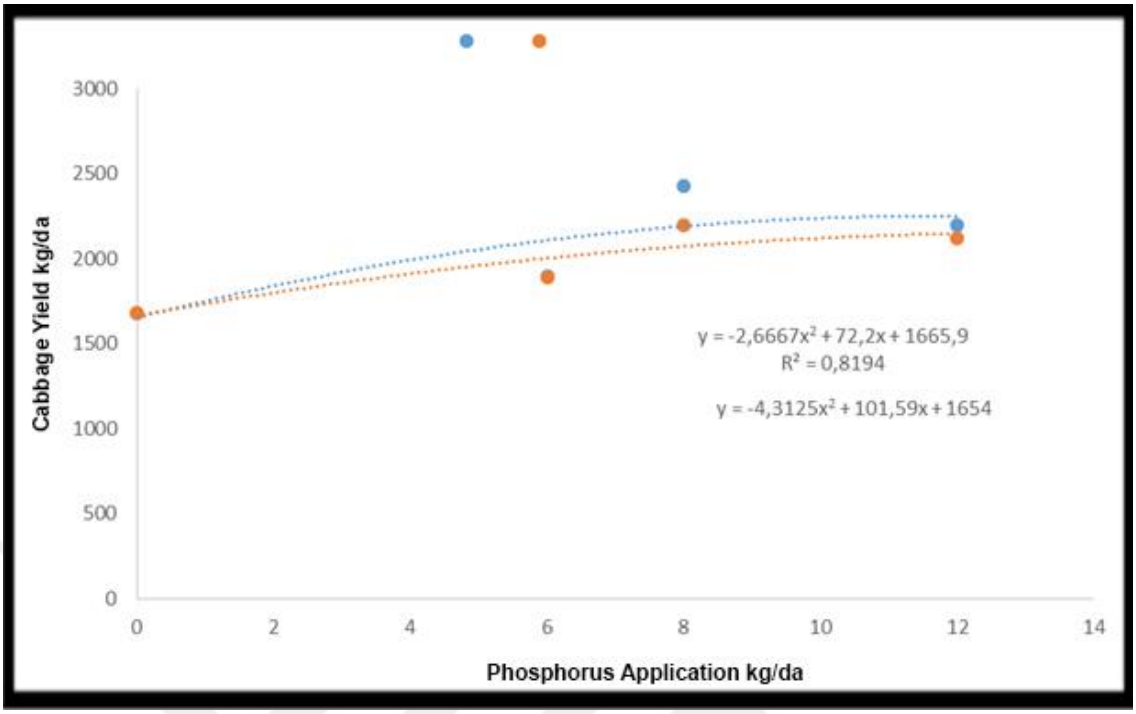


Figure 3.47. Effect of applications on yield of cabbage plant of 2017

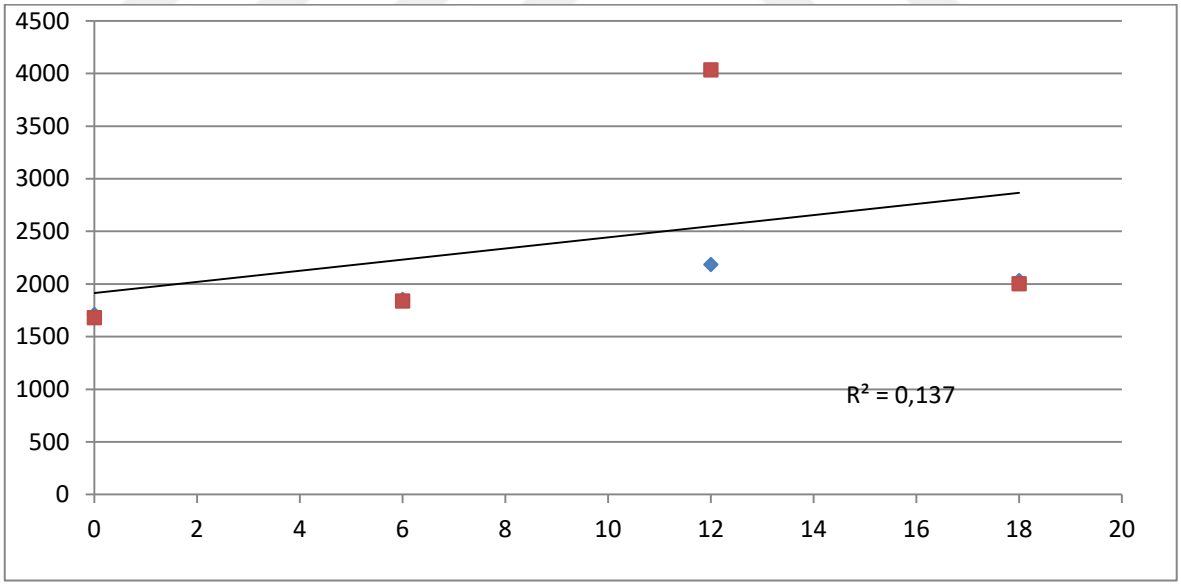


Figure 3.48. 2016- 2017 years average

Figure 3.49. Field experiment in which the effectiveness of phosphorus rock with biological fertilizer is compared to TSP mineral fertilizer in cabbage plant

For example, according to a clinging study in Germany, (organo-)mineral manures at present sold in Germany regularly surpass the point of confinement esteems for Cd set by the German Fertilizer Ordinance. Another vital perspective raised by this examination is the way that numerous P containing mineral manures show generally high boron concentrations and furthermore supply boron burdens to the dirt which are unmistakably exceeding plant take-up. Boron from anthropogenic sources has just been perceived as a potential ecological issue rather as of late (*see Chetelat and Gaillardet, 2005; Liu et al., 2012*). The arrangement of three tests that al-however hypothetical computation did not coordinate with the exploratory discoveries but rather %CCE of RPs in hatching study took after the comparative pat-tern as those qualities got in the hypothetical count (*see B.B. Basak and D.R. Biswas, 2016*). When we look at the literature we should apply the phosphorus fertilizer application in different variations according to soil types. It means that application in calcareous soil can not be the same as application in non-calcareous soil. According to the article, The sorption of P on calcareous paddy soils diminished withincreasing pH. At low pH esteems (2-3), the sorption of Pincreased, while it was diminished with expanding pH. The Psorption was likewise impacted by soil/arrangement proportion and more Pwas sorbed as more water was accessible. Discharge andretention of P in paddy soil may influence the water quality andnutrient status of soils. Compound equilibria with P-contain-ing minerals can control the broke up P fixation in soilsolution

and groundwater (*Mohsen Jalali, Narges Hemati Matin, 2013*). Effective groups were identified in the phosphorus solution and their properties, shelf life, durability were determined. Efficacy tests of microorganisms have been adhered to depending on individual and coexistence conditions, depending on different time, temperature and conditions. The most effective dose was *Bacillus megaterium (BM) + Pseudomonas aeruginosa (PA) + Pseudomonas putida (PP) + Acinetobacter baumannii (AB) + Micrococcus luteus (ML) + Burkholderia cepacia (BC) +Aspergillus niger (AN) + Epidermophyton floccosum (EF)* obtained from co-administration. As a result of the research, the comparison of the application of rock phosphate with microbial formulation in our country was compared with the triple super phosphate used in normal agriculture, and the photosynthetic activity, stoma permeability, chlorophyll and other efficacy parameteres were evaluated in different cultures grown in different geographical regions. In this study, it was determined that 8 kg/da rock phosphate + *Bacillus megaterium (BM) + Pseudomonas aeruginosa (PA) + Pseudomonas putida (PP) + Acinetobacter baumannii (AB) + Micrococcus luteus (ML) + Burkholderia cepacia (BC) +Aspergillus niger (AN) + Epidermophyton floccosum (EF)* formulations were more effective than chemical fertilizer applied on yield and yield components in wheat, maize, tomato, potato, grape, quince, orange and cabbage plants. According to the results obtained, the effect of fertilizer application on the yield and yield components of wheat, maize, tomato, potato, grape, quince, orange and cabbage plants were investigated as a result of two year field work and the efficient application was found to be more effective than control and the use of rock phosphate alone according to the usage parameter. A formulation was developed that has the potential to be used in all countries that restrict the use of phosphorus, especially in the territory of our country. This fertilizer formulation has also resulted in a technological fertilizer formulation that allows for the use of phosphorus sources that are limited and not found in organic agriculture.

4. CONCLUSION

In this project which investigated the usability of phosphate reserves existing in our country as a source of phosphorus fertilizer in agriculture, the selection of the most appropriate formulation was made by performing the provision, isolation and identification of the bacteria to be used in the study and the antagonistic effect tests of the formulation being the advantageous in terms of the fertilizer value which is the most effective in the solution of rock phosphate were carried out. The possible formulation identified accordingly was the most effective combination of *Bacillus megaterium* (BM) + *Pseudomonas aeruginosa* (PA) + *Pseudomonas putida* (PP) + *Acinetobacter baumannii* (AB) + *Micrococcus luteus* (ML) + *Burkholderia cepacia* (BC) + Aspergillus niger formulation when used together. Then, field experiments were made for a period of two years with an attempt to test the effects of the said formulation in agriculture in different regions (Erzincan, Konya, Kayseri, Antalya, Manisa, Mersin, Erzurum and Iğdır) and in different plants (grape, potato, corn, tomato, quince, orange, cabbage and wheat) (pH = 7.9, Total P2O5 ratio 29.6%) and at various doses (0, 6, 8 and 12 kg), thus by comparing its effects on the yield, yield parameters and chemical content, P fertilizer usage efficiency of rock phosphate in comparison with the implementation of chemical phosphorous fertilizer.

As a result of the study, the evaluations of overall yield and parameters such as foresentetic activity, stomatal permeability chlorophyll that effect the yields of different cultivated plants grown in different geographical regions were made after having compared the use of rock phosphate along with the microbial formulation which is available in our country with the triple superphosphate used in traditional agriculture.

As a result of the study, it has come into light that 8 kg / da rockphosphate + *Bacillus megaterium* (BM) + *Pseudomonas aeruginosa* (PA) + *Pseudomonas putida* (PP) + *Acinetobacter baumannii* (AB) + *Micrococcus luteus* (ML) + *Burkholderia cepacia* (BC) + *Aspergillus niger* was found to be more effective on the yield and yield components of wheat, corn, tomato, potato, grape, quince, orange, and cabbage plants when compared with the chemical fertilizer applied at the same ratio. The product obtained as a result of this study has the potential to be used as P fertilizer activity enhancer in organo-mineral

fertilizer mixtures, especially in organic agriculture in our country, and the necessary patent studies on the product will be evaluated commercially.

REFERENCES

1. By M. J. Hedley, R.e.w.a.p.h.n., plant-ınduced changes ın the rhızosphere of rape {brassıca nafus var. emerald) seedlıngs. *New Phytol. (1982)* 91, 45-56, 1982.

2. Cordell, D., The Story of Phosphorus: missing global governance of a critical resource. *Paper prepared for SENSE Earth Systems Governance, Amsterdam, 24th -31st August, 2008.*

3. C.D. Campbell *, S.J.G., D.J. Hirst a and P. Baptista, Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. *Journal of Microbiological Methods 30* (1997) 33–41, 1997.

4. C.R. Chena, b., *, L.M. Condrona, M.R. Davisc, R.R. Sherlocka, Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus radiata D. Don.). *Soil Biology & Biochemistry 34* (2002) 487±499, 2002.

5. Fraser, T., et al., Linking alkaline phosphatase activity with bacterial phoD gene abundance in soil from a long-term management trial. *Geoderma, 2015*. 257-258: p. 115-122.

6. José Igual, A.V., Emilio Cervantes, Encarna Velázquez, Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. https://hal.archives-ouvertes.fr/hal-00886151.

7. McDowell, R.W., et al., Mechanisms of phosphorus solubilisation in a limed soil as a function of pH. *Chemosphere, 2003*. 51(8): p. 685-692.

8. Paul, D. and S.N. Sinha, Isolation and characterization of phosphate solubilizing bacterium Pseudomonas aeruginosa KUPSB12 with antibacterial potential from river Ganga, India. *Annals of Agrarian Science, 2017*. 15(1): p. 130-136.

9. D.H.Pote , J.A.L., H.Zhang Does initial soil P level affect water-extractable soil P response to applied P? *Advances in Environmental Research 7* (2003) 503–509.

10. S. BANIK, a.B.K.D., Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. Plant and Soil 69, 353-364 (1982). 0032-079X/82/0693-0353501.80. Ms. 4727 9 1982 Mart&us Nijhoff/Dr W. *Junk Publishers, The Hague. Printed in the Netherlands*.

11. Peyami Battal, B.T., The Effects of Different Mineral Nutrients on the levels of Cytokinins in Maize (Zea mays L.). *Turk J Bot 25* (2001) 123-130 © *TÜBİTAK*.

12. Fikrettin ¸Sahin, R.Ç.F.K., Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. Plant and Soil 265: 123–129, 2004. © *2004 Kluwer Academic Publishers. Printed in the Netherlands*.

13. Teodor Hodisan , M.C., Claudia Cimpoiu , Agata Cot Separation, identification and quantitative determination of free amino acids from plant extracts. *Journal of Pharmaceutical and Biomedical Analysis 18* (1998) 319–323.

14. M.h.m.n. Senden, A.j.g.m.v.d.m., J. Lımborgh and H.th. Wolterbeek, Analysis of major tomato xylem organic acids and PITC-derivatives of amino acids by RP-HPLC and UV detection. Plant and Soil 142:81-89, 1992. © *1992 Kluwer Academic Publishers. Printed in the Netherlands*.

15. Lidia Giuffr6 de L6pez Carnelo, S.R.d.M., Liliana Marbh", Heavy metals input with phosphate fertilizers used in Argentina. *The Science of the Total Environment 204* (1997) 245-250.

16. Jones, N.C.a.R.J., Disruption of Maize Kernel Growth and Development by Heat Stress'. *Plant Physiol.* (1994) 106: 45-51.

17. Mehmet Nuri Aydoğan, ö.F.A., Isolation and Characterisation of Some Bacteria and Microfungus Solving Tricalcium Phosphate. *See discussions, stats, and author*

profiles for this publication at: https://www.researchgate.net/publication/291352695.

18. MacKenzie1, T.Q.Z.a.A.F., Phosphorus in zero tension soil solution as influenced by long-term fertilization of corn (Zea mays L.). *Canadıan Journal Of Soil Science*

.

19. Nakamura, K., et al., Enzyme production-based approach for determining the functions of microorganisms within a community. *Appl Environ Microbiol,* 2004. 70(6): p. 3329-37.

20. Qiao, L., et al., A novel surface-confined glucaminium-based ionic liquid stationary phase for hydrophilic interaction/anion-exchange mixed-mode chromatography. *J Chromatogr A,* 2014. 1360: p. 240-7.

21. Rodriguez, H., et al., Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium Azospirillum spp. *Naturwissenschaften,* 2004. 91(11): p. 552-5.

22. P. Manımohan, A.v.j.a.k.m.l., The genus Enfoloma in Kerala State, India. Mycol. Res. 99 (9): 1083-1097 (I995) *Printed in Great Britain.*

23. P.S. Hoodaa, V.W. Truesdalea, A.C. Edwardsb M.N. Aitkend and A.M.a. , P.J.A. Withersc , A.R. Rendella, Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Advances in Environmental Research 5* 2001 13□21 *Ž.*

24. I. R. Richards*, C.j.c.a.a.j.k.r., Effects of long-term fertilizer phosphorus application on soil and crop phosphorus and cadmium contents. Journal of Agricultural Science, Cambridge (1998), 131, 187–195. *Printed in the United Kingdom # 1998 Cambridge University Press*.

25. Hilda Rodríguez *, R.F., Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* 17 (1999) 319–339.

26. Sadana, U.S. and N. Claassen, Potassium efficiency and dynamics in the rhizosphere of wheat, maize, and sugar beet evaluated by a mechanistic model. *Journal of Plant Nutrition*, 1999. 22(6): p. 939-950.

27. Hammond, J.P., M.R. Broadley, and P.J. White, Genetic responses to phosphorus deficiency. *Ann Bot*, 2004. 94(3): p. 323-32.

28. İbrahim Erdal, F.h., mardin-mazıdağı hamfosfat atıklarının gübre olarak kullanılabilirliğinin belirlenmesi. *pamukkale üniversitesi mühendislik fakültesi pamukkale unıversity engineering college mühendislik bilimleri dergisi journal of engıneerıng scıences yıl cilt sayı sayfa*:1996 : 2 : 3.

29. Cutting, J.G.M., Determination of the Cytokinin Complement in Healthy and Witchesbroom Malformed Proteas. *J Plant Growth Regul* (1991) 10:85-89.

30. John W. Morris, P.D., Roy . Morris, and Joe B. Zaerr*, Cytokinins in Vegetative and Reproductive Buds of Pseudotsuga menziesii'. *Plant Physiol*. (1990) 93, 67-71 0032-0889/90/93/0067/05/$01 .00/0.

31. Oli, P., et al., Parboiled rice: Understanding from a materials science approach. *Journal of Food Engineering*, 2014. 124: p. 173-183.

32. Serpil Kahya, E.B., K. Tayfun Carlı, Polimeraz Zincir Reaksiyonu (PCR) Optimizasyonu. *Uludag Univ. J. Fac. Vet. Med.* 32 (2013), 1: 31-38.

33. Nahas, E., Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World Journal of Microbiology & Biotechnology* 12.567-572.

34. L. K. Hsıeh, T.-C.L., C. 0. and A.K.L.S. Chichester, Biosynthesis of Carotenoids in Brevibacterium sp. KY-4313. journal of bacteriology, May 1974, p. 385-393 Copyright 0 1974 *American Society for Microbiology*.

35. Ahemad, M. and M. Kibret, Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University - Science*, 2014. 26(1): p. 1-20.

36. Behera, B.C., et al., Phosphate solubilization and acid phosphatase activity of Serratia sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. J*ournal of Genetic Engineering and Biotechnology*, 2017. 15(1): p. 169-178.

37. Buggenhout, J., et al., The breakage susceptibility of raw and parboiled rice: A review. *Journal of Food Engineering*, 2013. 117(3): p. 304-315.

38. Cabello, M., et al., Effect of an arbuscular mycorrhizal fungus, Glomus mosseae, and a rock-phosphate-solubilizing fungus, Penicillium thomii, on Mentha piperita growth in a soilless medium. *J Basic Microbiol*, 2005. 45(3): p. 182-9.

39. Carroll P. Vance, C.U.-S.a.D.L.A., Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *Tansley review*.

40. G. A. Gilbert*, J.D.K., C. P. Vance & D. L. Allan, Acid phosphatase activity in phosphorus-deficient white lupin roots. *Plant, Cell and Environment* (1999) 22, 801–810.

41. Davies, P.J., *The Plant Hormones*: Their Nature, Occurrence, and Functions. Nature, occurrence and functions.

42. Germida, J.R.d.F.M.R.B.J.J., Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). *Biol Fertil Soils* (1997) 24:358–364.