GROWTH RESPONSES OF CULTURABLE SOIL MICROBE POPULATIONS TO DIFFERENT WHEAT CULTIVARS AND CARBON SOURCES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

BUKET KOÇAK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF
MASTER OF SCIENCE

IN
THE DEPARTMENT OF BIOLOGY

SEPTEMBER 1998

Approval of the Graduate School of Natural and Applied Sciences

Prof. Dr. Tayfur Öztürk
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Gürdal Alaeddinoğlu
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Biology.

Asst. Prof. Dr. Ayşegül Ozan
Supervisor

Examining Committee Members

Asst. Prof. Dr. Ayşegül Ozan

Dr. Vehbi Eser

Assoc. Prof. Dr. Sertaç Önde

Doç. Dr. Tülin Güray

Prof. Dr. Zeki Kaya

ABSTRACT

GROWTH RESPONSES OF CULTURABLE SOIL MICROBE POPULATIONS TO DIFFERENT WHEAT CULTIVARS AND CARBON SOURCES

Koçak, Buket

M.S., Department of Biology

Supervisor: Asst. Prof.Dr. Ayşegül Ozan

September 1998, 109 pages

Chemical composition of the root exudates show variations in terms of quantity and quality depending on the plant species. The purpose of this study was to investigate if these differences in microorganism profile occur at the cultivar level. The microorganisms numbers were used as an indication of how well the substrates in the exudates are metabolised by the populations. The results of this study suggested the possible presence of growth stimulatory and inhibitory metabolites in the root exudates of wheat cultivars investigated on soil microorganisms.

In this study Bezostaja 1, Bolal 2973, Gerek 79, Gün 91, Kıraç 66, Kırkpınar 79 and Lancer wheat cultivars differing in the degree of their drought tolerance were investigated.

For each of these cultivars the numbers of gram negative, general K strategist, general R strategist bacteria, actinomycetes and culturable soil fungi were determined and compared . The results indicated that soil microorganisms were altered under the influence of different wheat cultivars. Among the cultivars investigated two extreme influences occurred on microorganisms were represented by Bolal & Kıraç cultivars. Bolal supported the proliferation of the bacterial populations, Kıraç, however, had its most positive proliferative effect on soil fungi.

In the second part of this study the differences in the utilization of different carbon sources by the soil microorganisms were analysed. For each of the seven cultivars, growth responses of soil microorganisms were measured against added substrates in time dependent fashion. Glucose and glutamic acid were used as substrates. Also, responses of soil microbe populations to aldicarb ,a pesticide, were measured. Most of the time the bacterial populations responded maximally to glutamic acid compared to glucose & aldicarb. Aldicarb amendments positively affected the numbers of gram negative bacterial populations.

In the third part of the study the growth responses of soil microorganisms to other amino acids were measured. Besides glutamic acid, glycine, arginine and serine were tested. Among the four amino acids, serine was found to be the most effective on the actinomycete populations as well as the populations of bacteria grown in minimal salts and rich media; glutamic acid on the other hand exerted its stimulating effect on gram negative bacteria and soil fungi.

Key words: soil microorganisms, aldicarb, carbon sources, bacteria, fungi, wheat cultivars

ÖZ

KÜLTÜR EDİLEBİLEN TOPRAK MİKROBU POPULASYONLARININ FARKLI BUĞDAY KÜLTİVARLARINA VE KARBON KAYNAKLARINA KARŞI BÜYÜME TEPKİLERİ

Koçak, Buket
Yüksek Lisans, Biyoloji Bölümü
Tez Yöneticisi: Y. Dr. Ayşegül Ozan

Eylül 1998, 109 sayfa

Kök sızıntılarının kimyasal kompozisyonu bitki türlerine göre nitelik ve nicelik açısından farklılıklar gösterir. Bu çalışmanın amacı mikroorganizma profilindeki bu farklılıkların çeşit seviyesinde olup olmadığını araştırmaktır. Mikroorganizma sayıları kök sızıntılarındaki maddelerin populasyonlar tarafından nasıl metabolize edildiğinin bir göstergesi olarak kullanılmıştır. Bu çalışmanın sonuçları araştırılan buğday çeşitlerinin kök sızıntılarında toprak mikroorganizmaları üzerinde büyümeyi uyarıcı ve engelleyici metabolitlerin olma ihtimalini ortaya çıkardı.

Bu çalışmada kuraklığa mukavemet dereceleri bakımından farklılık gösteren Bezostaja, Bolal, Gerek, Gün, Kıraç, Kırkpınar ve Lancer buğday kültivarları çalışıldı.

Bu kültivarların her biri için gram negatif, genel K stratejist, genel R stratejist bakteriler, aktinomisetler ve kültür edilebilen toprak funguslarının sayıları belirlendi ve karşılaştırıldı. Sonuçlar toprak mikroorganizmalarının farklı buğday çeşitlerinin etkisi altında değiştiğini gösterdi. Araştırılan kültivarlar arasında mikroorganizmalar üzerinde oluşan iki uç etki Bolal ve Kıraç kültivarlarının tarımının yapıldığı topraklarda gözlendi. Bolal kültivarı bakteri popülasyonlarının çoğalmasını en üst düzeyde desteklerken, Kıraç kültivarı en pozitif çoğaltma etkisini toprak fungusları üzerinde gösterdi.

Bu çalışmanın ikinci bölümünde toprak mikroorganizmaları tarafından değişik karbon kaynaklarının kullanımında gösterdiği değişiklikler analiz edildi. Yedi kültivarın her biri için mikroorganizmaların eklenen substratlara karşı büyüme tepkileri zamana bağlı şekilde ölçüldü. Substrat olarak glukoz ve glutamik asit kullanıldı. Aynı zamanda toprak mikrop popülasyonlarının, bir böcek ilacı olan aldikarba karşı tepkileri de ölçüldü. Çoğunlukla bakteri popülasyonları glukoz ve aldikarbla karşılaştırıldığında maksimum tepkiyi glutamik aside gösterdi. Aldikarb ilaveleri gram negatif bakteri populasyonlarının sayılarını pozitif yönde etkiledi.

Çalışmanın üçüncü bölümünde toprak mikroorganizmalarının diğer amino asitlere büyüme tepkileri ölçüldü. Glutamik asitten başka glisin, arginin ve serin denendi. Dört amino asidin arasında serin hem aktinomiset popülasyonları hem de minimal tuz ve zengin ortamda büyüyen bakteriler üzerinde en etkili bulundu; diğer taraftan glutamik asit uyarıcı etkisini gram negatif bakteriler ve toprak fungusları üzerinde ortaya çıkardı.

Anahtar Kelimeler: Toprak mikroorganizmaları, aldikarb, karbon kaynakları, bakteriler, mantar, buğday kültivarları

ACKNOWLEDGEMENTS

I express my deepest gratitude to my supervisor Asst. Prof. Dr. Ayşegül Ozan for her valuable supervision and her encouragement throughout this study.

I thank Prof. Dr. Gülay Özcengiz for providing her facilities, Prof. Dr. Gürdal Alaeddinoğlu for kindly providing aldicarb, and Dr. Ayşe Ergüven for her help in statistical analysis.

Special thanks go to my mother Mrs. Gülşen Koçak, my father Mr. Abdullah Koçak, my brother Bülent Koçak, my fiancee Bahadır Özazar and my grandmother Ayşe Bulut for their support and understanding.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

AS: Aldicarb

ATP: Adenosine triphosphate

C: Carbon

CFU: Colony forming unit

CO_2: Carbon dioxide

CoA: Coenzyme A

DAPI: Diamidino phenyl indole

dH_2O: Distilled water

DNA: Deoxyribo nucleic acid

DVC: Direct microscopic viable count

g: gram

H_2: Hydrogen gas

H_2S: Hydrogen sulfide

IAA: Indoleacetic acid

LISA: Low-input sustainable agriculture

lt: liter

M: Molar

mg: miligram

ml: mililiter

mm: milimeter

MPN: Most probable number

N: Nitrogen

N_2O: Nitrous oxide

O: Oxygen

O_2: Oxygen gas

OAA: Oxaloacetic acid

P: Phosphorus

PAHs: Polycyclic aromatic hydrocarbons

PCBs: Polychlorinated biphenyls

PCR: Polymerase chain reaction

PEP: Phosphoenol pyruvate

PLP: Pyridoxal phosphate

PTS: Phosphotransferase system

RFLPs: Restriction fragment length polymorphisms

RNA: Ribo nucleic acid

SCSU: Sole carbon source utilization

S: Sulphur

TCA: Tricarboxylic acid

TM: Türk Malı

VAM: Vesicular arbuscular mycorrhizal

µL: microliter

°C: Centigrade

α: Alpha

β: Beta

CHAPTER 1

INTRODUCTION

1.1. Soil Microorganisms

Soil is generally a favorable habitat for the proliferation of microorganisms with microcolonies developing on soil particles. Numbers of microorganisms in soil habitats normally are much higher than those found in freshwater or marine habitats. Microorganisms found in soil include bacteria, fungi, algae, protozoa and viruses (Atlas and Bartha, 1992). Bacteria are the most numerous of the microorganisms in soil. Indeed, they are the most common of all the living organisms on the face of the earth. Both energy source and carbon source are useful for describing basic physiological differences among bacteria as well as among organisms generally. Physiological groupings for bacteria other than those based on energy and cell carbon sources are found in the literature. One of the best known is the autochthonous and zymogenous seperation proposed by Winogradsky. Autochthonous organisms are defined as those growing slowly in soil containing no easily oxidizable substrate. Zymogenous species are those showing bursts of activity when fresh residues are added to soil (Paul and Clark, 1989). Slow but constant activity is characteristic of autochthonous organisms most of which are Gram-negative rods and actinomycetes. Winogradsky contrasted zymogenous or opportunistic soil organisms with autochthonous microorganisms. The former generally are not able to utilize humic compounds, but exhibit high levels of activity and rapid growth on easily utilizable substrates that become available in the form of plant litter, animal droppings, and carcasses. Intermittent activity with inactive resting stages is characteristic of such zymogenous organisms. *Pseudomonas, Bacillus, Penicillum, Aspergillus,* and

Mucor are some of the bacterial and fungal genera of typical zymogenous organisms. It is diffucult to ascribe generalized adaptive features to the indigenous soil microbiota. Soils have many microhabitats, and at a particular location there may even be several microenvironmental situations that would favor different indigenous populations. In the rich litter horizons of surface soils, indigenous microorganisms tolerate and grow on high concentrations of organic nutrients. Bacteria found in soils may be obligate aerobes, facultative anaerobes, microaerophiles, or obligate anaerobes. Individual soils may favor bacterial populations with a particular type of metabolism. For example, anoxic conditions in flooded soils favor proliferation of facultative and obligate anaerobes. Although it may not be possible to describe the general features of indigenous soil bacteria, the abiotic parameters of some soils restrict the microbial populations that can develop there. For example, some soils have extremely alkaline pH values, while others are extremely acidic. In such soils, the indigenous microbial populations must possess adaptive features that allow them to grow there (Atlas and Bartha, 1992) .

Permafrosts constitute one of the extreme soil environments. From the 1950s to the 1970s, a significant amount of work was done on the microbial flora in both Arctic soils and the underlying permafrost in northern Canada and Alaska. A wide variety of species and physiological types of microorganisms, including bacteria like *Bacillus* spp., *Azotobacter* spp., sulfate reducers, and some thermophiles, (*Streptomyces* spp.), yeasts, fungi, and protozoa, were found in arctic soils (Shi *et al* ., 1996). In the course of the past few years, Russian researchers have produced an impressive body of information on the microbial communities in the northeast Siberian permafrost (Gilichinsky, 1994). Large numbers of viable microorganisms, including fungi, yeast, algae, actinomycetes, and bacteria have been found in most (90%) of the samples studied (Shi *et al.*, 1996).

Many bacterial genera occur in soil. A higher proportion of Gram-positive bacteria is found in soil than in marine or fresh water habitats, but in absolute

numbers Gram negative bacteria predominate in soil as well as in aquatic habitats. A higher proportion of indigenous soil bacteria utilize substrates such as carbohydrates than bacterial populations found in the hydrosphere. Common bacterial genera found in soil include *Acinetobacter, Agrobacterium, Alcaligenes, Arthobacter, Bacillus, Brevibacterium, Caulobacter, Cellulomonas, Clostridium, Corynebacterium, Flavobacterium, Micrococcus, Mycobacterium, Pseudomonas,Staphylococcus, Streptococcus, and Xanthhomonas.* There are wide differences in the relative proportions of individual bacterial genera found in particular soils (Atlas and Bartha, 1992).

As determined by the plate count, members of the genus *Arthobacter* are the numerically predominant bacteria in soil. They are characterized by their pleomorphism and Gram variability.

The genera, *Streptomyces, Pseudomonas, and Bacillus* follow *Arthobacter* as commonly occuring bacteria in soil. Any one of the three may at various times account for 5 to 20% of the total bacterial colony counts (Paul and Clark, 1989). Actinomycetes are bacteria, but they are usually considered seperately in enumerations of soil populations because they greatly differ from conventional concept of bacteria. They typically form long filaments resembling those of molds, but with diameters of bacterial dimensions. This filamentous growth habit is a considerable advantage for a microorganism in soil. Under dry conditions, the filament can bridge the gap between one particle of soil and another. This type of growth also maximizes surface area for a given weight and may give the actinomycetes a nutritional advantage. These organisms are found in soil in very large numbers and produce a gaseous substance called **geosmin**, which gives the soil its characteristic musty odor (Tortora *et al.*, 1994). Actinomycetes can compromise a significant proportion of the bacterial population in the soil about 10% - 33%. The genera *Streptomyces* and *Nocardia* are the most abundant actinomycetes found in the soil. *Micromonospora, Actinomyces* and many other actinomycetes are indigenous to soils but generally are present in low numbers. Actinomycetes are relatively resistant to desiccation and can survive under

conditions of drought in desert soils. They favor alkaline or neutral pH and are sensitive to acidity (Atlas and Bartha, 1992). Actinomycetes of 29 genera were found in Yunnan, China. Therefore, Yunnan deserves to be called a kingdom of actinomycetes (Xu *et al.*, 1995).

There is a positive correlation between actinomycete diversity and vegetation. Thus, primeval forest soil is a massive treasure-house or gene house of actinomycetes (Table 1.1.). The drier and poorer the soil is and the cooler the climate is, the fewer actinomycetes there are and the higher the percentage of *Streptomyces* strains observed. The genus *Streptomyces* appears to be the most important in ecological function. It represents up to 90% of all soil actinomycete diversity and is an important component of the soil actinomycete population (Xu *et al.*, 1995). Many streptomyces produce antibiotics, variously antibacterial, antifungal, antialgal, antiviral, antiprotozoal, or antitumor (Paul and Clark, 1989).

Important photoautotrophic bacterial populations in soil include the cyanobacterial species *Anabaena, Calothrix, Chroococcus, Cylindrospermum, Lyngbya, Microcoleus, Nodularia, Nostoc, Oscillatoria, Phormidium, Plectonema, Schizothrix, Scytonema , and Tolypothrix.* Some of these species, such as *Nostoc* provide both fixed forms of nitrogen and organic carbon in some soil habitats. Availability of fixed forms of nitrogen is an important limiting factor in soil for microbial activities and growth of higher plants. *Azotobacter* is an important heterotrophic free-living soil bacterium, capable of converting atmospheric nitrogen to fixed forms of nitrogen. Some anaerobic *Clostridium* species also fix nitrogen in soil; *Rhizobium* and the slow growing *Bradyrhizobium* fix atmospheric nitrogen within the nodules of certain plant roots. Several chemolithotrophic bacteria perform transformations of inorganic compounds that are essential for the maintenance of soil fertility.

Fungi constitute high proportion of the microbial biomass in soil. Most types of fungi can be found in soil, either as indigenous or as allochthonous organisms. Soil fungi may occur as free-living organisms or in mycorrhizal

association with plant roots. Fungi are found primarily in the top 10 cm. of the soil and are rarely found below 30 cm. of the soil. They are most abundant in well-aerated soils.

Table 1.1. Comparisons of actinomycete populations among various types of vegetation

Vegetation type	No. of areas Studied	Avg. count of actinomycetes (10^3 CFU/g)	*Streptomyces* % of population	Avg. count of genera
Primeval forest	8	482.8	79	9.0
Secondary forest	16	1.199.6	88	6.7
Wasteland	11	893.2	88	5.9
Nonirrigated farmland	7	1.359.4	94	5.4
Vegetable farmland	8	5.963.1	88	6.5
Paddy farmland	6	1.211.9	76	5.7

The most frequently isolated fungi from soils are members of *fungi imperfecti*, such as species of *Aspergillus, Geotrichum, Penicillum, and Trichoderma*, but numerous ascomycetes and basidiomycetes also occur in high numbers. Some soil fungi, especially those found in association with plant roots, are somewhat diffucult to isolate and identify (Atlas and Bartha, 1992).

Most fungi in soil are opportunistic. They grow and carry out active metabolism when conditions are favorable, which implies adequate moisture, adequate aeration, and relatively high concentrations of utilizable substrates. Many soil fungi metabolize carbohydrates, including polysaccharides, and even allochthonous fungi that enter the soil often can grow and metabolize the major components of plant residues. Relatively few fungal species, however , are able to degrade lignin. Dormancy is a typical condition of soil fungi. Some fungi have

been shown to remain dormant but viable for decades. In the absence of available substrates, they are inactive (Atlas and Bartha, 1992).

1.2. Growth Responses of Soil Microorganisms to carbon sources

When utilizable nutrients are added to soil, the microbial populations and their activity rapidly increase until the nutrients are depleted, and then the microbial activity returns to the lower, baseline levels (Tortora *et al.*, 1994). Plants, the major source of organic matter upon which soil microorganisms are dependent, are literally covered with microorganisms. Soil microbial populations respond to the release of organic materials near the plant root increasing their numbers and changing the characteristics of the microbial community (Prescott *et al.*, 1993). Organic materials released from roots include amino acids, keto acids, vitamins, sugars, tannins, alkaloids, phosphatides, and secondary metabolites. Roots surrounded by microorganisms excrete much more organic material than roots grown in aseptic conditions. Although a few of these materials inhibit microorganisms, most stimulate microbial growth. The influence of materials released by the plant into the soil is evidenced by the observation that bacterial populations within the rhizosphere have markedly different nutritional properties than bacteria in root-free soil. Many rhizosphere bacteria require amino acids for maximal growth, and it is likely that root exudates supply these acids. Initially, carbohydrate exudates and mucilaginous materials support the growth of large populations of microorganisms within the grooves of the epidermal plant cells, on the root surface, and within mucilaginous layers surrounding the roots. As the plant matures, autolysis of some of the root material takes place as part of normal root development and simple sugars and amino acids are released into the soil, stimulating the growth of *Pseudomonas* and other bacteria with high intrinsic growth rates. The plant cover of the soil is an important factor in determining the types and numbers of microorganisms in that soil (Atlas and Bartha, 1992) . The largest fraction of all organic carbon entering the soil is that contributed by plant residues. Plants contain 15-60% cellulose, 10-30% hemicellulose, 5-30% lignin, and 2-15% protein. Soluble substances such as sugars, amino sugars, organic

acids, and amino acids, can constitute 10% of the dry weight. They are readily leached from plant residues and are quickly utilized by soil organisms (Paul and Clark, 1989). Soil microorganisms are largely responsible for the transformation of the structural polymers of plants. As a consequence of their activity, carbon dioxide is reintroduced to the atmosphere, humic materials are formed, and simpler organic compounds are made available to other populations (Atlas and Bartha, 1992).

Biogenic polymers, recycled primarily by microbial degradation, in soil include cellulose, hemicelluloses, and chitin (Atlas and Bartha, 1992). The decomposition of lignin is primarily attributed to fungi. White-rot fungi are the most active lignin-degrading microorganisms, resulting in the degradation of all wood components to carbon dioxide and water.

Amino acids comprise about 20% of the soil carbon but 30-40% of the soil nitrogen. Amino acids in the free state are rapidly degraded. Microbial enzymes, such as pronase and subtilisin, carry out terminal amino acid chain removal and are more powerful in nature than animal and plant proteases, such as trypsin and papain. Microbial attack of carbohydrates and proteins results in the synthesis of microbial products, and depending on the carbon:nitrogen:sulfur:phosphorus ratios in the synthesis of SO_4^{2-} and NH_4^+ as well as CO_2 (Paul and Clark, 1989). Carbohydrates translocated by permeases are subsequently phosphorylated by ATP-dependent kinases. Another pathway for carbohydrate utilization is the phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS), which catalyses concomitant internalization and phosphorylation of various carbohydrates (Lauret *et al.*, 1996).

Analysing sole-carbon source utilization (SCSU) patterns by microbial samples shows promise as a means of assessing soil microbial community structure. SCSU examines the functional capabilities of the microbial population, and the resulting data can be analyzed using multivariate techniques to compare metabolic capability of communities. Organic soil horizons exhibit higher

functional diversity than mineral soil, as the former likely contains a wider range of organic substrates. Presumably, the metabolic capabilities of the unique microbial community associated with each soil horizon reflect these differences and could be enhanced by other factors such as variation in moisture and nutrient levels between organic and mineral soil. Mineral soil samples shows considerable variability when compared to organic samples. Mineral soil microbial communities may show considerable patchiness due to site-to-site differences such as nutrient levels or drainage (Staddon *et al.*, 1996).

A soil microbial community represents a continuum of microorganisms with various C requirements, with obligatory oligotrophs adapted to low C concentrations at one extreme and obligatory copiotrophs to high C concentrations at the other extreme. The appearance of bacterial colonies on agar media is also multiphasic, the first phase consisting of mostly copiotrophic bacteria and later phases of facultative and obligate oligotrophs (Hu and Bruggen, 1997). Carbohydrate carbon, if available, will be utilized, rather than the amino acid carbon, thus preserving the carbon skeleton of the amino acid for protein synthesis.

1.3. Method of Quantitating Soil Microorganisms

Culture Techniques

Culturing of a soil microorganism involves transfer of its propagules to a substrate conducive to growth. All cultural techniques are selective and designed to detect microorganisms with particular growth forms or biochemical capabilities. Most techniques depend on the use of soil suspensions, with the degree of dilution required being related to the initial number of organisms in the soil. Serial dilutions are made on a known weight of wet or dry soil.

The most frequently used method for the measurement of bacterial populations is the **plate count.** An important advantage of this method is that it

measures the number of viable cells. Mycologists usually prefer to use the term **cfu** (colony forming unit) in lieu of propagule (Paul and Clark, 1989) . The plate count is based on three assumptions: that each bacterium grows and divides to produce a single colony, that the original inoculum is homogeneous, and that no aggregates of cells are present. To ensure that colony counts will be within countable range (between 25-250 per plate), the original inoculum is diluted several times in a process called **serial dilution** (Tortora *et al.*, 1994) . Media adjustments for different groups of organisms include the use of antibiotics, to eliminate bacteria not fungi. Other modifications include acidification to favor fungi, certain dyes to favor Gram-negative bacteria, and diverse selective media for groups such as actinomycetes, nitrogen fixers, or sulfur oxidizers. The plate count of bacteria usually represents only 1-5%, and at the most, 50% of the number determined by direct microscopy.

Most studies have used cultivation techniques, such as plate counts or most probable number (MPN) protocols to enumerate microbial populations in soils. However, it became apparent that many microbes from soil environments do not respond readily to cultivation techniques and therefore population data cannot be considered as accurate numbers. Depending on the soil under study, the culturable portion of microorganisms underestimates the resident population of active and quiescent microbes by at least one to two orders of magnitude (Hartmann *et al.*, 1997).

Indirect quantification of microbial populations through their physiological activity is a widely used practice in soil microbiology. Microbial biomass is estimated by several methods, such as respiration; metabolic heat production; adenylate energy content; adenosine triphosphate (ATP) content, enzymatic activities such as dehydrogenase and phosphatase activity, or soil fumigation techniques. These methods provide a measure for the active or activatable population, but they cannot provide information on the exact population levels. In particular, spatial discrimination down to the micrometer

level is not possible even by using specific oligonucleotide primers for (PCR) polymerase chain reaction (Hartmann *et al.*, 1997).

Conventional Staining Methods for Microorganisms

For direct enumeration of microorganisms, DNA-binding fluorochromes such as acridine orange and 4', 6-diamidino-2-phenylindole (DAPI) have been used for many years. Cell numbers derived from these DNA-binding fluorochromes usually show a reasonable correlation with viable counts when applied to growing laboratory cultures but can exceed plate counts by several orders of magnitude when used to examine bacterial populations in natural environments such as soil, sediment, and aquatic habitats. However, the whole microbial community, including the part that cannot be cultured, is of significant interest for the understanding of processes in soils and therefore is examined by direct observation.

The direct microscopic viable count (DVC) method has been employed successfully to determine viable bacteria in environmental samples. In the DVC or Kogure method, samples are incubated with yeast extract and nalidixic acid for a period that is sufficient to induce growth. Yeast extract provides nutrients, while nalidixic acid, a DNA gyrase inhibitor, blocks DNA synthesis. Thus, cell replication is inhibited, while cell growth and enlargement are promoted. The result is the formation of cell filaments or enlarged cell forms in the case of substrate-responsive cells. Subsequent acridine orange staining of these preparations, followed by microscopy, allows enumeration of responsive cells to provide an estimate of the viable population (Hartmann *et al.*, 1997).

Molecular Detection Methods for Microorganisms in Soil

Immunological Techniques

Immunological detection methods are based on the ability of antibodies to recognize specific three-dimensional structures, such as parts of proteins or polysaccharides, of biological macromolecules. In soil microbiology, they are becoming increasingly important for tracking of specific microorganisms and for microbial community analysis.

Detection methods include agglutination, immunofluorescence staining, immunogold labelling, and ELISA.

Immunoseperation techniques have also been successfully applied to isolate specific species from soil (Mavingui *et al.*, 1990).

Nucleic Acid Probes

Ribosomal RNA-directed probes have been used to detect bacteria in a wide range of complex habitats, e.g., aquatic microbial communities attached to surfaces, sediments, and sewage sludge flocs. Recently, the amount of information reported in literature related to investigations of soil are increasing (Fleske *et al.*, 1998; van Elsas *et al.*, 1998; Heuer *et al.*, 1998; Watts & Wellington, 1998).

New Microscopic Techniques and Image Analysis

The introduction of image processing and analysis broadened the field of microscopic examinations in microbial ecology a few years ago. The benefit provided by these computer-assisted methods ranges from simple contrast enhancement to automated cell counting and determination of biomass. Any computing analysis of photomicrographs needs a digitized image, which can be obtained by converting images recorded with a video camera. A similar approach

has been used to estimate cell sizes of bacteria in different soils. For the biomass determination of fungal hyphae in soil and on synthetic polymers, adequate software routines are required (Hartmann *et al.*, 1997).

Flow Cytometric Analysis of Microbial Populations

Flow cytometry is an appropriate tool to study microbial populations provided in the form of a suspension of single cells. Flow cytometry requires no prior cultivation of microorganisms and offers great advantages in characterizing mixed microbial communities because several thousand single cells can be analyzed in a few minutes, yielding much better statistical information than microscopic counting. However, a study based on the recovery of inoculated bacteria from soil pointed out that further development of the method was required (Page and Burns, 1991).

By using techniques for *in situ* detection of single microbial cells, studies of microbial populations in their microhabitats including interactions with other organisms and their environment became possible. Because soil habitats are complex and diverse, few applications have yet been reported. Inherent problems are still the high percentage of quiescent cells, especially in nutrient-poor soil habitats, and the high numbers of microorganisms not yet described (Hartmann *et al.*, 1997).

1.4. Plant – Microbe Associations

Not only do microorganisms exhibit relationships of neutralism, commensalism, synergism, mutualism, amensalism, competition, and parasitism among populations, but they also exhibit these types of interactions with plants. Some of these interactions are beneficial to both plants and the microbial populations, whereas others are detrimental to plants or microbial populations (Atlas and Bartha, 1992) The root surface itself is a recognized critical site for interactions between microbes and plants and called as the rhizoplane. Plant roots

provide suitable habitats for the growth of microorganisms, and particularly high numbers of many different microbial populations are found on and surrounding plant roots. Interactions between soil microorganisms and plant roots satisfy important nutritional requirements for both the plant and the associated microorganisms. The structure of the plant root system contributes to the establisment of the rhizosphere microbial population (Atlas and Bartha, 1992). Rhizosphere bacteria comprise many genera and typically reach population sizes 10-100 times greater than those in nonrhizosphere soil, sometimes exceeding one billion colony forming units (cfu) per gram of root tissue and associated rhizosphere soil (Chanway, 1997). The interactions of plant roots and rhizosphere microorganisms are based largely on interactive modification of the soil environment by processes such as water uptake by the plant system, release of organic chemicals to the soil by the plant roots, microbial production of plant growth factors, and microbially mediated availability of mineral nutrients. Within the rhizosphere, plant roots have a direct influence on the composition and density of the soil microbial community, known as the rhizosphere effect. The actual extent of the rhizosphere effect is dependent on the particular plant and its physiological maturity. There is a higher proportion of Gram-negative, rod-shaped bacteria and a lower proportion of Gram-positive rods, cocci, and pleomorphic forms in the rhizosphere than in root-free soil (Atlas and Bartha, 1992).

Just as plant roots have a direct effect on the surrounding microbial populations, microorganisms in the rhizosphere have a marked influence on the growth of plants. In the absence of appropriate microbial populations in the rhizosphere, plant growth may be impaired. Microbial populations in the rhizosphere may benefit the plant in a variety of ways, including increased recycling and solubilization of mineral nutrients; synthesis of vitamins, amino acids, auxins, and gibberellins, which stimulate plant growth; and antagonism with potential plant pathogens through competition and development of ammensal relationships based on production of antibiotics. Microorganisms synthesize auxins and gibberellin-like compounds, and these compounds increase the rate of seed germination and the development of root hairs that aid plant growth.

Arthobacter, *Pseudomonas*, and *Agrobacterium* populations found in the rhizosphere have been reported to be capable of producing organic chemicals that stimulate growth of plants (Atlas and Bartha, 1992).

Allelopathic (antagonistic) substances released by microorganisms in the rhizosphere may allow plants to enter amensal relationships with other plants. Such allelopathic substances surrounding some plants can prevent invasion of that habitat by other plants, and this may represent a synergistic relationship between a plant and its rhizosphere microbial community.

Microorganisms in the rhizosphere influence the availability of mineral nutrients to the plants, sometimes using limiting concentrations of inorganic nutrients before they can reach plant roots, and in other cases increasing the availability of inorganic nutrients to the plant. Rhizosphere microorganisms increase the availability of phosphate through solubilization of materials that would otherwise be unavailable to plants. The principal mechanism of increasing phosphate-availability is the microbial production of acids that dissolve apatite, releasing soluble forms of phosphorus. Iron and manganese may be more available to plants because of rhizosphere microorganisms that produce organic chelating agents, thus increasing the solubility of iron and manganese compounds.

Although increased uptake of minerals due to rhizosphere microorganisms is beneficial, the abundant microbial populations in the rhizosphere can sometimes create a deficiency of required minerals for the plants. For example, bacterial immobilization of zinc and oxidation of manganese cause the plant diseases "little leaf" of fruit trees and " grey speck" of oats, respectively. Microorganisms in the rhizosphere may immobilize limiting nitrogen, making it unavailable for the plant.

Although diverse and complex, the majority of interactions in the rhizosphere are mutually beneficial to both plants and microorganisms and are synergistic in character. A further exploration and optimization of these

interactions may lead to significant improvements in crop production (Atlas and Bartha, 1992) . The practical significance of plant inoculation with beneficial asymbiotic rhizosphere bacteria was first demonstrated in Russia during the early and mid 20th century, when greenhouse and field crops were routinely inoculated with bacteria belonging to the genera *Bacillus* and *Azotobacter.* These inoculants were thought to exert a positive influence on plant growth by solubilizing phosphorus (*Bacilus*) or fixing atmospheric nitrogen (*Azotobacter*) (Chanway, 1997) (Table 1.2.).

Some fungi enter into a mutualistic relationship with plant roots called **mycorrhizae** in which the fungi actually become integrated into the physical structure of the root. Mycorrhizal associations exist for prolonged periods with the

Table 1.2. Postulated mechanisms by which asymbiotic soil bacteria stimulate plant growth directly

Mechanism	**Stimulatory effect on plant growth**
Root- associated nitrogen fixation	Biomass and nitrogen content
Production of auxin, cytokinin, or giberellin	Shoot or root biomass; root branching; induce reproductive cycle
Inhibition of plant ethylene synthesis	Root length
Phosphorus solubilization	Biomass and phosphorus content
Sulfur oxidation	Biomass and foliar nutrient content
Increased root permeability	Biomass and nutrient content
Incrased nitrate	Biomass and nitrogen content
Induction of plant systemic resistance to pathogens	Reduced incidence of disease symptoms and mortality in the presence of pathogens

maintenance of a healthy physiological interaction between the plant and the fungus (Safir, 1980). The mycorrhizal associations of fungi and plant roots represent a diverse relationship – both in terms of structure and physiological function- that leads to a nutrient exchange favorable to both partners. Enhanced uptake of water and mineral nutrients, particularly phosphorus and nitrogen, has been noted in many mycorrhizal associations; plants with mycorrhizal fungi are therefore able to occupy habitats they otherwise could not (Atlas and Bartha, 1992).

Symbiotic Nitrogen Fixation in nodules: One of the most important mutualistic relationships between microorganisms and plants involves the invasion of the roots of suitable host plants by nitrogen-fixing bacteria, resulting in formation of a nodule within which the bacteria are able to fix atmospheric nitrogen. The symbiotic fixation of nitrogen is extremely important for the maintenance of soil fertility. In agricultural practices, it is utilized to increase crop yields.

The nitrogen-fixing (diazotrophic) associations of Rhizobia with leguminous plants are of great importance both in global nitrogen cycling and in agriculture.

Pathogenic Associations: Microbial diseases of plants, whether caused by viruses, bacteria, fungi, or protozoa, are of ecologic and economic importance. In a broad sense, microbial diseases of plants cause malfunctions that result in the reduced capability of the plant to survive and maintain its ecological niche. The development of plant diseases due to microbial pathogens normally follows a pattern of initial contact of the microorganisms with the plant, entry of the pathogen into the plant, growth of the infecting microorganisms, and development of plant disease symptoms (Atlas and Bartha, 1992).

1.5. Microorganism-Wheat Interactions

Microbial populations exert their influence on wheat growth. Materials exuded by the roots of wheat plants include a wide range of potential substrates, inhibitors & stimulants. Among soil microorganisms the compounds released by axenicly grown wheat roots, there are volatile compounds (CO_2, ethanol, isobutanol, isoamyl alcohol, acetoin, isobutyric acid), low- molecular weight compounds (sugars, amino acids, vitamins, organic acids, and nucleotides) and high-molecular weight compounds (polysaccharides and enzymes) (Prescott *et al.*, 1993).

The rhizosphere of wheat seedlings contains a significant proportion of bacteria that produce indoleacetic acid (IAA), a plant growth hormone that can increase the growth of plant roots. In mature wheat plants, a lower proportion of microorganisms in the rhizosphere is capable of producing IAA. This may be in response to a decline in root exudate production but it also beneficially corresponds to a decreased need for the growth hormone by the plant. Bacterial populations in the rhizosphere of young wheat plants have been shown to inhibit the growth of pea and lettuce plants. As the wheat plant matures, the proportion of these bacteria decreases and they are replaced by a higher proportion of microorganisms capable of producing growth-promoting substances similar to gibberellic acid (Atlas and Bartha, 1992).

The effect of five mycorrhizal fungi on the growth of 10 wheat cultivars under three phosphorus regimes was assessed in a greenhouse study by Hetrick *et al.*, 1996. Six of the cultivars responded positively, while four responded negatively or were nonresponsive to mycorrhizal inoculation. The responses of the individual cultivars were consistent regardless of inoculum source, suggesting that mycorrhizal responsiveness is an inherited trait rather than a response to individual fungi (Hetrick *et al.*, 1996).

Even though there are positive interactions between wheat and microbial populations, which favor the growth of each other, pathogenic interactions are also present. Leaf rust of bread wheat (*Triticum aestivum L.)*, caused by *Puccinia recondita* is found nearly whereever wheat is grown, and is the most regularly occuring among the other rusts. Wheat cultivars that are susceptible to leaf rust regularly suffer yield reductions of 5-15% or greater, depending on the stage of crop development when the initial rust infections occur. Genetic resistance is the most economical and preferable method of reducing yield losses due to leaf rust. The research on leaf rust has indicated that the responses of wheat cultivars to the pathogen varies (Kolmer, 1996; Liu and Kolmer, 1997) .

Durum wheat (*Triticum turgidum)* cultivars developed by the International Maize and Wheat Improvement Center (CIMMYT) are currently grown on more than 8 million hectares world-wide. Stripe rust (caused by *Puccinia straformis* Westend.*)* is an important disease that can cause significant losses to durum production in cool and humid regions. Frequent stripe rust epidemics have been reported in Iran, Yemen, Egypt, and Turkey. Although a few cultivars in India are known to be resistance to the disease, the area under durum wheat cultivation may have greatly diminished, partly because most cultivars are highly susceptible. Durum wheat germ plasm has been evaluated for stripe rust resistance in various countries, and resistant sources have been reported in land races from Israel and Jordan (Ma *et al.*, 1997). The responses of wheat cultivars to microprojectile bombardment system based genetic modification have also shown variations. The transformation frequency was generally caused by the difference in the *in vitro* culture response with genotype, rather than the efficiency of the introduction of the transgene into wheat cells by particle bombardment (Takumi and Shimada, 1997).

Septoria tritici blotch of wheat (*Triticum aestivum L.*), caused by the fungus *Mycosphaerella graminicola*, is a serious disease in all wheat growing areas of the world and causes severe yield losses. A high level of genetic variation has been reported within *M. graminicola* populations based on restriction

fragment length polymorphisms (RFLPs) , suggesting the potential for the selection of individuals with increased virulence to resistant wheat cultivars. Location-specific adaptation has been demonstrated among isolates of *M.graminicola*, indicating that isolates were more virulent to wheat cultivars in which they had been previously exposed (Ahmed *et al.*, 1996). Virulence genes in the pathogen are necessary, to match the corresponding resistance genes. However, the adaptation of the pathogens to their hosts is influenced by a complex genetic information of the pathogen and the host (Bartos *et al.*, 1996).

Fusarium head blight is one of the severe diseases of small grain cereals. Symptoms of disease are similar in all small grain cereal crops, however, in field experiments with artificially inoculated cereals, differences between cereal species can be observed. *F.avenaceum*, a common pathogen of wheat in Poland may reduce yields and result in the accumulation of significant amounts of moniliformin- a toxic secondary metabolite in the grain (Golinski *et al.*, 1996). The occurrence of moniliformin in grain originating from wheat, rye, triticale, and oat heads infected by *F.avenaceum* was recently reported (Table 1.3.). The concentration of moniliformin in cereal grain damaged by F.avenaceum is still not elucidated, despite the frequent colonization of cereal heads with Fusarium head blight symptoms (Lew *et al.*, 1993). Different wheat cultivars have been tested in inoculated field experiments, for their susceptibility to infection by different Fusarium spp. (Golinski *et al.*, 1996).

As it can be seen from literature records the microorganism-wheat interactions have been studied with the focus on pathogenic relations. This is easily justifiable when the commercial value of the species is considered.

Table 1.3. Occurrence of *Fusarium avenaceum* on wheat and triticale heads with *Fusarium* head blight symptoms in the period 1985 through 1989 (Lew *et al.*, 1993)

	Percentage of heads infected by *F.avenaceum* in total heads collected in a given year	
Year	**Wheat %**	**Triticale %**
1985	36	nt
1986	21	39
1987	21	23
1988	36	83
1989	18	40
Average of 5 seasons	26	46

nt= Sample not available

1.6. Importance of Soil Microorganisms in Sustainable Agriculture

The world's population continues to grow and to require much more food. Attempts by modern high output agriculture to meet this need have led to serious environmental problems. A more sustainable balance is now required and is being sought in a variety of ways (Aldwell, 1997) . When the world move from high-input conventional agriculture, which is production based, to sustainable systems that rely more heavily on nutrient cycling and soil microbial ecology, the elucidation of the complex interactions occuring in soils will be necessary. The best known definition of sustainable development is that of Bruntland (1987), – the needs of the present generation are to be met without compromising those of future generations. For agriculture to be sustainable, a number of essential elements must be met which include: maintenance of soil fertility, economic viability, environmental compatibility and social acceptibility (Aldwell, 1997).

Feasibility of sustainable agriculture is based on the knowledge of the effect of management practices on soil properties and how they affect soil-crop relationships in order to make sound management decisions.

Crop rotation is a key practice within agricultural systems since it affects soil productivity, crop behavior, and need for inputs and is closely related to the economic sustainability of the system. However, long-term conventional tillage-based cropping generally diminishes soil productivity . Conventional cropping initially produces a more intense mineralization of labile fractions, leaving the more recalcitrant fractions as remnant. Microbial biomass N and microbial biomass C are very sensitive to changes in soil management and diminish with the years under cropping (Studdert *et al.*, 1997) . Crop rotation and other ancient agricultural practices have been inappropriately replaced by the use of synthetic fertilizers and pesticides. Today, it is recognized that the old traditional methods have potential for sustainable agriculture (Nieto-Cabrera *et al.*, 1997) . Agricultural practices in the Great Plains are changing slowly from very high-input and intensive systems to approaches requiring less use of fertilizer, energy, and pesticides. Conservation tillage has become commonplace and low-input sustainable agriculture (LISA) is being adopted. Under LISA, legume cover crops are employed to augment soil organic matter, reduce soil erosion, and fix atmospheric nitrogen (O'Leske *et al.*, 1997) .

Sustainable rural development aims to meet the expectations of society, such as a clean environment, healthy food, and a 'exquisite' landscape. The maintenance of biotic diversity, habitats and species in rural areas is one of the focal points of sustainable agriculture and rural development. Although some elements of biotic diversity (natural enemies of pests) have already been specified as important in conventional types of agriculture, biotic diversity received special importance when the production function of agriculture was changed and supplemented by other functions (Kiss *et al.*, 1997).

Microorganisms play a vital role in maintaining and enhancing soil fertility, detoxifying pesticides and other pollutants, and in biological control of agricultural pests. Farmers need to understand the soil and how it functions. Soil consists of five components: mineral particles, air, water, organic matter, and living organisms. Of these components, soil organisms posses the capability to most quickly balance the soil. The Farm For Profit Program in U.S.A works with soil organisms to increase their number and activity which helps them : develop soil structure, cycle nutrients, breakdown organic plant and animal residues, and stabilize soil humus. Soil organisms break down the bulk of organic matter which exists in or enters the soil. That breakdown makes the nutrients stored in the organic matter available to plants. This process forms the basis of nutrient cycling. Throughout the process of decay, different groups of organisms compete for the energy and nutrient sources stored in organic matter. Soil microorganisms convert energy and elements into intermediate by-products that are critical to the development of balanced, stable soil. They include: organic acids that help dissolve soil minerals, hormones, enzymes and other biological chemicals that influence both plant growth and nutrient cycling, fatty acids and waxes that bind soil particles together to form aggregates, and cellulose which consists of stable organic molecules that promote stability of the soil.

Mycorrhizal research is one such area deserving extensive investigation for sustainable agriculture, primarily because mycorrhizal fungi are a crucial link between roots and soil. Vesicular Arbuscular Mycorrhizal (VAM) fungi are generally known to benefit plant health, the net benefit to plant health increasing as stress (particularly, nutrient and water) increases. VAM fungi contribute greatly to phosphorus uptake to nitrogen uptake directly and to nitrogen uptake both directly and indirectly (by increasing N-fixation rates due to improved nutrition) in associations of legumes (Barea *et al.*, 1996).

Rice (*Oryza sative L.)* is the staple in the diet of over 40% of the world's population making it the most important food crop currently produced. One of the most important factors in the generation of high yields from modern rice crops is

nitrogen fertilizer. Without the addition of (fertilizer) nitrogen the yield of the present varieties is drastically limited. While biological nitrogen fixation in wetland rice fields contributes significantly to the long-term fertility of these systems, it is not enough to produce maximum yields. Decreasing the amount of industrially produced fertilizer nitrogen needed in agricultural systems is an important goal of agricultural scientists in general. In the case of sustainable rice production, in particular, one important aim is to replace industrially fixed nitrogen with biologically fixed nitrogen. According to a National Research Council Report (1994), an estimated 100-175 million metric tons of biologically fixed nitrogen is produced annually. Most of this nitrogen is fixed by the legume/*Rhizobium* symbiosis. This is significantly more than the 10 million metric tons produced by lightining and 80 million tons produced in 1989 by industrial processes. Therefore, it is obvious that (symbiotic) biological nitrogen fixation has great potential for supplying nitrogen to crops (Stoltzfus, 1997) .

The limitation of symbiotic nitrogen fixation due to P deficiency restricts the development of a sustainable agriculture, particularly in Mediterranean and tropical soils. Low phosphorus availability is a major limiting factor for plant growth in more than 60% of tropical soils. This affects particularly symbiotic legumes which usually have higher P requirements than non-symbiotic plants (Vadez *et al.*, 1997).The ability to predict long-term plant-availability of soil P provides an additional management tool for sustainable agriculture. Quantifying the long-term availability of soil P provides some measure of potential return on a capital investment of P fertilization in low-input agriculture (Schmidt, 1997). For the expression of the high yielding capacity of modern wheat cultivars a higher P supply is necessary than for low yielding cultivars. Accordingly, concern has been expressed about the low productivity of such cultivars at low nutrient suply and the adjustment of higher available P levels in soils for high yielding cultivars has been advocated (Horst *et al.*, 1996) . The limitation of symbiotic nitrogen fixation due to P deficiency restricts the development of a sustainable agriculture, particularly in Mediterranean and tropical soils (Vadez *et al.*, 1997).

1.7.Environmental problems caused by pesticide , herbicide applications and high-input agriculture

Before man started large scale industrial activities, the concentrations of the organic chemicals on the surface of this planet remained more or less constant. Today the world is faced with certain industrial chemicals that do not readily participate in the global cycles of carbon, nitrogen or sulfur. Such compounds cause problems of disposal and may, if they escape containment, lead to adverse effects on the environment. Chemicals exhibiting transitory or permanent accumulation have been termed "pollutants" or "environmental pollutants", exspressions which stress their undesired effects on the environment .

The majority of the novel pollution phenomena involve organic chemicals called xenobiotics, chemicals synthesized by humans that have no close natural counterparts. These xenobiotic chemicals include pesticides, plastics, and other synthetics, some of which persist because transport mechanisms and catabolic pathways for them have not evolved (Atlas and Bartha, 1992) .

Agricultural uses of pesticides result in accumulation of these chemicals in groundwater, lakes, streams, and estuaries. Although the concentrations of the chemicals required to cause acute toxicity in humans would generally be higher than that of found in the environment, the recalcitrance of the compound in local areas pose a potential danger in time (Charpalamadugu and Chaudry, 1991) .

The use of high levels of nitrogen fertilizers in crop production has several drawbacks. Most nitrogen fertilizer is produced via the Haber-Bosch process. This process requires large amounts of natural gas, coal, or petroleum, all non-renewable energy sources. In addition, it produces CO_2, a gas implicated in the greenhouse effect. The chemical production of nitrogen fertilizer is also expensive, and in developing countries the additional costs often exceed the means of farmers, limiting the yields potential of their crops. Once chemical fertilizers are applied, additional problems can arise. Roughly one third of the

nitrogen applied is used by the crop. The non-assimilated nitrogen from farming systems has been implicated in nitrate contamination of ground water supplies, a potential health hazard. In addition, excess nitrogen can also lead to production of nitrous oxide (N_2O), a potent "greenhouse" gas. Therefore, crop systems requiring large additions of fertilizer nitrogen are non-sustainable systems, since they require the use of non-renewable natural resources and can contribute to health hazards and environmental pollution (Stoltzfus, 1997) .

Major environmental problems arise within the European Union (EU) from the excessive use of nitrogen compounds. Amongst these are: acidification of forests and natural ecosystems through atmospheric deposition of nitrogen (N) compounds, eutrophication of inland waters and coastal seas mainly by organic N and nitrates, contamination of groundwater by nitrates, and a contribution to the global warming problem by dinitrogen oxide, N_2O. (Van Der Voet *et al.*, 1996) . Since the goal of obtaining maximum profits may conflict with the goal of minimizing environmental damages, the agricultural research community is challenged to develop profitable cropping systems that incorporate reduced tillage as well as reduced dependence on fertilizer and herbicide inputs (Kelly *et al.*, 1996) .

Intensification of agriculture over the last few decades (fertilisation, pest management, specialisation and concentration in production) is regarded to have caused a rapid decline in plant and animal species diversity (Gutsche and Rossberg, 1997) . However,management of soil microorganisms becomes critical to the maintenance of good soil health because microorganisms are involved in many important functions such as soil formation, toxin removal, and elemental cycles of carbon, nitrogen, phosphorus, and others (Borneman *et al.*, 1996). Even though the promotion of microbial diversity alone is said not to eliminate the need for improvements in farming skills and high dependence on fertilizers and pesticides, it , however, contributes to the development of diverse systems in terms of plant and animal species able to sustain production on both marginal lands as well as in more fertile areas.

1.8. Xenobiotics and Soil Microbes

Once applied in the environment, xenobiotics are subjected to photochemical, chemical, and biological effects capable of causing transformations in the compound's chemical structure. Biological and non-biological processes may work together to degrade xenobiotics. In nature it is diffucult to distinguish between the two modes of degradation in most cases. Though some reactions are clearly non-biological, such as photolysis, others, such as hydrolysis, can be either non-biological or biologically maintained.

Mineralization or ultimate biodegradation of an organic molecule in water and soil is almost always a consequence of microbial activity. Whether or not a xenobiotic is adsorbed, absorbed, activated, inactivated, persistent, short-lived, mobile, stationary, or will eventually constitute a residue problem may depend upon its transformation by soil microorganisms.

Microbial degradation of pesticides in the soil is a natural process. It is one of the main mechanisms not only in detoxification of the pesticides but also responsible for their removal from the environment. Processes that cause a rapid breakdown of pesticide contribute to maintaining a safe environment.The rate, extent and mode of degradation of pesticides are biologically controlled. However, these processes also affect the performance of the pesticide. Any interference with soil microbial activity may affect both, the persistence and the effectiveness of the pesticide. Also, in certain cases, the pesticide may only be partially degraded to one or more toxic products introducing, in turn, further contamination to the environment (Goulding *et al.*, 1988) .

In the transformation of pesticides, microbial communities play a significant role in bringing about complete mineralization. In fact, mixed microbial populations can account for the complete mineralization of a chemical if an easily utilizable carbon and energy source is supplied, apparently via a cometabolic pathway. Among the microbial communities, bacteria and fungi are

the major transformers/degraders of pesticides and their breakdown products. Until recently, research into pesticide degradation by microorganisms has focused primarily on bacteria: fewer studies have been performed with fungi, actinomycetes, cyanobacteria, etc. The major reasons for this may be because bacteria are easier to culture and grow than fungi; they are more amenable to genetic manipulations (Kumar *et al.*, 1996) .

The development of bacteria to degrade chlorinated hydrocarbons under anaerobic conditions, therefore, has potential for biotechnological breakthrough. The demonstration that white-rot fungi, which are known to degrade lignins, also can metabolize the toxic, very resistant, organic polychlorinated biphenyls (PCBs) is a further example of the diversity of soil microflora. It also is an example of the application of biotechnology in its broader sense to environmental problems (Paul and Clark, 1989) .

The lignolytic enzymes catalyze the critical first transformation step of the pollutants, the prerequisite for transformation of xenebiotics in soil is the transport into, or the production of, the lignolytic enzymes in soil. Nevertheless, it is not known if lignolytic enzymes of white rot fungi are present and active in soil (Lang *et al.*, 1997) . The development of effective, low-cost fungal inocula that are engineered to maintain inoculum potential during transport and application is one of the keys to the commercialization of fungal soil remediation (Lestan and Lamar, 1996).

Creosote, a widely used wood preserving agent, is a mixture of approximately 200 compounds, of which 85% are polycyclic aromatic hydrocarbons (PAHs), 10% are phenolics, and 5% are N-S-O heterocycles. When present in groundwater, creosote compounds pose a threat to public health because many are highly toxic and/or carcinogenic. The biodegradation of PAHs has been reported to be catalyzed by a wide variety of bacteria, fungi, and algae (Hosein *et al.*, 1996).

The tfdA gene encodes 2,4-dichlorophenoxyacetic acid (2,4-D)/α-ketoglutarate dioxygenase; this catalyzes removal of the acetate side chain of 2,4-D, yielding 2,4-dichlorophenol. Thus far, the tfdA gene is uniquely associated with 2,4-D degradation and is most often found with the other tfd genes that encode the enzymes necessary to complete the degradation of 2,4-D. The evidence for tfdA or TfdA-like activity has been found in diverse phylogenetic groups of bacteria (Hogan *et al.*, 1996).

Recent studies have shown that pesticides and herbicides can decrease microbial respiration, biomass, and diversity. Genetic diversity is essential to life, since it permits adaptation through the formation of new organisms by genetic transfer and mutations (Borneman *et al.*, 1996) . The soil microbial community can change its characteristics upon exposure to complex organic molecules. After the community's repeated exposure to a given chemical, faster rates of degradation may occur. A microbial community can become so efficient at rapid herbicide decomposition that herbicide effectiveness is diminished (Prescott *et al.*, 1993) .

Herbicides and foliar insecticides applied at field rates seldom reach the soil at sufficient concentration to cause direct injury to soil organisms. The nitrifying bacteria are the most susceptible, and inhibition of nitrification is occasionally observed following a foliar application of a biocide. Rhizosphere populations of nonpathogenic and asymbiotic organisms may be either increased or decreased, depending on the pesticide employed and its rate of application (Paul and Clark, 1989).

1.9. Effects of Aldicarb on Soil Microorganisms

Between the 1940s and 1970s, pesticide use increased almost 40-fold, with new products such as organochlorines (e.g. chlordane) becoming prominent. The carbamate aldicarb is manufactured with the trade name Temik^{TM} as an insecticide, as well as an acaricide and nematicide (Moore *et al.*, 1998).

Carbamates possess the broadest spectrum of biological activity. 2,4-dichlorophenoxy acetic acid (2,4-D), carbamates (such as aldicarb), and trifuralin are the ones which constitute the major pesticides currently in use in Turkey. The development of accelerated biodegradation of carbamates, resulting in reduced performance of pesticide, a concern for carbamate insecticides such as, carbayl (Racke and Coats, 1988), aldicarb (Suet *et al.*, 1987) and carbofuran (Chaudry and Ali, 1988) which are used against several soil-borne pests of root crops. Aldicarb solubility in water is about 0.6% (w/v) at room temperature and the compound is considered to be sufficiently soluble in soil (Charpalamadugu and Chaudry, 1991).

Aldicarb being one of the most potentially toxic compounds has been used on tobacco, sugar beet, sugar cane, potatoe, and peanut for the control of aphids, thrips, mealybugs, white flies, mites, and nematodes (Charpalamadugu and Chaudry, 1992). The systematic properties of aldicarb enabled it to be used for the indirect control of viral diseases as well as for phytophagous nematicide. In general, it exerts its effect as an insecticide, nematocide, acaricide, as well as being a growth regulator (Hassall, 1990).

Although microbial degradation of aldicarb (AS) is poorly understood, it has been shown to be oxidized to ASO and ASO_2, and hydrolyzed to AS oxime and nitrile (Ou *et al.*, 1985). Attempts have been made to isolate microorganisms responsible for AS degradation in soil. Several fungal isolates of *Fusarium* and *Penicillum*, and bacterial isolates of *Arthobacter, Pseudomonas, Nocardia, Achromobacter,* and *Bacillus* have been isolated from AS treated soil (Read, 1987). The fungal isolates of *Fusarium* and *Penicillum* was shown to metabolize AS slowly. Bacterial isolates, however, collectively degraded pesticide rapidly. The degradation of AS appeared to be concentration dependent. Concentrations of pesticide higher than 800 and 5000 ppm was shown to inhibit bacterial and fungal growth, respectively (Charpalamadugu and Chaudry,1992). The hydrolysis of AS under anaerobic conditions stimulated methanogenesis, products of AS

degradation were utilized as a source of energy by the methanogenic bacteria (Kiene and Capone, 1986).

The heavy use of AS creates problem locally in southern Turkey since, in this region AS is used as the major pesticide against cotton pests. It is therefore aimed to substantially reduce the pollution by using biological treatment systems, to be more specific by biodegradation. Through enrichment experiments thirty morphologically distinct bacterial strains were isolated in pure form which can utilize Temik^{TM} AS as the sole source of carbon and energy. The characterization studies showed that the most efficient bacterial isolate were aerobic, Gram negative, slightly curved rods that occurred singly and in pairs (Halıcıgil, 1995).

1.10. Biological Control: Alternative to Xenobiotics

The high application rates of pesticides to much of the agricultural land could be lowered and in some cases eliminated through better biological control mechanisms. These include a variety of techniques, such as integrated pest management, altered tillage practices, and the development of more pest-resistant plants. Genetically altered microorganisms utilized as biological control agents and newer methods of breeding plants would be part of a management scenario based on a better understanding of soil microbiology and biochemistry (Paul and Clark, 1989).

As a consequence of increased public concern for environmental damage caused by continued use of conventional synthetic agrichemicals, sustainable agriculture has gained great importance. Control or integrated management of weeds and plant diseases is viewed as a component of sustainable agriculture. During the past decade considerable research activities in agriculture have been directed toward biological control of plant diseases and weeds (Norman, 1998) .

The first requirement in terms of biological control of soilborne pathogens is to be able to apply and subsequently establish adequate antagonistic organisms

at the proper place for example, on the root system which is to be protected. All the biological products presently available against soilborne pathogens are listed in Table 1.4. The commercial preparations containing the antagonists may be added to soils and particularly soilless substrates to prevent colonization by the pathogens, of the soil and root system, thereby reducing their infection and survival rates. Treating the crop remains also contributes to reducing the rate of survival of the pathogens as well as decreasing their potential role as a substrate base for pathogen's development. The pretreatment of soils with a non-pathogenic *Fusarium spp.* isolate has been reported to efficiently protect moderately susceptible species grown in containers against wilting (Fokkema, 1996) .

Table 1.4. Biological control products presently available for use against soilborne pathogens

Trade name	**Microorganisms used**	**Target species**
Soil Gard	*Gliocladium virens*	*Rhizoctania,Pythium*
Mycostop	*Streptomyces griseoviridis*	*Fusarium, Pythium*
Gliomix	*Gliocladium* spp.	growth promoter
Vaminoc	VA- mycorrhizal inoculant	growth promoter
Rotstop	*Phlebiopsis gigantea*	*Heterobasidion annosum*
Trichopel, Trichoseal Trichoject	*Trichoderma* spp.	seedling disease, silverleaf
Polygandron	*Pythium oligandrum*	Pythium
Kodiak/Epic	*Bacillus subtilis*	*Pythium, Rhizoctania, Fusarium*
Nogall (Galltrol, Bacterose) and others	*Agrobacterium Radiobacter* K 1026, K 84	*Agrobacterium tumefaciens*
Biocoat	*Pseudomonas fluorescens*	growth promoter *Fusarium*
Dutch Trig	*Verticillium dahliae*	Dutch elm disease

There is evidence that siderophore-producing pseudomonads can be used in the biological control of soilborne plant pathogens. In alkaline soils of low iron availability, the pseudomonad may deprive the pathogen of its iron environment (Paul and Clark, 1989). Some of the microorganisms found in soil are insect pathogens, which are potentially useful for pest control. *Bacillus thuringiensis*, for example, is a soil bacterium that is pathogenic to the larvae of many insects; it is now used in their control. It produces intracellular crystals of toxic glycoproteins when it sporulates. Commercial preparations containing endospores and crystalline toxin of this bacterium are sold in gardening supply shops to be sprayed on plants (Tortora *et al.*, 1994) .

Some bacteria also reduce disease severity by inducing systemic resistance to plant pathogens. Control of *Fusarium* on pine seedlings by bacterial inoculation was first described 4 decades ago by Krasilnikov (1958), but subsequent reports of biological control agents for tree species are comparatively rare. Development of inoculants with biocontrol properties may be very useful application for root-associated bacteria in forest regeneration. However, the potential of such inoculants in forestry is only just beginning to be explored (Chanway, 1997) .

The biopesticide concept was introduced and over the following century research on potential biological control products would diversify to include other microbial parasites and antagonists, nematodes and macrobiologicals, that is , predators and parasitoids. Data collected by Lisansky suggest that there were, in 1993, 177 microbial products (most of these formulations of *Bacillus thuringiensis)* and over 107 registered macrobiological products, e.g. predators, parasitoids and nematodes. The concept of applying a biological pesticide which has a long term, suppressive effect on a pest in a particular site is more popular in research on the biological control of plant diseases where fungi or bacteria may establish on vulnerable plant surfaces like roots or leaves and act as competitors, antagonists or parasites which prevent invasion by disease organisms (Waage, 1996).

Microbial metabolites have become the focus research for natural product alternatives to conventional pesticides. Soil microorganisms produce a wide range of bioactive compounds that are potentially phytoxic to higher plants. Several microbial metabolites with herbicidal potential have been isolated (Hokkanen and Lynch, 1995). Phenolic compounds constitute one of the most widespread group of secondary metabolites of higher plants. They exert an allelochemical influence in metabolic aspects of insect-host-plant specifity and they play an important as plant defensive chemicals with protective functions against insect pests (Havlickova *et al.*, 1996).

The application of beneficial bacteria for biological control of plant diseases or bioremediation on a commercial scale requires the release of large numbers of wild-type or genetically modified strains into the environment. Concern about the ecological safety of such applications has been raised (Natsch *et al.*, 1996). Biological control of leaf diseases by epiphytic bacteria is one of the goal of numerous biocontrol research programs (May *et al.*, 1996). Crop rotation has been a successful method for suppressing plant-parasitic nematodes (McSorley and Gallaher, 1997).

In addition to microbial toxins that suppress weeds, biological interactions include those that limit harm from plant pathogens. The effectiveness of pathogenic infections may be diluted by allelochemicals that diffuse from the host plant; alternatively, microbial allelochemicals (antibiotics) can limit damage from disease organisms (Einhellig, 1996). Beneficial effects of allelopathy were reported (Rice, 1986). Alfalfa residue added to soil was reported to stimulate growth of cucumber, lettuce and several other species. However, exploitation of allelopathy in weed management strategy and in growth regulation remains a major challenge to agricultural science. Crop residues may be managed to help suppress certain weeds in agroecosystems (Mallik and Watson III, 1997). Shilling *et al.* found rye mulching effective for weed suppression in an agroecosystem. Sorghum residues incorporated into soil inhibited weeds and enhanced growth of snapbeans (Shilling *et al.*, 1985).

Agriculturalists have become aware that sustainable agriculture practices are regarded as the savior of the next milenium. Sustainable agriculture practices takes its roots from optimal management of soil microorganisms. Soil microorganisms with their diverse metabolic processes play the major role in the biogeochemical cycling of minerals. Managing the soil microbes cuts down and even eliminates the chemical fertilizer costs and keeps the soils fertile. Therefore the selection of the cultivars to be grown in certain areas should depend on how well they interact with the soil microbes.

Current literature contains a wast amount of information on the interactions of microbes and plants. Up to now wheat-microorganism interactions have been focused on pathogenic relations due to the commercial importance of the crop. The main concern of this study was to investigate if there are differences between wheat cultivars on influencing soil microorganisms. This was achieved by doing initial counts and measuring the response of soil microorganism populations associated with cultivars to different carbon sources. The results of this study were discussed in terms of the selection of cultivars to be used in sustainable agriculture practices.

CHAPTER 2

MATERIALS & METHODS

2.1. Materials

KH_2PO_4 (from Merck)

$MgSO_4.7H_2O$ (from Merck)

$CaCl_2.2H_2O$ (from Merck)

NaCl (from Merck)

$FeCl_3$ (from Merck)

KNO_3 (from Merck)

Glucose (from Sigma)

Glutamic acid (from Sigma)

Serine (from Sigma)

Arginine (from Sigma)

Glycine (from Sigma)

Aldicarb (from Temik, kindly provided by Prof. Dr. Gürdal Alaeddinoğlu)

Rose Bengal (from Sigma)

Crystal Violet (from Carlo Erba Reagent)

Pimaricin (from INC Biomedicals)

Chloramphenicol (from Sigma)

Agar (from INC Biomedicals)

Nutrient Agar (from Difco laboratories 23)

dH_2O

pH meter

Disposable petri dishes

150ml. Erlenmeyer flasks

Orbital shaker (G24 Environmental Incubator Shaker New Brunswick Scientific Co.Inc.)

Incubator (NEL NR 900)

Laminar Flow Hood (from Labcaire System Ltd.)

2.2. Methods

The soil samples were obtained from the experimental sites of (CRIFc) Central Research Institute for Field Crops, Breeding & Genetics Department, Wheat Unit in Haymana, Ankara, in 1996. In the experimental sites Gün, Gerek, Bolal, Bezostaja, Kıraç, Kırkpınar and Lancer wheat cultivars were planted as single rows side by side with unplanted seperation lanes between the cultivar rows. The soil samples were obtained shortly after harvest from 20 cm depth. Three samplings were done for each cultivar rows and lanes between the rows. The soil samples were transferred to brown paper bags and placed in plastic bags. Several holes were punched on plastic bags to ensure proper aeration during transport. The soil samples were stored at room temperature in paper bags for about four months before experiments, for the equilibration of the soil microorganisms. Soils were dried and the experiments were carried out on soil microbe populations resistant to desiccation.

2.2.1. Preparation of Soil Suspensions

The soil samples were taken from fields where seven different wheat cultivars were planted, which are namely Bezostaja, Bolal, Gerek, Gün, Kıraç, Kırkpınar and Lancer, at Wheat Research Farm of CRIFc in Haymana, Turkey. The investigated seven wheat cultivars differ in the degree of their drought tolerance. Kırkpınar, Bezostaja, and Gün cultivars have low degree of drought tolerance, Bolal cultivar has semi degree of drought tolerance and Lancer, Kıraç, and Gerek cultivars have high degree of drought tolerance.

The collected soil samples were placed into 150 ml sterilized Erlenmeyer flasks capped with aluminum foil (10 g soil per flask) followed by addition of 20 ml sterile distilled water. The flasks were placed on an orbit shaker at 200 rpm

30°C for 1 hour and later serial dilutions were made on the known weight of dry soil up to 10^{-5} fold.

2.2.2 Medium Preparation

The media used in this study were prepared according to Blum and Shafer (1988).

2.2.2.1 Preparation of BMS Medium

1 g KH_2PO_4, 0.4 g $MgSO_4.7H_2O$, 0.13 g $CaCl_2.2H_2O$, 0.1 g NaCl, 0.01 g $FeCl_3$, 0.5 g KNO_3, 1 g glucose and 1lt dH_2O were put into the glass bottle and mixed. The pH of the medium is adjusted to pH 7.0. 1.5 g agar per 100 ml of distilled water were added and the medium in the bottle is autoclaved for sterilization. After autoclaving, 20 µl of pimaricin that is required for prevention of fungal growth was added into the bottle in a laminar flow hood under sterile conditions. Then the medium was poured into the disposable petri dishes again in a laminar flow hood. Foaming and air bubbles on the surface of the agar medium in the plates were prevented with bunsen burner flame. After the agar medium was solidified, the plates became ready for inoculation of the soil suspensions.

2.2.2.2 Preparation of ACT Medium

The same processes were repeated for the preperation of ACT medium with the omission of glucose.

2.2.2.3 Preparation of FGI Medium

The medium ingradients were the same as ACT plus 0.05 g Rose Bengal dye. Here, instead of pimaricin (fungal growth inhibitor) , 2 ml of 0.16 M chloramphenicol was added per liter of medium to supress of bacterial growth.

2.2.2.4 Preparation of BNA Medium

23 g nutrient agar (from Difco laboratories), 1 lt of distilled water were put into the appropriate volume of a glass bottle and the pH was brought to the pH 7.0. Then the medium in the bottle was sterilized in autoclave. After sterilization, the fungal growth inhibitor pimaricin (20 µl per 1000 ml dH_2O) was added into the medium in a laminar flow hood.

2.2.2.5 Preparation of GNB Medium

23 g nutrient agar, 0.004 g crytal violet (selective dye for Gram negative bacteria) and 1 lt of distilled water were put into a bottle. The pH of the medium was adjusted to the pH 7.0. Followed by the sterilization in autoclave, 20 µl of pimaricin was added.

2.2.3 Preparation of Chloramphenicol "bacterial growth inhibitor"

100% ethanol is diluted to 80% ethanol solution. Then 0.05 g of chloramphenicol powder was added per ml of 80% ethanol solution.

2.2.4 Enumeration of Soil Microorganisms

For initial counts, soil samples representing within and between the rows of the wheat cultivars were removed from paper bags. They were mixed throughly. In addition to soil samples taken from wheat cultivation sites, a soil from an unrelated site was included in the study. This soil was sample from Çubuk, Ankara from the hill slopes under wild vegetation and termed as "wilderness" soil. The soil was seperated from peble stones and large root particles by sieving through 5 & 2 mm sieves. Siefted soil was mixed again throughly and 10 gr subsamples were weighed. These 10 gr soil samples were transferred to 150 ml erlenmayer flasks containing 20 ml of sterile distilled water. The samples were kept at orbital shaker (New Brunswick) at 30° C for one hour.

Followed by the removal from orbital shaker incubator the solutions were allowed to stand about half an hour for precipitation of large soil particles. Then, the suspensions were serially diluted ten times each stepwise between 10 and 10^6 fold.

100µl diluted soil suspension on selective agar media (described above) were spread with a specifically shaped alcohol & flame sterilized glass spreaders and kept in an incubator (NEL NR 900) at 28°C for 6 days in darkness before colony count. At each successive serial dilution 3 replicate inocula were employed.

After the initial counts were taken; soil samples belonging to 7 different wheat cultivars were again placed into 150 ml sterilized Erlenmeyer flasks (10 g soil per flask) . Soil samples were amended with glucose or glutamic acid (0.5 mg/g soil). The carbamate pesticide "aldicarb" was amended in solution form (1 ml 4000 ppm/flask = 0.4 mg/g soil) (Table 2.1.). The flasks were capped with aluminum foil and the amendments were mixed into the soil by shaking the flasks. To moisten the soil, 1 ml of sterile distilled water was added into the flasks except for the flasks containing aldicarb. Control soil received only water. Then flasks containing soil samples with the amendments were incubated in the dark for 3, 5, 10, 15, and 22 days. 20 ml of sterile distilled water was added to each flask at 3, 5, 10, 15 and 22 days. The flasks were placed on an orbital shaker (G24 Environmental Incubator Shaker New Brunswick Scientific Co.Inc.) at 200 rpm. 30°C for 1 hour and serial dilutions were made up to 10^{-6} fold stepwise. At each successive serial dilution, similarly, 100µl of inocula were placed in each of three replicate plates. Then the petri plates were kept in an incubator (NEL NR 900) at 28°C for 6 days in the dark before counting the colonies. After 6 days incubation, single colonies on selective media were counted & each colony was equated with a single propagule in the soil suspension and the term "cfu" (colony forming unit) was used.

In the third part of the study, "Bolal" wheat cultivar was chosen for analysing the effects of amino acids on the microbial growth. Here, other than

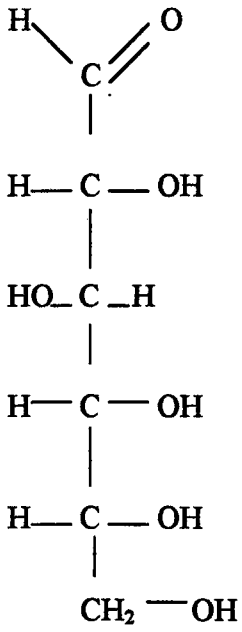
glutamic acid , which is an acidic amino acid, three more amino acids were used. These amino acids were glycine (neutral nonpolar), serine (neutral polar), and arginine (basic). Structural formula of amino acids and other carbon sources used in the study are given in Figure 2.1. The amounts, moles and amount of carbon that the carbon sources used in the study are presented at Table 2.1. Soil samples from Bolal cultivar were supplied with these amino acids and the samples were incubated at room temperature in dark for 3, 5, 10, and 15 days and the same procedures described in the second part of the study were repeated.

Table 2.1. Mass, number of moles and the amount of carbon for each carbon source investigated in the experiments

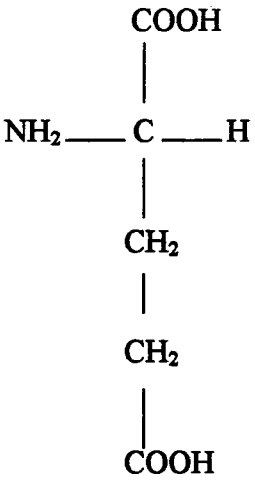
CARBON SOURCES	AMOUNT	MOLES	AMOUNT OF CARBON
Glucose	5 mg	2.78×10^{-5}moles	2.0×10^{-3}gram
Glutamic acid	5 mg	3.4×10^{-5}moles	2.04×10^{-3}gram
Serine	5 mg	4.76×10^{-5}moles	1.71×10^{-3}gram
Arginine	5 mg	2.87×10^{-5}moles	2.07×10^{-3}gram
Glycine	5 mg	6.67×10^{-5}moles	1.6×10^{-3}gram
Aldicarb	4 mg	2.1×10^{-5} moles	1.76×10^{-3} gram

2.4. Statistical Analysis of Data

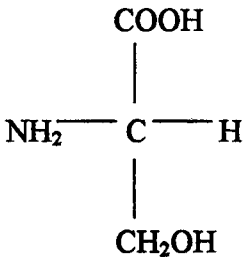
The data obtained from colony counts transferred into Microsoft Excel files in Office 97 Version. Statistical analysis of the data was carried out by using the method of two-way ANOVA with three replications. The results were interpreted according to Sokal (1981).



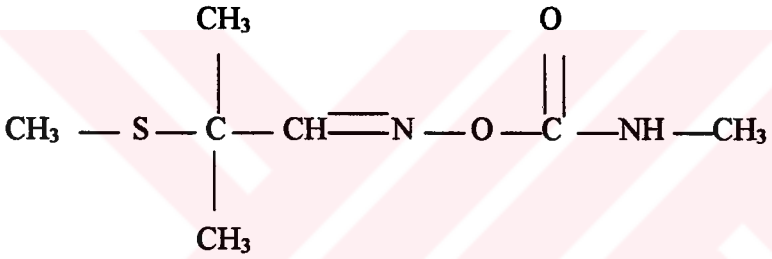
D-Glucose



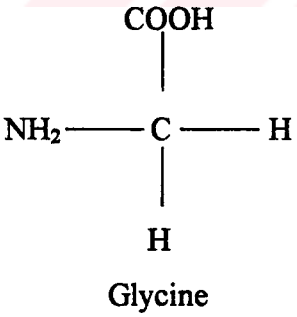
Glutamic acid



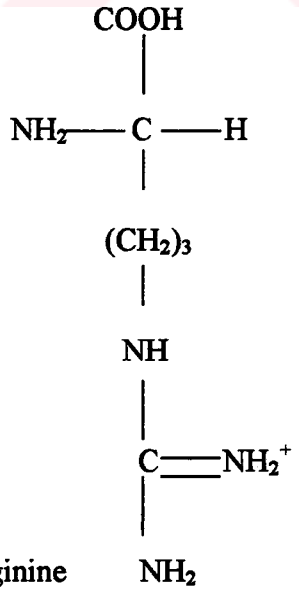
Serine



Aldicarb



Glycine



Arginine

Figure 2.1. Structural formula of carbon sources used in the study.

Figure 2.2 The wheat field of (CRIFc) in Haymana from which the soil samples were taken

CHAPTER 3

RESULTS

3.1. Features of colonies growing on the surface of selective media

In this study, five different selective media, which are namely BMS , BNA, ACT , GNB and FGI were used. BMS is a defined mineral salts medium. It favored mainly the growth of general K strategist bacteria which depend upon physiological adaptations to the environmental resources or carrying capacity of the environment and reproduce more slowly than R strategists. Thus, they tend to be successful in resource limited situations. The representative colonies growing on BMS agar medium were shown in Figure 3.1.1

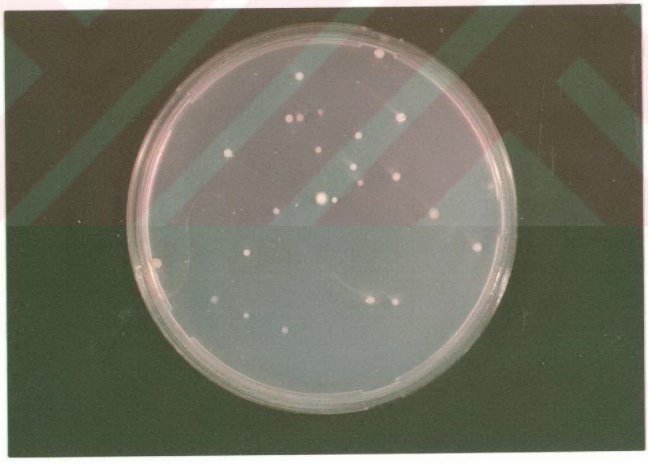


Figure 3.1.1 Bacterial colonies grown on BMS medium

BNA, however, is a rich medium containing complex carbohydrates, proteins, vitamins and amino acids. This rich medium might have favored the growth of R strategist bacteria. Sergei Winogradsky's zymogenous (opportunistic)

soil populations closely correspond to the concept of R strategy. They tend to be prevail in situations that are not resource limited, that is, where nutrients are not severely limiting. Figure 3.1.2 exemplifies the bacterial populations cultured on BNA media.

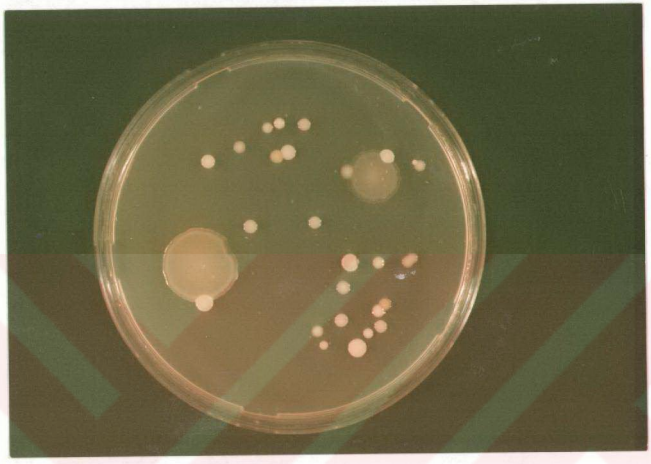


Figure 3.1.2 Bacterial colonies grown on BNA medium

ACT media promoted the growth of actinomycete populations, which are aerobic, gram-positive bacteria that form branching filaments or hyphae and asexual spores, in our soil samples taken from seven different wheat cultivar rows. The representative colonies of actinomycete populations were shown in Figure 3.1.3 .

GNB media allowed only the growth of Gram-negative bacteria which contain lipopolysaccharide as part of their cell wall complex. Lipopolysaccharide occurs in the cell walls of Gram-negative bacteria and occurs in a relatively constant proportion. Crystal violet favored the growth of Gram-negative bacteria by inhibiting the growth of Gram-positive bacteria without affecting Gram-negative organisms. Figure 3.1.4 shows representative Gram-negative bacterial colonies on the agar medium.

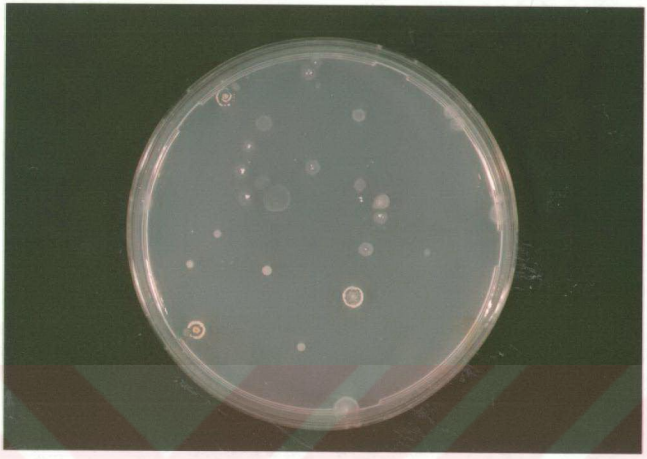


Figure 3.1.3 Actinomycete colonies grown on ACT medium

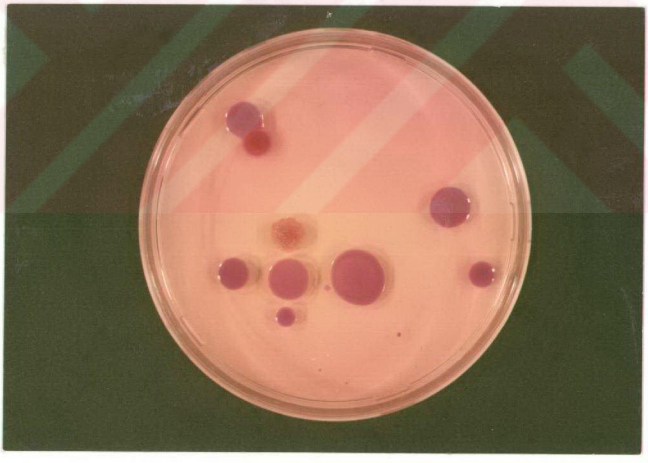


Figure 3.1.4 Gram negative bacterial colonies grown on GNB medium

FGI media were used for enumeration of soil fungi. Of the soil organisms, the fungi as a group are the organotrophs primarily responsible for the

decomposition of organic residues. Figure 3.1.5 represents fungal populations in the soil samples taken from the rows of the wheat cultivars investigated.

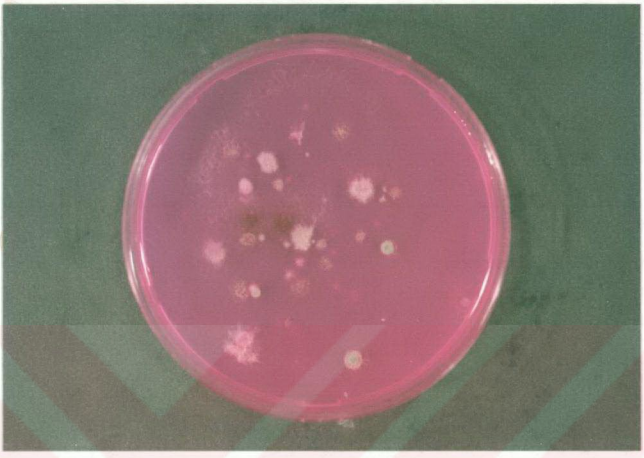


Figure 3.1.5 Fungal colonies grown on FGI medium

3.2. Initial Counts of Microbial Populations associated with Seven Different Wheat Cultivars

The comparison of the initial counts obtained from the soil samples taken from seven different wheat cultivar rows were presented as bar graphics.

The numbers of bacterial populations cultured in BMS medium were graphed in logarithmic scale. The greatest number of the bacterial populations on BMS media was recorded in Bolal wheat cultivar row. The lowest number of colonies was recorded in Kıraç wheat cultivar row (Figure 3.2.1).

Actinomycete population numbers were also presented in logarithmic scale. Similarly, Bolal was the most effective cultivar on the growth of actinomycete populations compared to other wheat cultivars. The lowest number

of actinomycetes was again recorded in soil samples taken from Kıraç wheat cultivar row (Figure 3.2.2).

The bacterial populations grown in BNA medium was maximum in Bolal wheat cultivar which is in agreement with the previous cases. Also the lowest number similarly was recorded for Kıraç (Figure 3.2.3).

Gram-negative bacteria reached the highest number in soil associated with Lancer wheat cultivar (Figure 3.2.4) whereas interestingly the greatest fungal population was present in Kıraç cultivar. This result suggested that Kıraç cultivar could promote fungal populations instead of bacterial populations in the soil as far as the culturable populations were concerned (Figure 3.2.5).

The counts representing between the rows of wheat cultivars often times were lower than that of within row counts. This trend was reverse in the case of Gram negative populations (Figure 3.2.4).

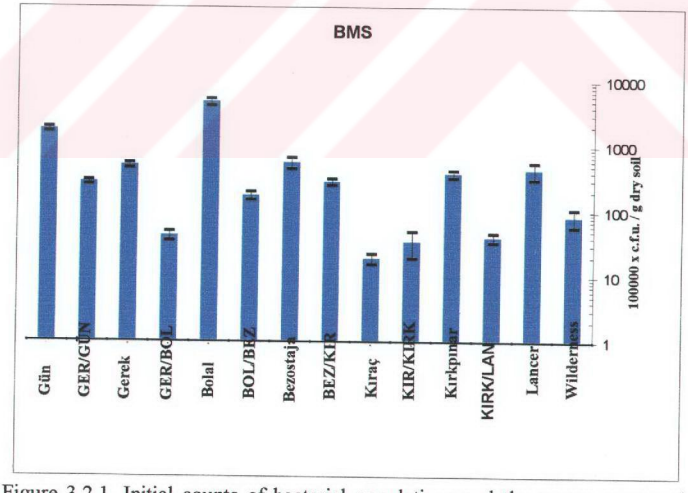


Figure 3.2.1. Initial counts of bacterial populations and the counts representing between the rows of the cultivars cultured in BMS medium

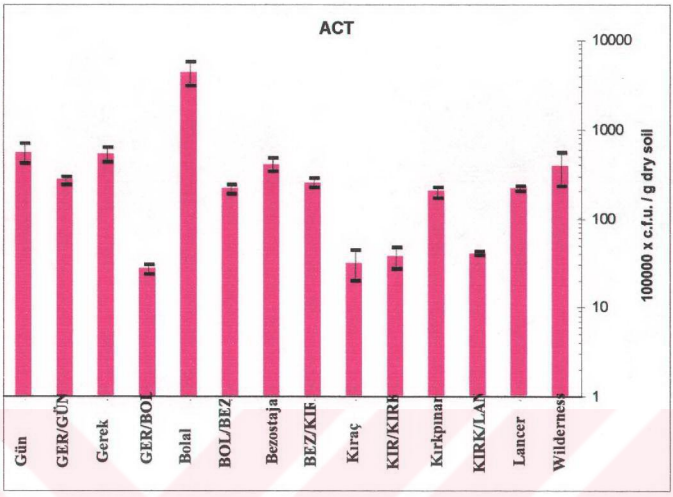


Figure 3.2.2. Initial counts of actinomycete populations and the counts representing between the rows of the cultivars cultured in ACT medium

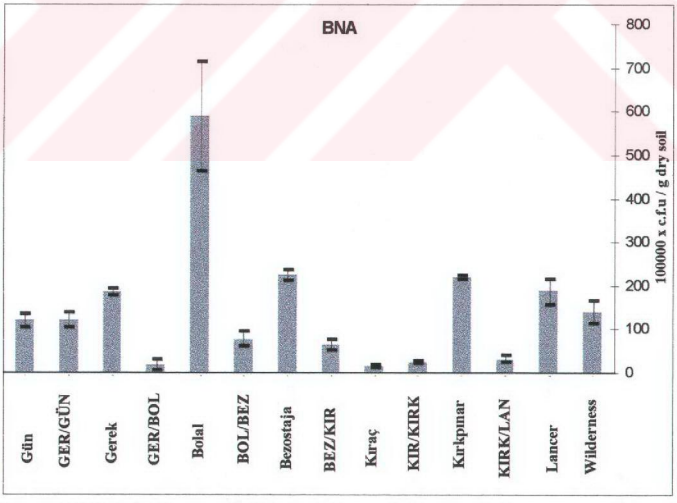


Figure 3.2.3. Initial counts of bacterial populations and the counts representing between the rows of the cultivars cultured in BNA medium

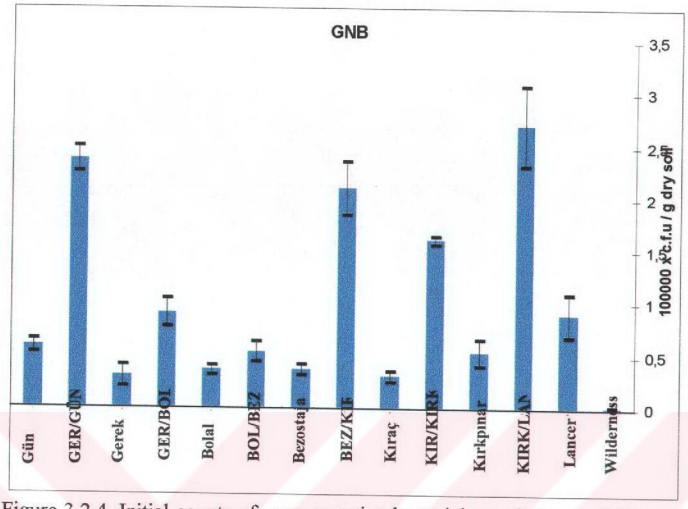


Figure 3.2.4. Initial counts of gram negative bacterial populations and the counts representing between the rows of the cultivars cultured in GNB medium

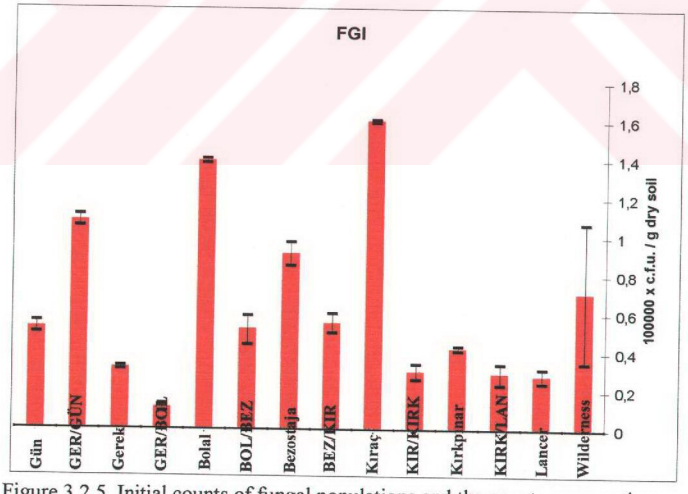


Figure 3.2.5. Initial counts of fungal populations and the counts representing between the rows of the cultivars cultured in FGI medium

3.3. Growth Responses of Soil Microbial Populations in Wheat Cultivar Rows to Different Carbon Sources

Growth responses of soil microbial populations to amendments of aldicarb, which is a carbamate pesticide, glucose and glutamic acid were measured. Effect of moisture on the growth of microbe populations for soil under different wheat cultivars was assessed with the control set, in which 1ml of dH_2O was added to flasks with other amendments.

In this part of the study, colony counts were obtained for the soil samples collected from the soil associated with seven different wheat cultivars, which are Kırkpınar, Gerek, Gün, Bolal, Lancer, Kıraç & Bezostaja. They were mixed with the amendments and held at room temperature in the dark for 3, 5, 10, 15 and 22 days.

For Kırkpınar wheat cultivar, the soil bacterial population cultured on BMS medium gave the highest colony counts with glutamic acid amendment. At 22 day of the entire period the greatest peak was reached. Glucose addition did not promote the growth of bacterial populations as efficiently. A gradual increase was observed until 5^{th} day. On the following days the numbers gradually declined till the end of the 22 days period (Figure 3.3.1.).

The bacterial populations grown on BNA medium as in the bacterial populations cultured on BMS medium increased their number most effectively with glutamic acid addition. Even though gradual decrease and gradual increase were observed up to 15 day, a sharp increase was observed between the days 15 and 22 on which the highest peak was recorded. Here, glucose similarly did not stimulate the growth of bacterial populations cultured on BNA medium as efficient as glutamic acid. Aldicarb caused a gradual increase in the population number between the days 10 and 15 followed by a gradual decline until the end of the period (Figure 3.3.2.).

Actinomycete populations responded well to glutamic acid addition. The highest colony counts of the whole period were recorded at day 10. Glucose stimulated a minor increase in the numbers of actinomycete populations only at early days of the entire period but on remaining days actinomycete population decreased gradually. Aldicarb amendment suppressed the growth of actinomycete populations (Figure 3.3.3.).

For the gram negative bacteria in soil under the influence of Kırkpınar cultivar, glutamic acid growth stimulation manifested itself early on the day 3, continued to increase at day 5 and 10. Numbers declined sharply at day 15 (Figure 3.3.4.). Remarkable growth promoting effect of Aldicarb on Gram negative bacterial population was observed between the days 5 and 10. Even though glucose was used somewhat efficiently and caused increase in the numbers $Gram^-$ negative bacterial population, the growth reached with glucose addition was lower than the growth attained with aldicarb amendment (Figure 3.3.4.).

The colony counts representing fungal populations were the highest with glutamic acid addition on day 15. Response of fungal populations to glucose preceded the response to glutamic acid. Although response to glutamic acid was late, the colony counts were the highest (Figure 3.3.5.).

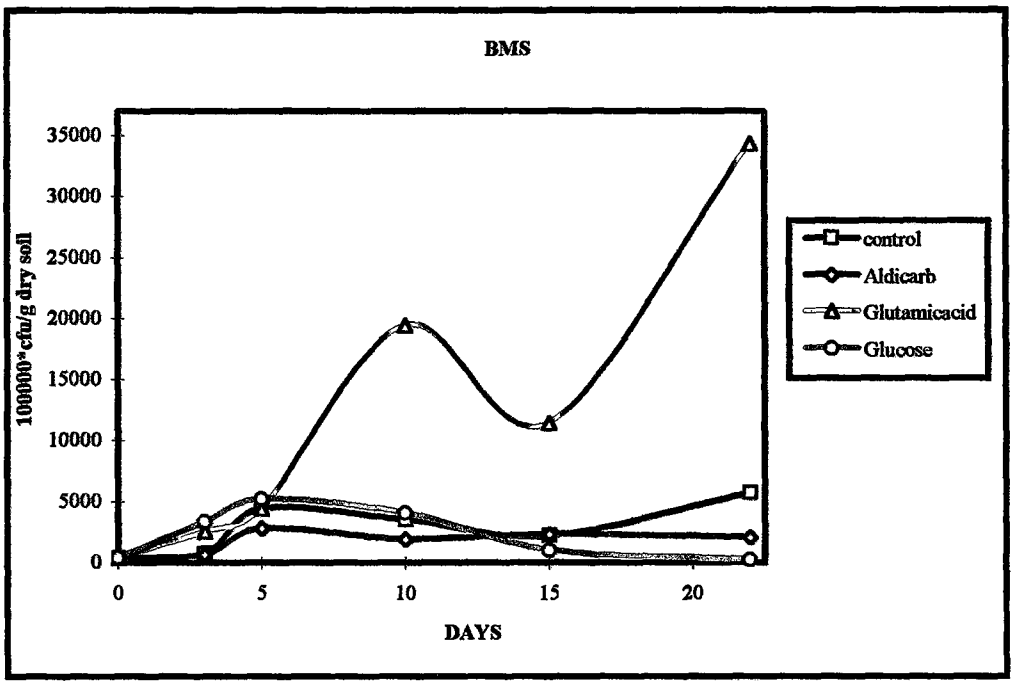


Figure 3.3.1. Growth responses to different carbon sources of bacterial populations associated with Kırkpınar wheat cultivar grown in BMS medium

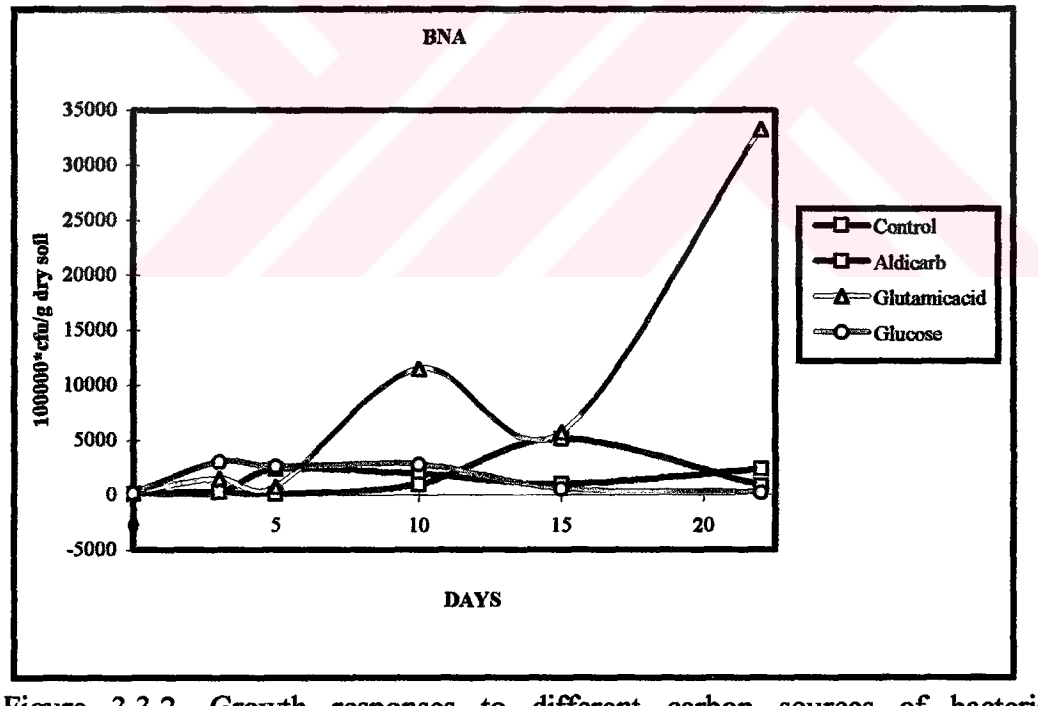


Figure 3.3.2. Growth responses to different carbon sources of bacterial populations associated with Kırkpınar wheat cultivar grown in BNA medium

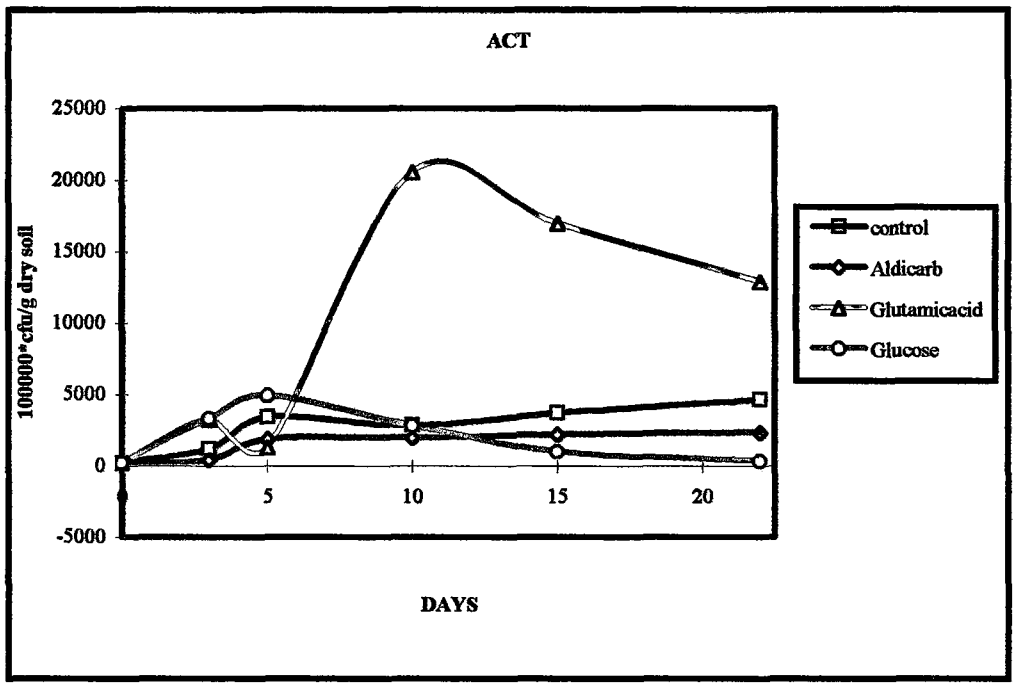


Figure 3.3.3. Growth responses to different carbon sources of actinomycete populations associated with Kırkpınar wheat cultivar grown in ACT medium

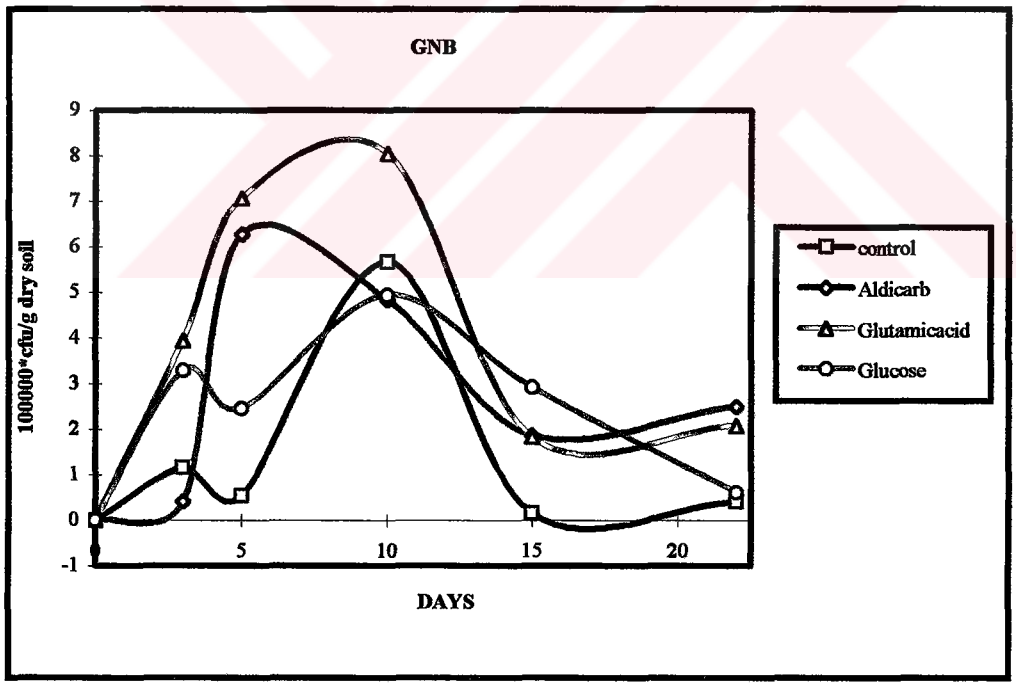


Figure 3.3.4. Growth responses to different carbon sources of gram negative bacterial populations associated with Kırkpınar cultivar grown in GNB medium

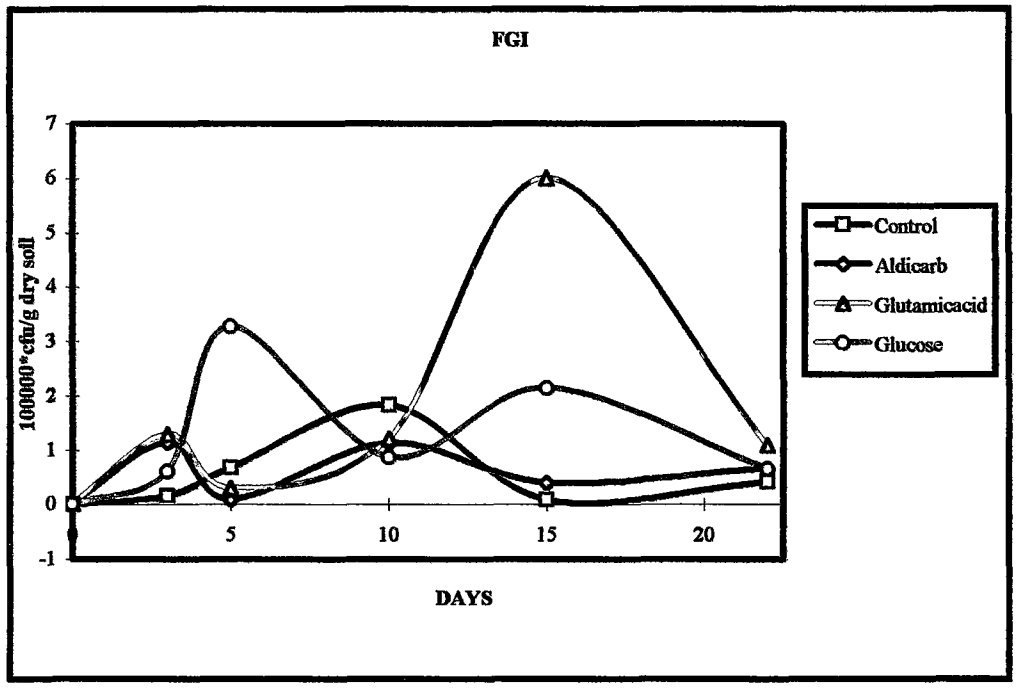


Figure 3.3.5. Growth responses to different carbon sources of fungal populations associated with Kırkpınar wheat cultivar grown in FGI medium

In soil associated with Gerek wheat cultivar, the counts of bacteria growing on BMS agar media were again the highest with glutamic acid. First response to glutamic acid was recorded on day 10, the sharp decrease at day 15 was followed by a second response on day 22. Glucose did not exhibit a good proliferative effect on the bacteria cultured on BMS medium. In the first 5 days of the whole period a small increase in the population occurred. On remaining days the counts were low (Figure 3.3.6.).

The bacterial growth on BNA agar media was maximum with glutamic acid amendment. Interestingly, the curve which shows the growth response of bacterial population on BNA medium to glutamic acid shows almost the same characteristics with the curve exhibiting the growth response of bacterial populations cultured on BMS medium to glutamic acid. Similarly, glucose did not stimulate the bacterial growth at high levels. It caused only a small increase on day 3. On the other hand, in aldicarb added samples, the bacterial populations showed stimulation at day 10 (Figure 3.3.7.).

In the soil associated with Gerek wheat cultivar, the actinomycete populations exhibited phasic response. There was small response to glucose on day 5 & aldicarb on day 15 (Figure 3.3.8.).

For the Gram$^-$ negative bacteria, Glutamic acid amendment again caused a continuous increase in numbers accompanied by subsequent decline at day 22. Glucose stimulation started at day 3, exhibited gradual increase at day 10 and 15. The numbers decreased at day 22. Aldicarb addition caused an increase in Gram negative bacterial populations at day 10 and continued with a low rate of increase during the experimental period. In this particular soil sample the moisture effect was pronounced at day 10 (Figure 3.3.9.).

The fungal population under the influence of Gerek wheat cultivar responded to glutamic acid most efficiently. The response was phasic. Some populations gave an early small response. In the second phase the numbers recorded at day 15 were about 4 times greater than the numbers recorded at day 3. Glucose was somewhat stimulatory on growth of fungal population as it was recorded on day 5 & 10 (Figure 3.3.10.).

In soil associated with Gerek cultivar, it was observed that all maximum peaks were reached by glutamic acid amendment for bacterial populations. Similarly, the extended response to glutamic acid occurred in fungal populations with a phasic pattern. Among seven different wheat cultivars investigated, the highest response to glutamic acid application was observed in fungal populations associated with Gerek cultivar.

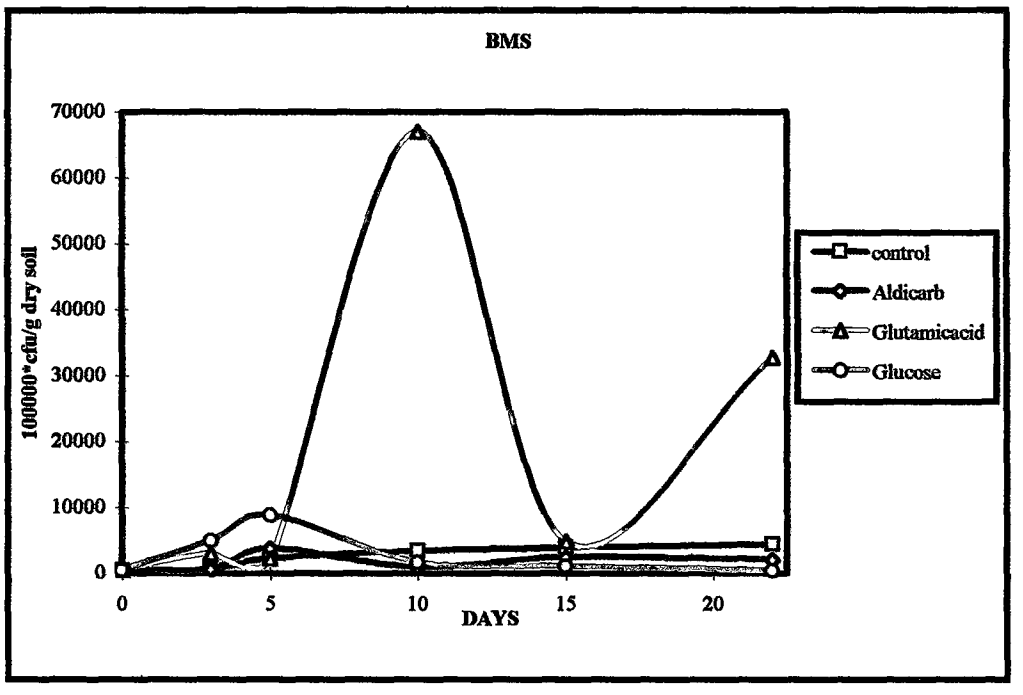


Figure 3.3.6. Growth responses to different carbon sources of bacterial populations associated with Gerek wheat cultivar grown in BMS medium

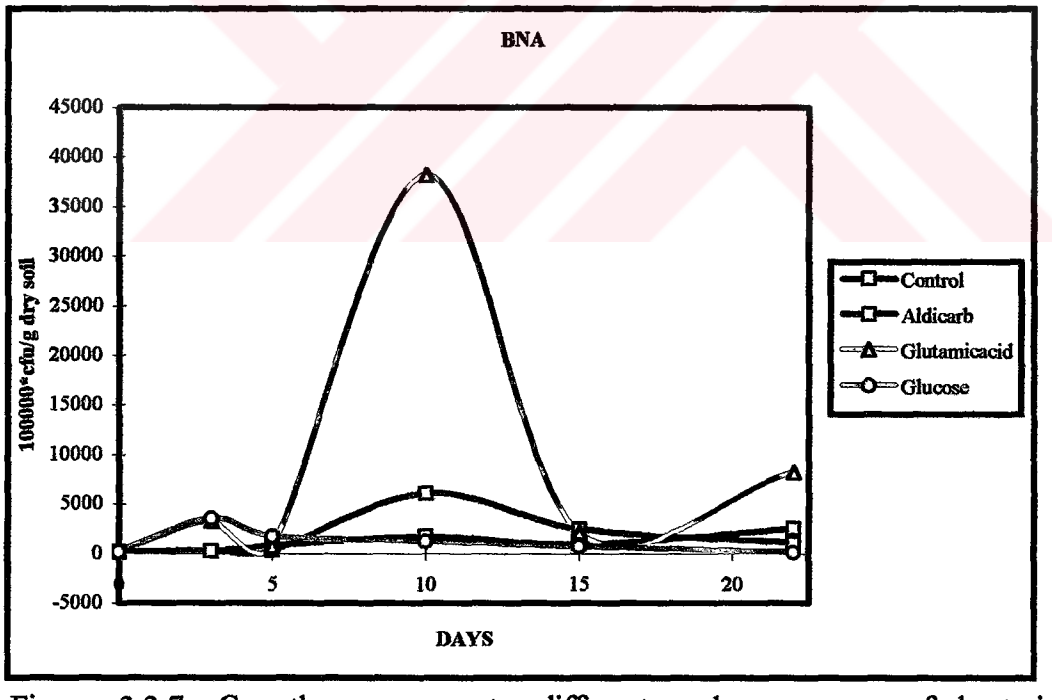


Figure 3.3.7. Growth responses to different carbon sources of bacterial populations associated with Gerek wheat cultivar grown in BNA medium

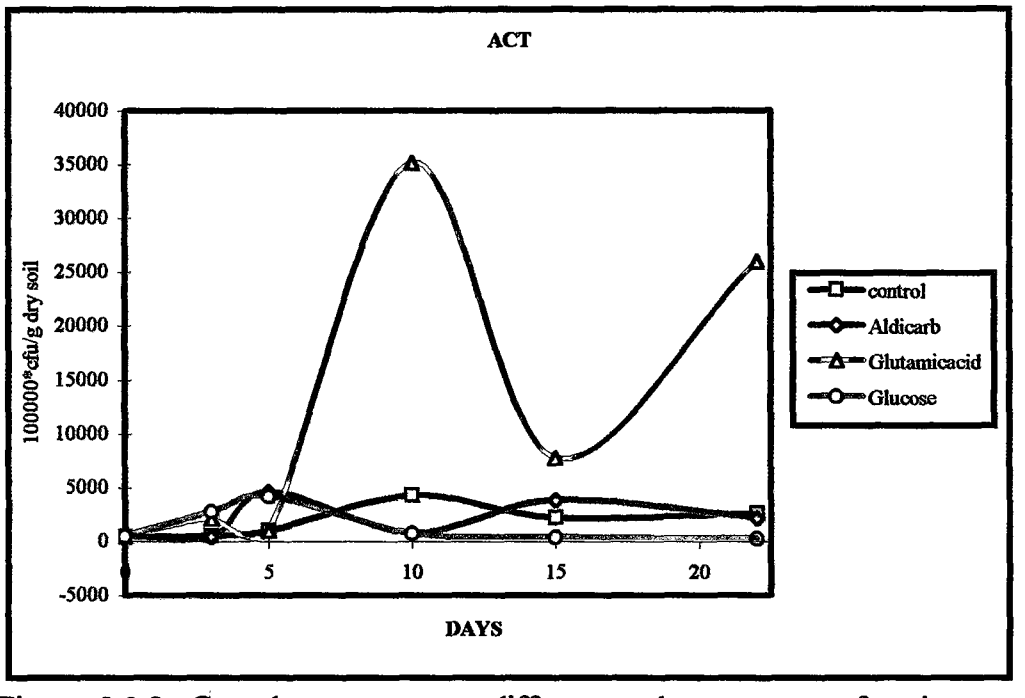


Figure 3.3.8. Growth responses to different carbon sources of actinomycete populations associated with Gerek wheat cultivar grown in ACT medium

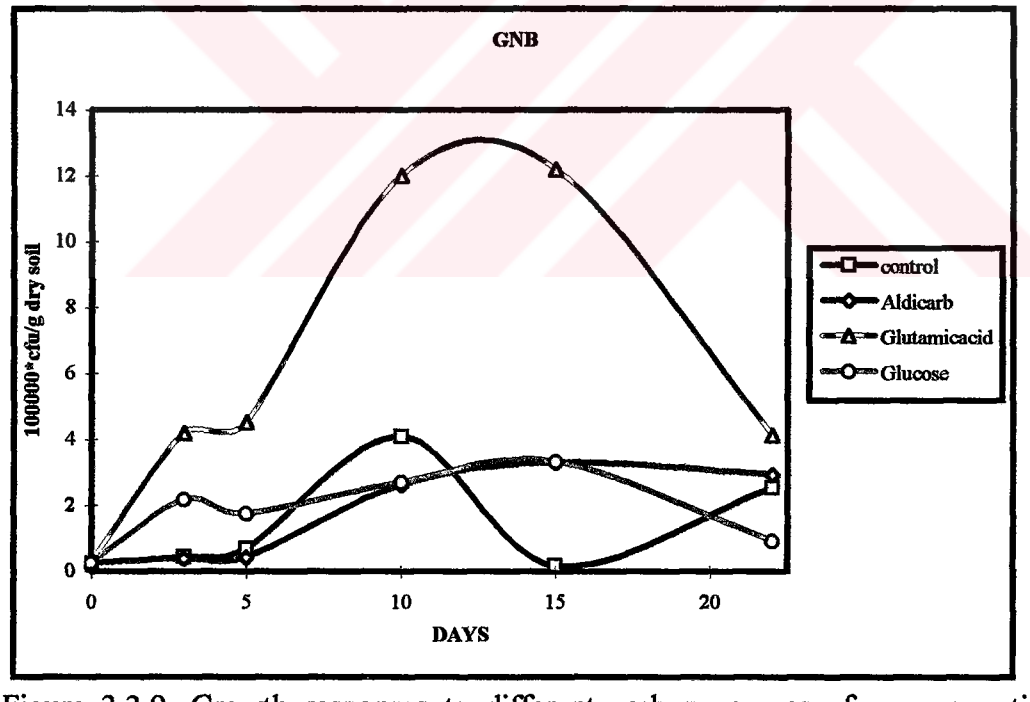


Figure 3.3.9. Growth responses to different carbon sources of gram negative bacterial populations associated with Gerek cultivar grown in GNB medium

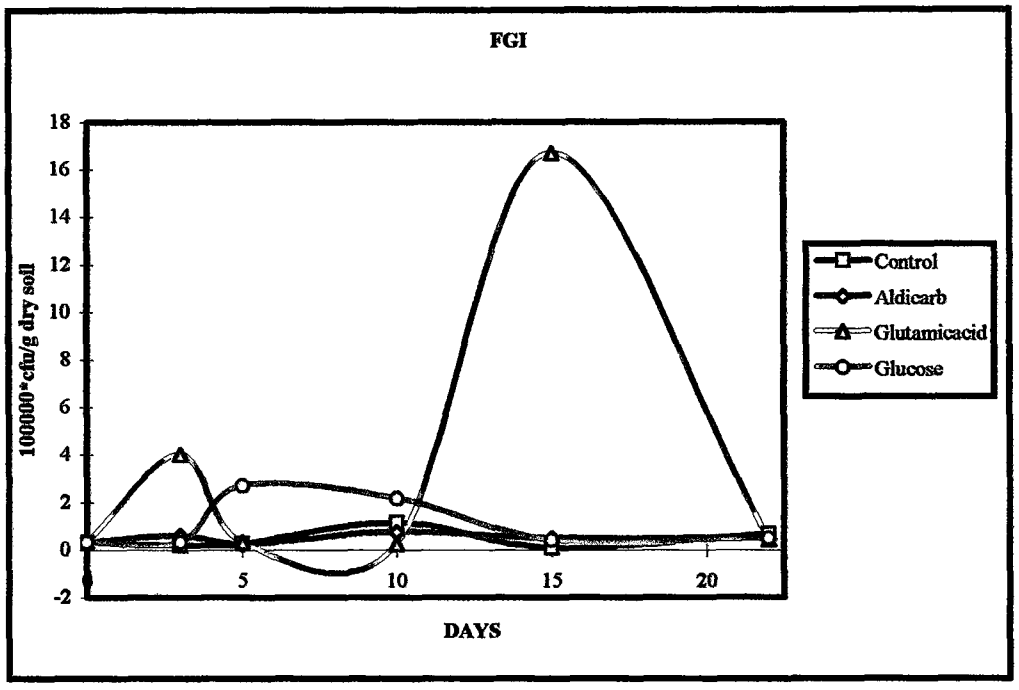


Figure 3.3.10. Growth responses to different carbon sources of fungal populations associated with Gerek wheat cultivar grown in FGI medium

In soil under the influence of Gün cultivar, the bacterial counts obtained on BMS agar media were the highest with Glutamic acid addition. Response to glucose was pronounced at day 5. Aldicarb addition caused a small phasic response in day 5 and 15 (Figure 3.3.11.).

The bacterial population cultured on BNA agar medium showed a continuous response to glutamic acid with increasing trend. The highest numbers were recorded at day 22. Glucose response ceased at day 22. Aldicarb caused a stimulation at day 5. At that point the response to aldicarb was better than that of glucose (Figure 3.3.12.).

The growth response curves of actinomycete populations were similar to the curves of bacterial population growth on BMS media. Glutamic acid enhanced the growth starting from the initial point up to day 15 on which the peak was recorded, followed by a decline. Glucose was metabolised in earlier days of the entire period. At early days aldicarb exhibited growth stimulation (Figure 3.3.13.).

Gram$^-$ negative bacterial populations associated with Gün were stimulated by glucose addition maximally. The response to this substrate peaked at day 3 and ceased starting from day 15. Phasic response with high number of bacterial counts occurred with glutamic acid. At day 10 the stimulation of aldicarb was remarkable. (Figure 3.3.14.). Only the Gün cultivar among the seven wheat cultivars investigated supported the highest number of Gram negative bacterial populations in terms of response to added substrates.

The most apparent observation for the growth of fungal populations associated with Gün cultivar was obtained with glutamic acid compared to other carbon sources used in the study. Between the days 5 and 10 the substrate caused a sharp rise in the number of fungal populations. However, glucose stimulation on fungal growth was not pronounced. Aldicarb promoted the fungal growth at a low rate (Figure 3.3.15.).

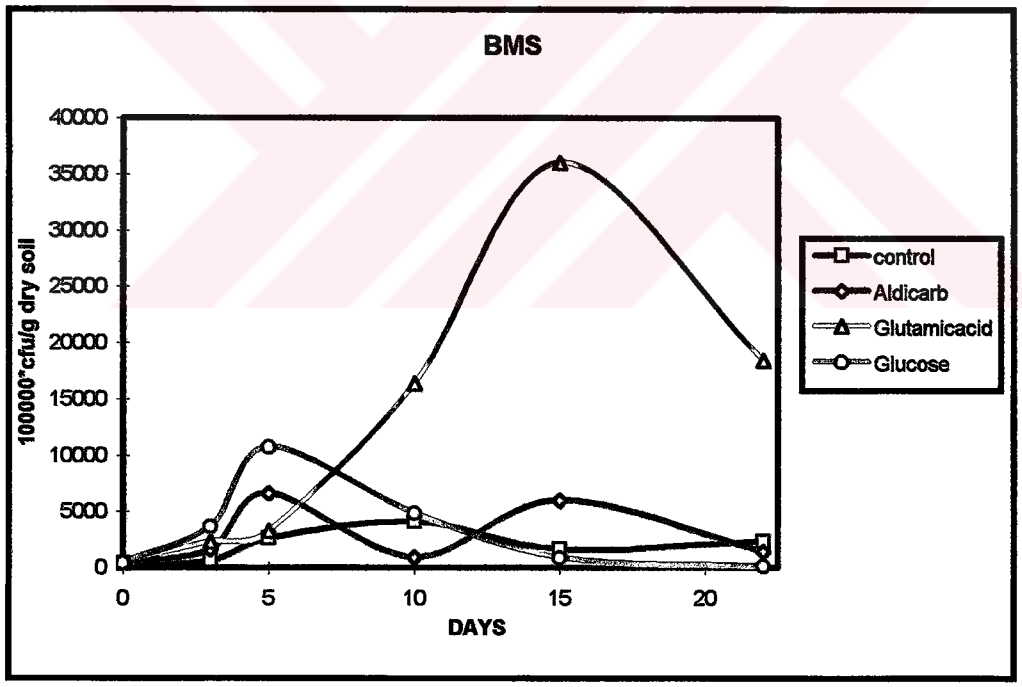


Figure 3.3.11. Growth responses to different carbon sources of bacterial populations associated with Gün wheat cultivar grown in BMS medium

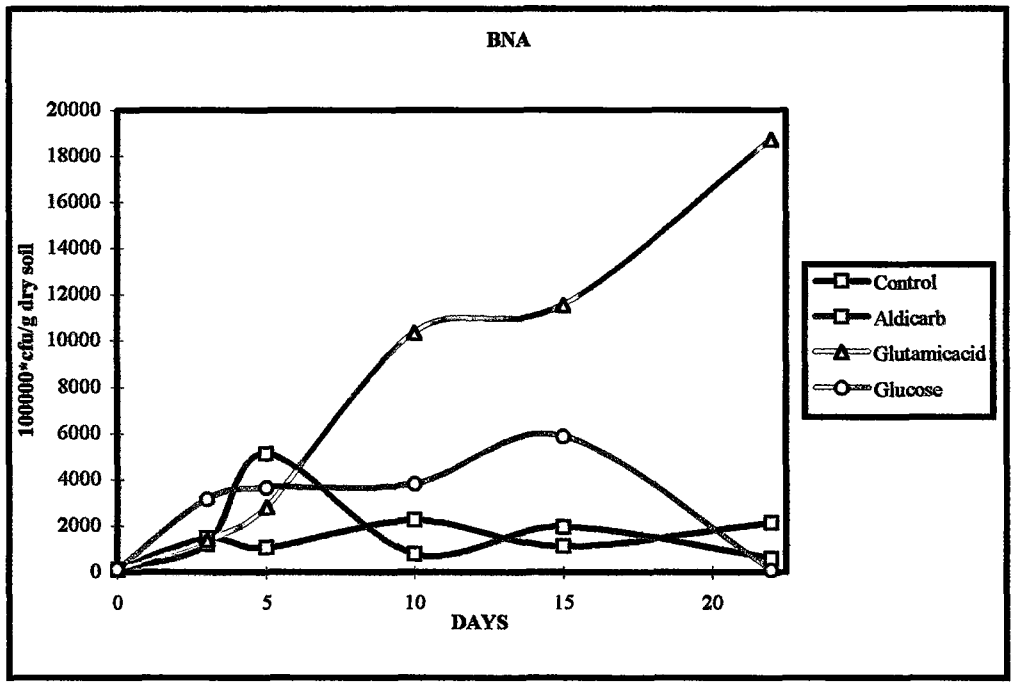


Figure 3.3.12. Growth responses to different carbon sources of bacterial populations associated with Gün wheat cultivar grown in BNA medium

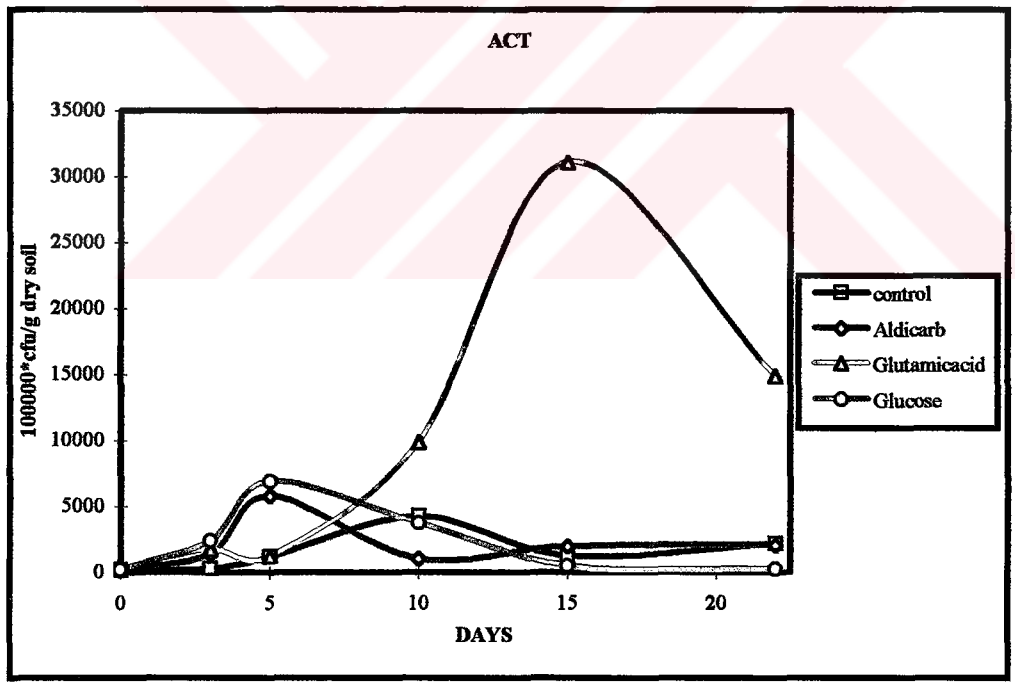


Figure 3.3.13. Growth responses to different carbon sources of actinomycete populations associated with Gün wheat cultivar grown in ACT medium

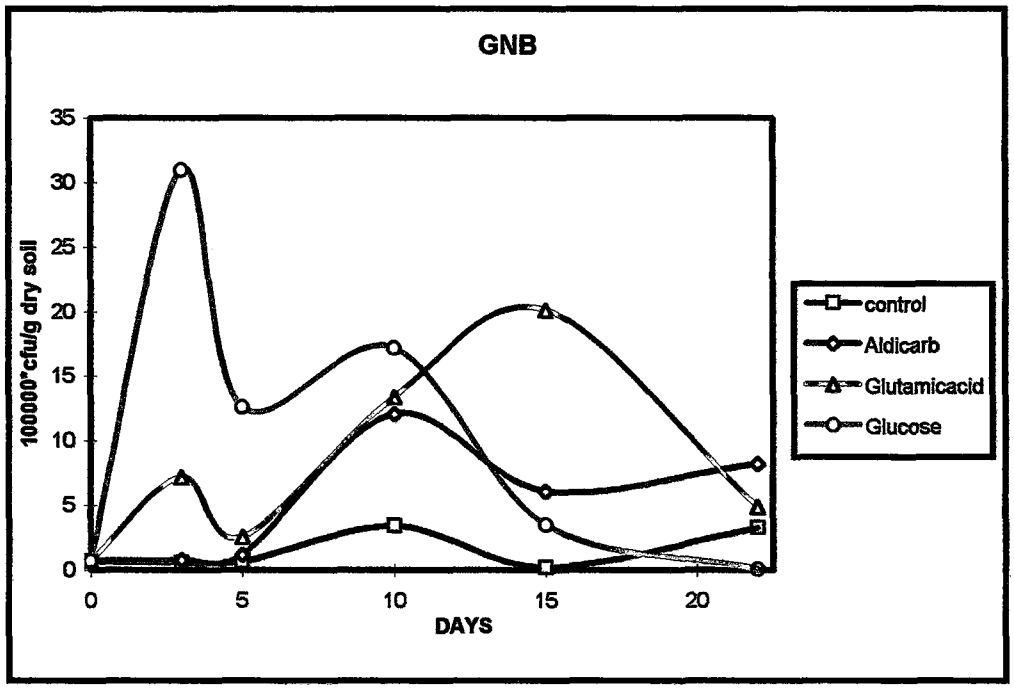


Figure 3.3.14. Growth responses to different carbon sources of gram negative bacterial populations associated with Gün wheat cultivar grown in GNB medium

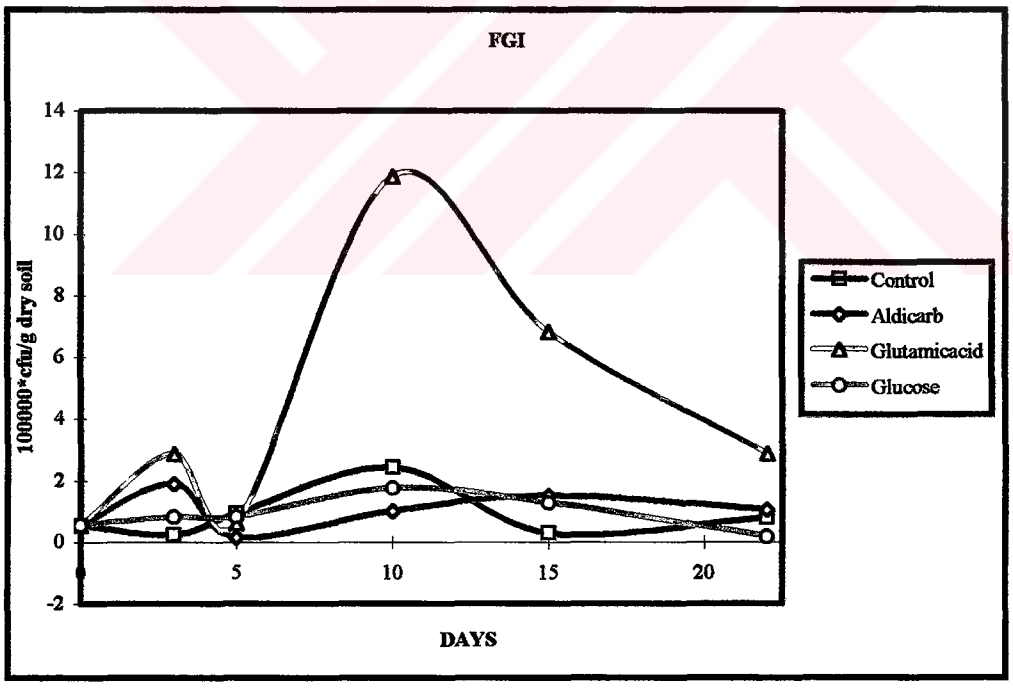


Figure 3.3.15. Growth responses to different carbon sources of fungal populations associated with Gün wheat cultivar grown in FGI medium

In soil under the influence of Bolal wheat cultivar the bacterial population grown in BMS media increased continuously with glutamic acid addition starting from day 10. Similarly, the bacterial counts of glucose added samples increased at day 5 and 10 followed by a decline. The bacterial population cultured in BMS media exhibited a small stimulation at day 15 (Figure 3.3.16.).

Bacterial counts on BNA medium responded glutamic acid and glucose amendment the best at day 10 with glutamic acid being the most stimulatory on bacterial populations. A phasic stimulation was recorded at day 5 and 15. Stimulation caused by aldicarb was 6 times smaller than that of glutamic acid (Figure 3.3.17.).

Actinomycete populations associated with Bolal responded glucose at day 5. Glucose utilization appeared to be completed at day 15. Response to glutamic acid however, lasted at day 22 with increase. Response to aldicarb was small and short lived (Figure 3.3.18.).

The growth of Gram negative bacterial population associated with Bolal was most responsive to glutamic acid. Glucose effect was not pronounced. Interestingly the Gram negative bacterial populations here did not exhibit growth stimulation in the presence of aldicarb (Figure 3.3.19.).

The best response by fungal population was to glucose amendment on day 10 on which the highest peak was recorded. Glutamic acid was used more efficiently after day 10. Aldicarb appeared to inhibit the fungal growth between the days 5 and 10 (Figure 3.3.20.).

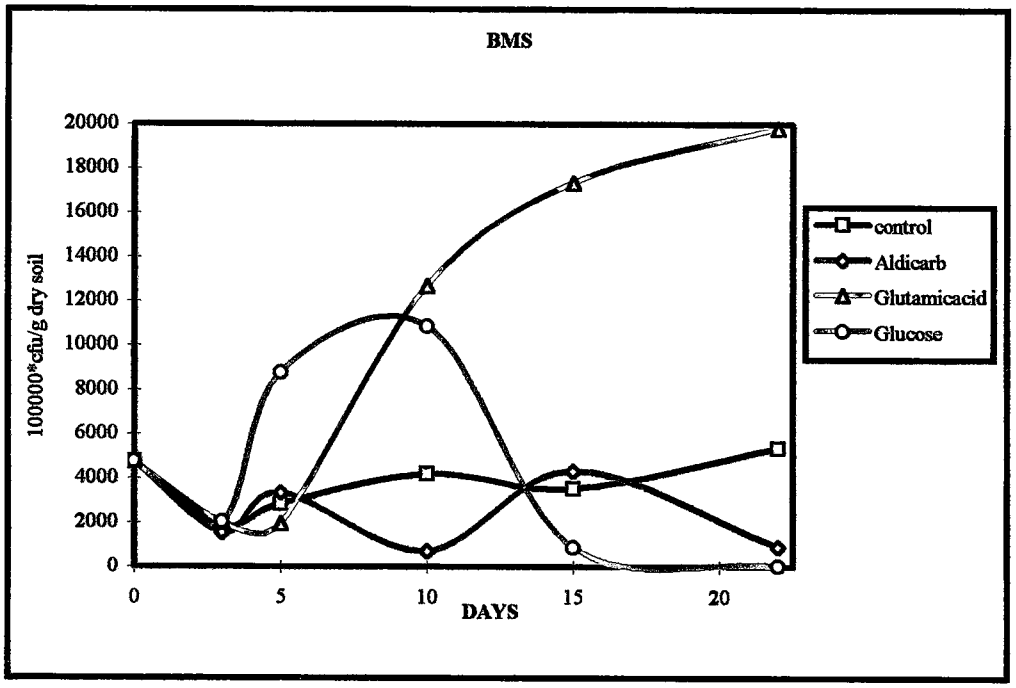


Figure 3.3.16. Growth responses to different carbon sources of bacterial populations associated with Bolal wheat cultivar grown in BMS medium

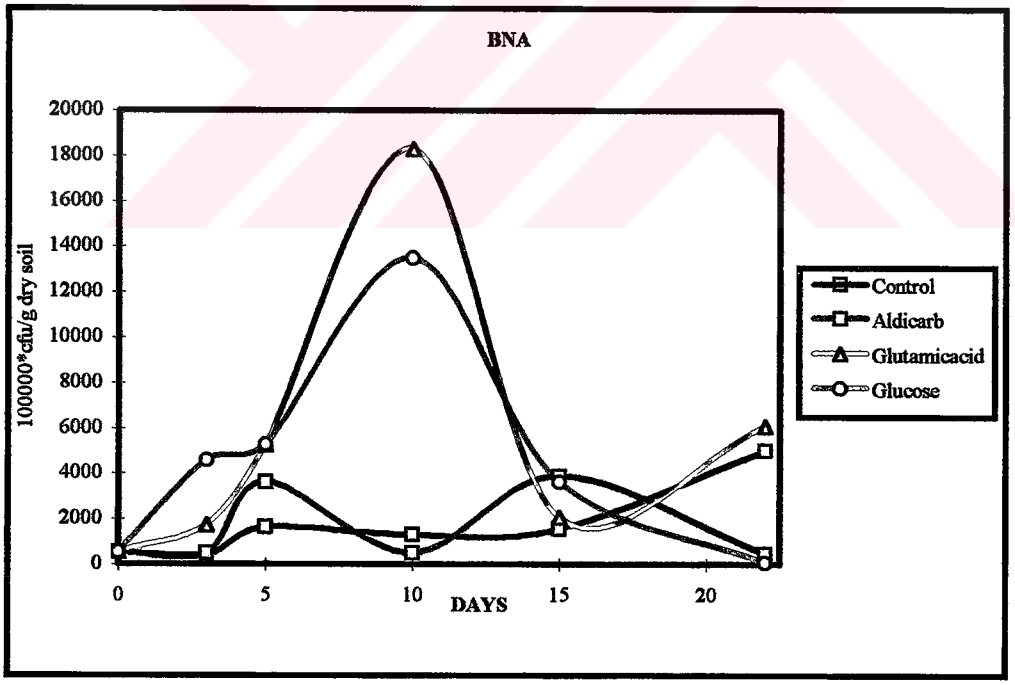


Figure 3.3.17. Growth responses to different carbon sources of bacterial populations associated with Bolal wheat cultivar grown in BNA medium

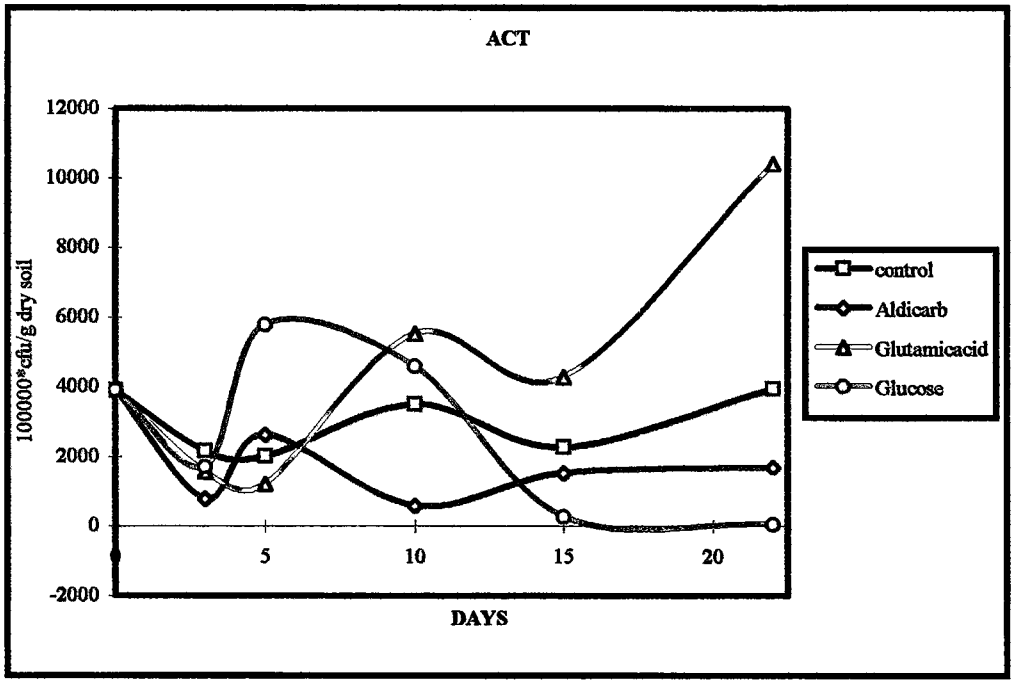


Figure 3.3.18. Growth responses to different carbon sources of actinomycete populations associated with Bolal wheat cultivar grown in ACT medium

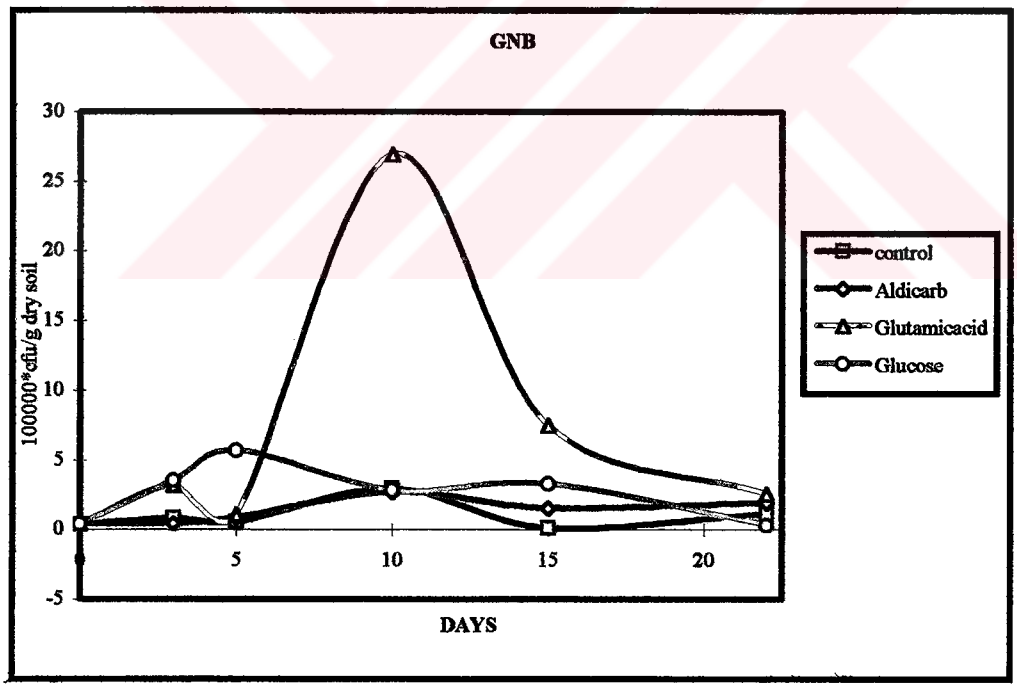


Figure 3.3.19. Growth responses to different carbon sources of gram negative bacterial populations associated with Bolal wheat cultivar grown in GNB medium

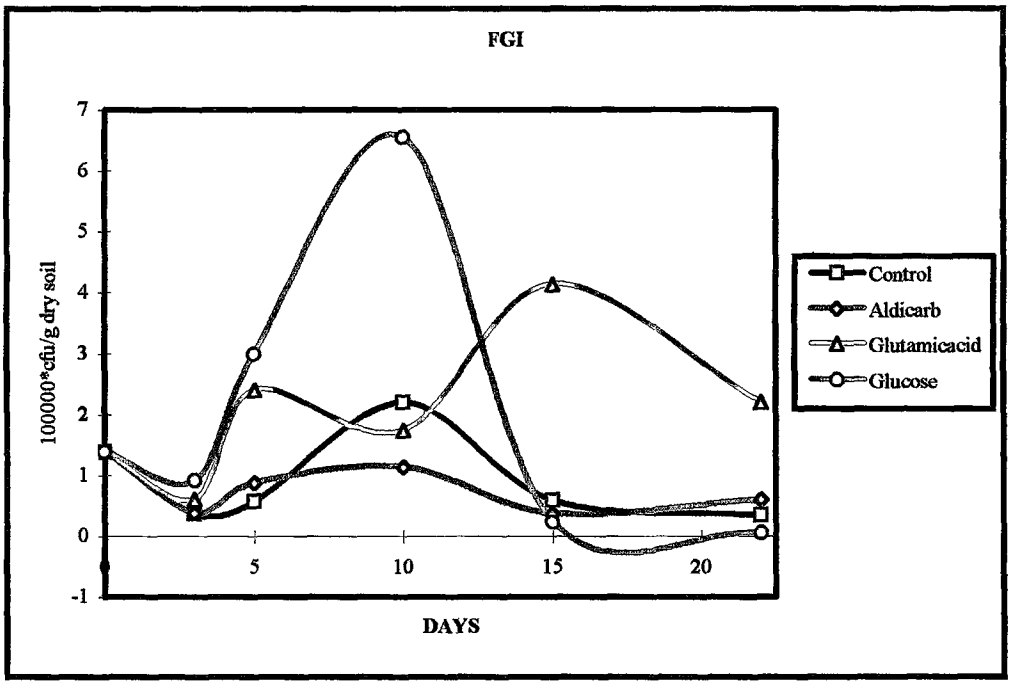


Figure 3.3.20. Growth responses to different carbon sources of fungal populations associated with Bolal wheat cultivar grown in FGI medium

In soil associated with Lancer, for the bacterial populations cultured in BMS media, appearently, glucose was not a preferred substrate. The response to glutamic acid exhibited a delay. The highest counts were recorded on day 22 (Figure 3.3.21.).

The trend observed in the response to glutamic acid in BMS media repeated somewhat similarly in BNA medium. Here, the glucose utilization was 3 times better. Aldicarb had a growth stimulating effect at day 15 (Figure 3.3.22.).

Actinomycete population showed a phasic response to glutamic acid. There were quick and late responding populations. At day 15 a short lived aldicarb effect was observed as in BNA. Glucose appeared to be not stimulatory (Figure 3.3.23.).

For the Gram$^-$ negative bacteria associated with Lancer, interesting substrate utilization patterns were observed. There were quick and slow responding populations to glutamic acid amendment. Effect of glucose was

observed at the highest at day 5 then upon utilization of the substrate the response died. The Gram negative bacterial populations showed a late but good response to aldicarb amendment with the highest number recorded at day 22 (Figure 3.3.24.).

For the fungal population aldicarb exhibited a suppressive effect. Fungal population in this cultivar utilized glucose more efficiently than glutamic acid (Figure 3.3.25.).

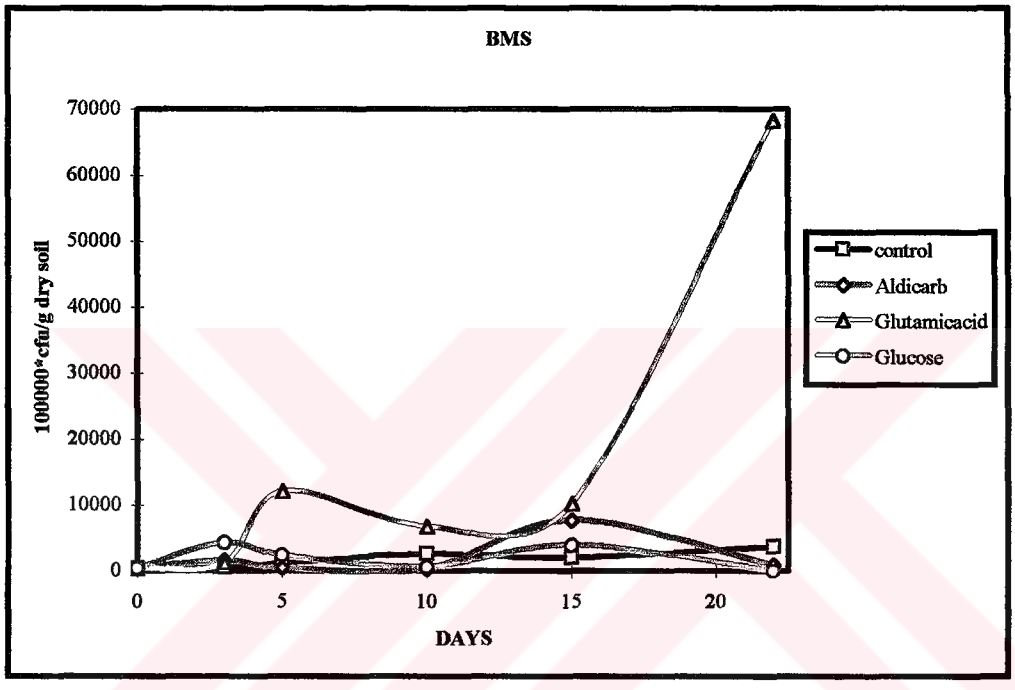


Figure 3.3.21. Growth responses to different carbon sources of bacterial populations associated with Lancer wheat cultivar grown in BMS medium

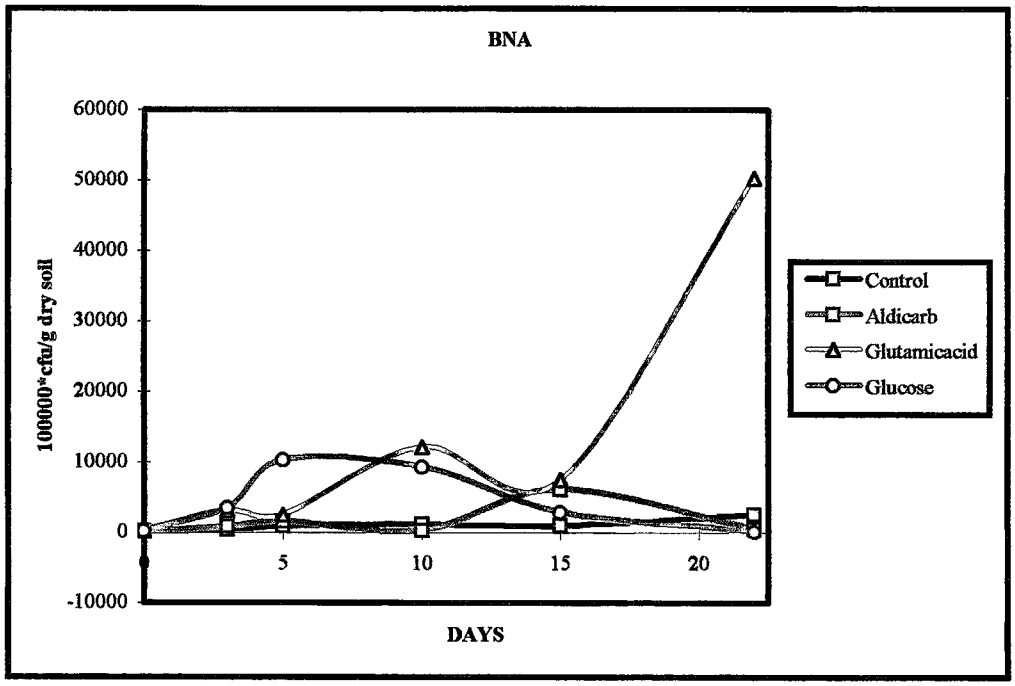


Figure 3.3.22. Growth responses to different carbon sources of bacterial populations associated with Lancer wheat cultivar grown in BNA medium

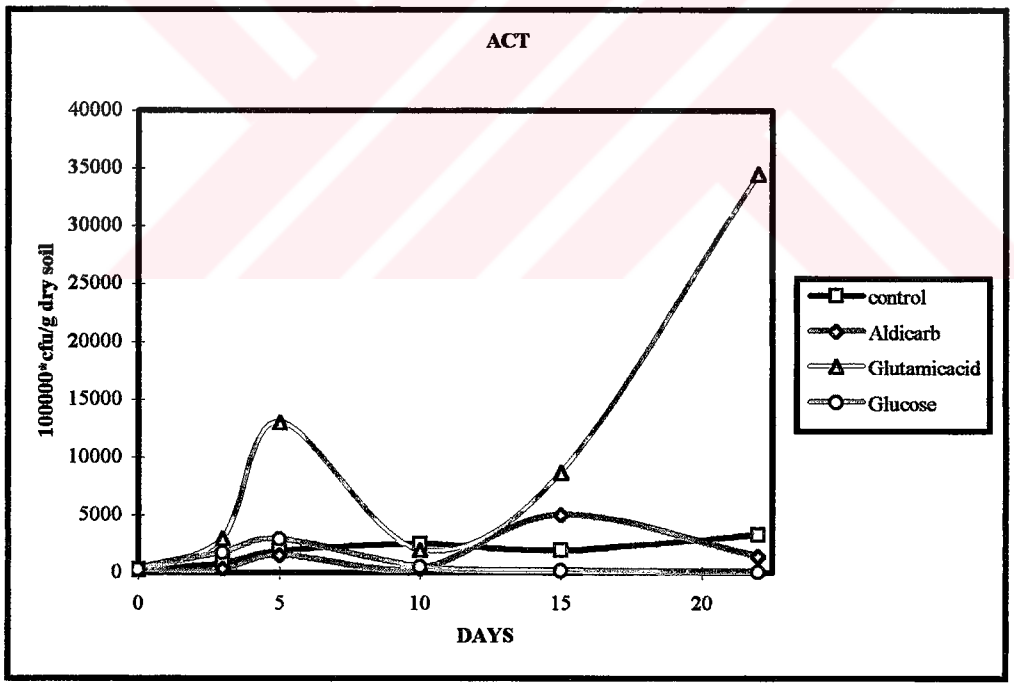


Figure 3.3.23. Growth responses to different carbon sources of actinomycete populations associated with Lancer wheat cultivar grown in ACT medium

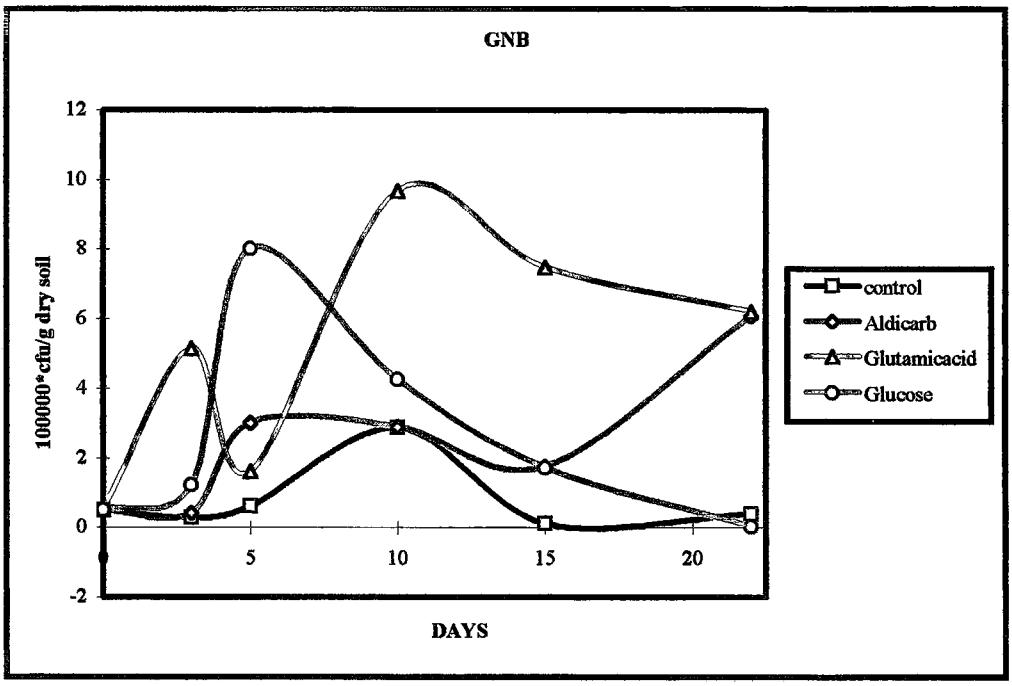


Figure 3.3.24. Growth responses to different carbon sources of gram negative bacterial populations associated with Lancer cultivar grown in GNB medium

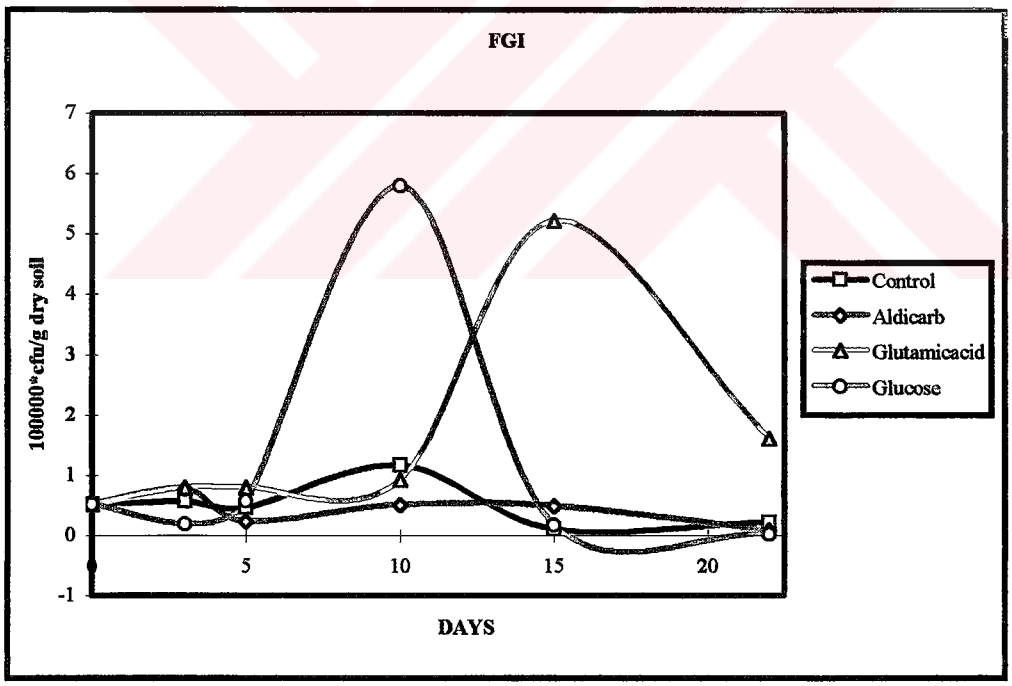


Figure 3.3.25. Growth responses to different carbon sources of fungal populations associated with Lancer wheat cultivar grown in FGI medium

In soil under the influence of Kıraç cultivar the bacterial populations grown in BMS media continuously increased except for a minor decrease at day 5 with glutamic acid addition.While glucose was not stimulatory at all aldicarb appeared to inhibit the growth (Figure 3.3.26.).

Counts of bacteria in complex BNA medium indicated the presence of two types of populations. The ones responding glutamic acid early and the others late. There was a wide spectrum glucose utilization with a slow rate. Aldicarb was not utilised in addition it appeared to impair growth (Figure 3.3.27.).

The actinomycete populations associated with Kıraç wheat cultivar utilized glutamic acid the best. Glucose and aldicarb did not exhibit growth stimulating effect (Figure 3.3.28.).

The gram negative bacterial populations responded early and well to glucose amendment. The aldicarb utilization was surprisingly comparable to glutamic acid utilization. More interestingly, the aldicarb utilization appeared to be occuring earlier than glutamic acid utilization. This indicated the presence of population with wide metabolic capabilities (Figure 3.3.29.).

FGI population associated with Kıraç utilized glutamic acid in phasic pattern. There was a good late response to glucose. Aldicarb appeared not to have a pronounced stimulatory effect (Figure 3.3.30.).

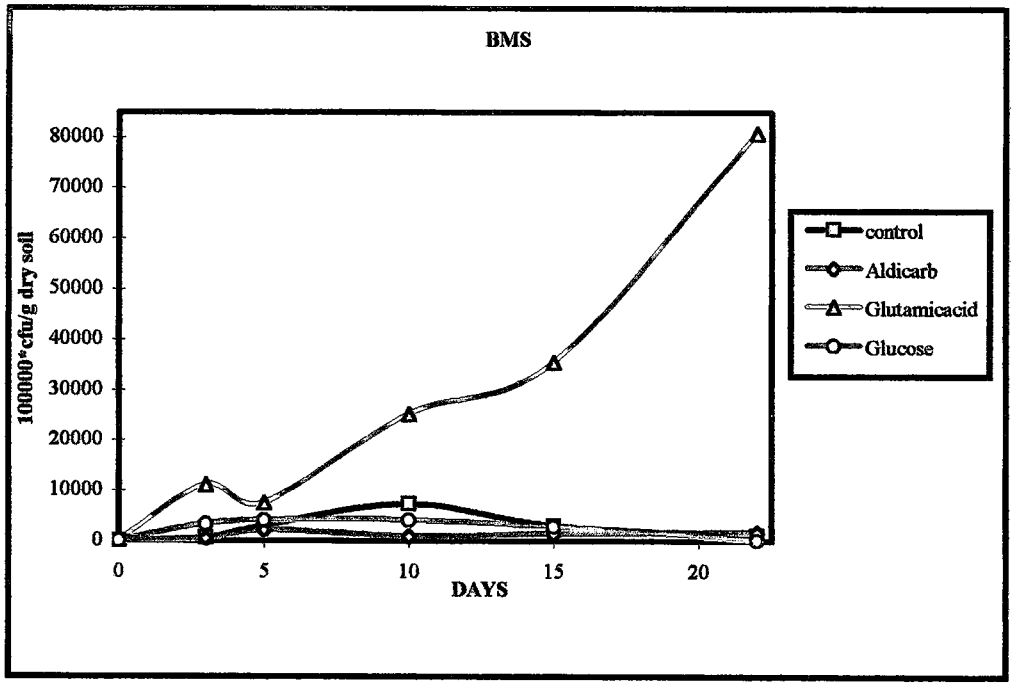


Figure 3.3.26. Growth responses to different carbon sources of bacterial populations associated with Kıraç wheat cultivar grown in BMS medium

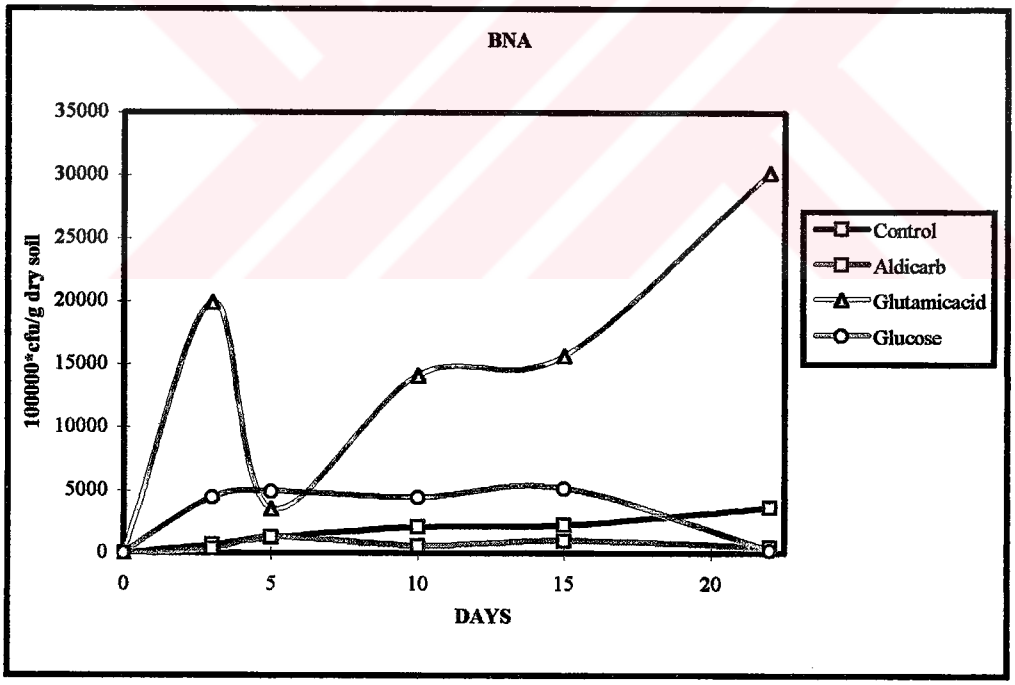


Figure 3.3.27. Growth responses to different carbon sources of bacterial populations associated with Kıraç wheat cultivar grown in BNA medium

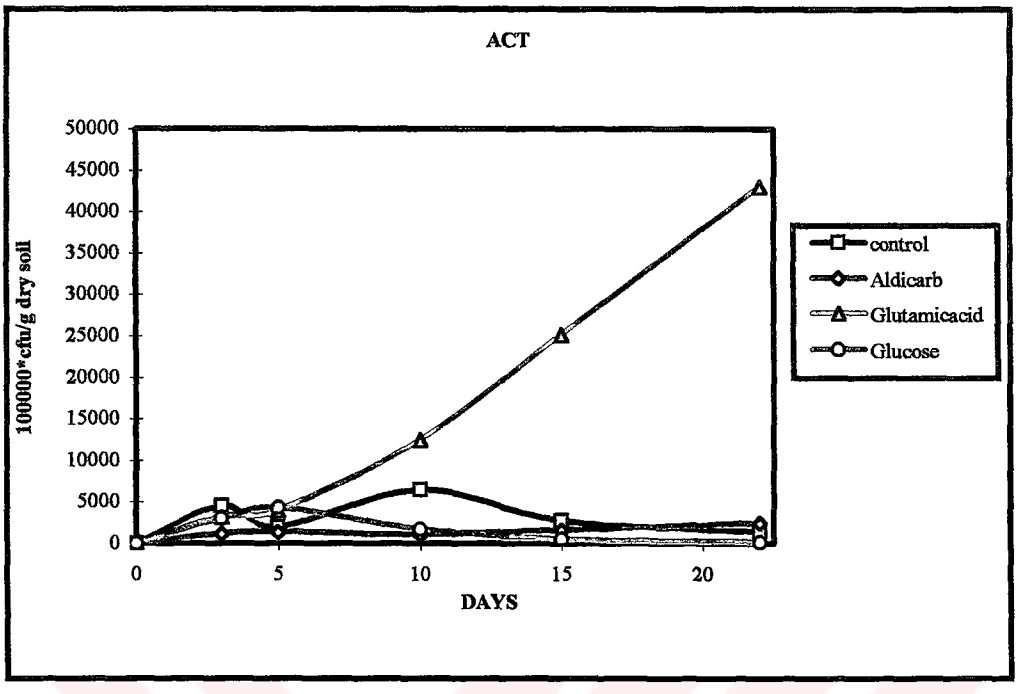


Figure 3.3.28. Growth responses to different carbon sources of actinomycete populations associated with Kıraç wheat cultivar grown in ACT medium

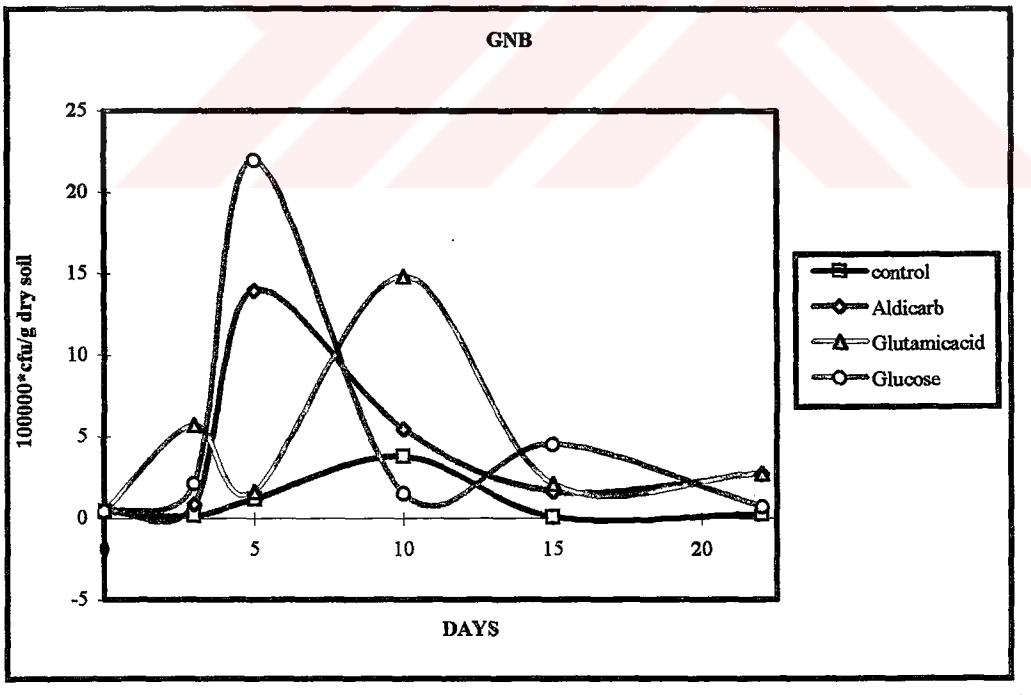


Figure 3.3.29. Growth responses to different carbon sources of gram negative bacterial populations associated with Kıraç wheat cultivar grown in GNB medium

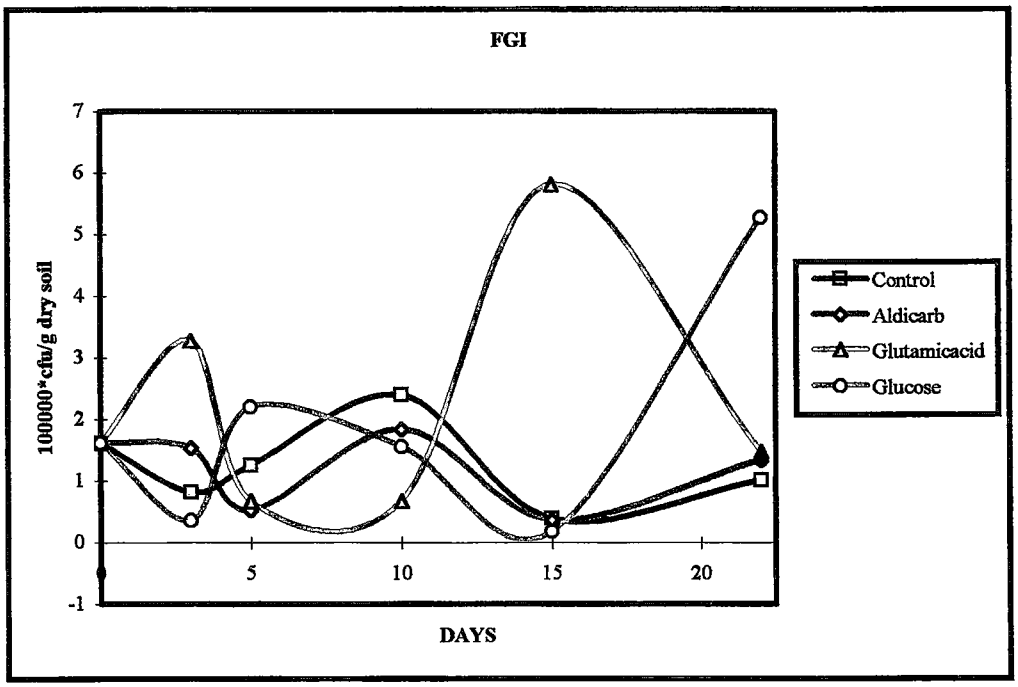


Figure 3.3.30. Growth responses to different carbon sources of fungal populations associated with Kıraç wheat cultivar grown in FGI medium

The bacterial populations associated with Bezostaja gave the best response to glutamic acid. The populations did not appear to utilize glucose. Aldicarb exhibited a suppressive effect (Figure 3.3.31.).

The pattern observed which was encountered in the case of BMS medium recurred when bacteria were cultured in BNA. Here, there was a small early response to glucose (Figure 3.3.32.).

Appearently actinomycete populations were also responding best to glutamic acid with a similar pattern observed in two previous media. This cultivar seemed to support the proliferation of amino acid utilizing populations for carbon source (Figure 3.3.33.).

Gram$^-$ negative bacterial populations exhibited phasic response. Glucose, however, was utilized by Gram negative bacteria associated with Bezostaja which was not seen in three previous bacterial populations. Interestingly, the Gram

negative bacterial population associated with Bezostaja did not respond to Aldicarb amendment (Figure 3.3.34.).

In fungal populations of Bezostaja soil, glucose addition caused a sharp rise between day 15 and day 22. The results indicated the presence of two groups of populations in terms of glucose utilization. Glutamic acid stimulated the growth comparably good with glucose at day 5 and then exhibited a decline (Figure 3.3.35.).

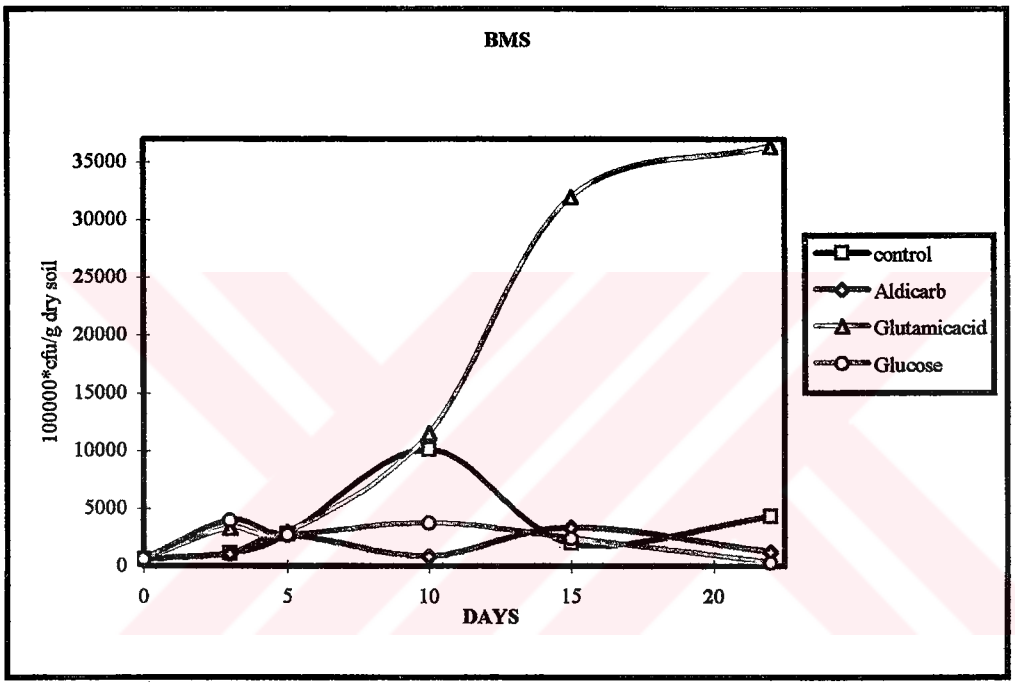


Figure 3.3.31. Growth responses to different carbon sources of bacterial population associated with Bezostaja wheat cultivar grown in BMS medium

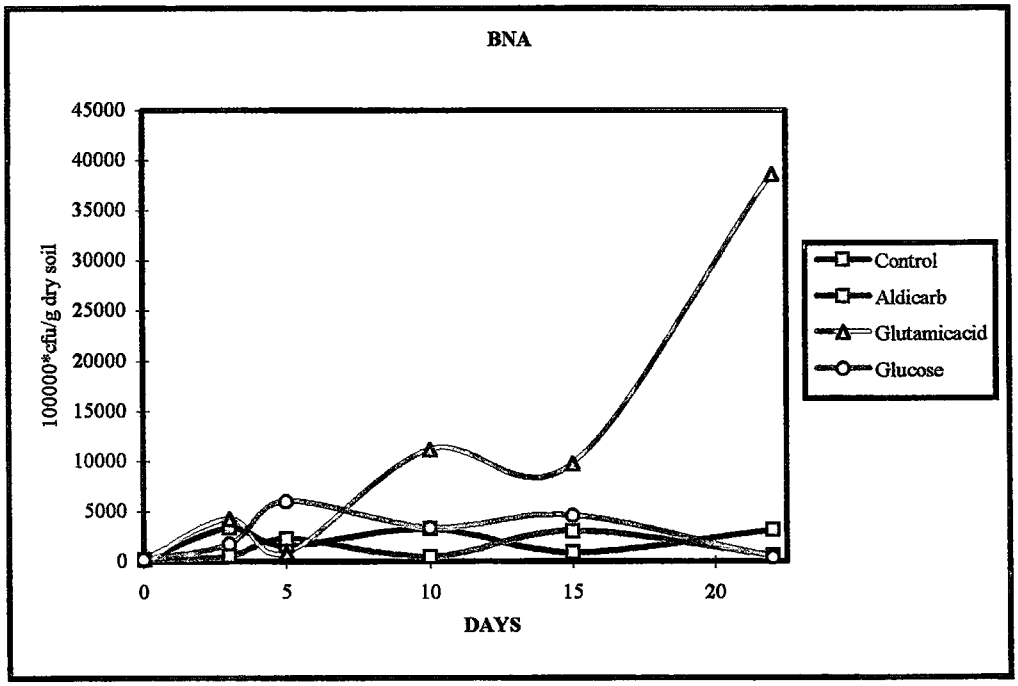


Figure 3.3.32. Growth responses to different carbon sources of bacterial populations associated with Bezostaja wheat cultivar grown in BNA medium

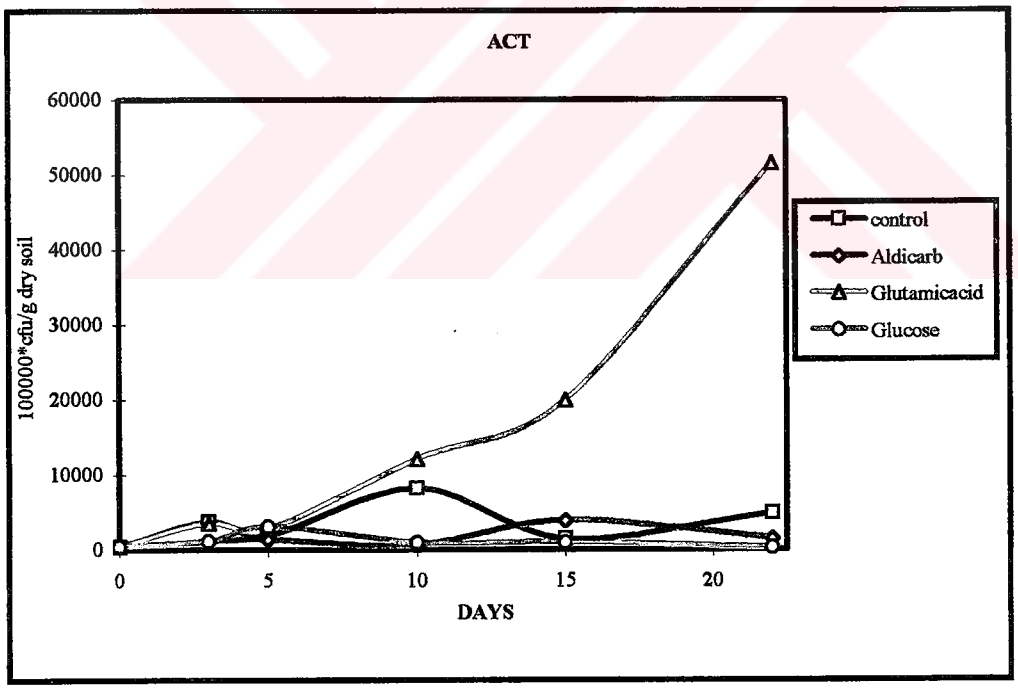


Figure 3.3.33. Growth responses to different carbon sources of actinomycete populations associated with Bezostaja wheat cultivar grown in ACT medium

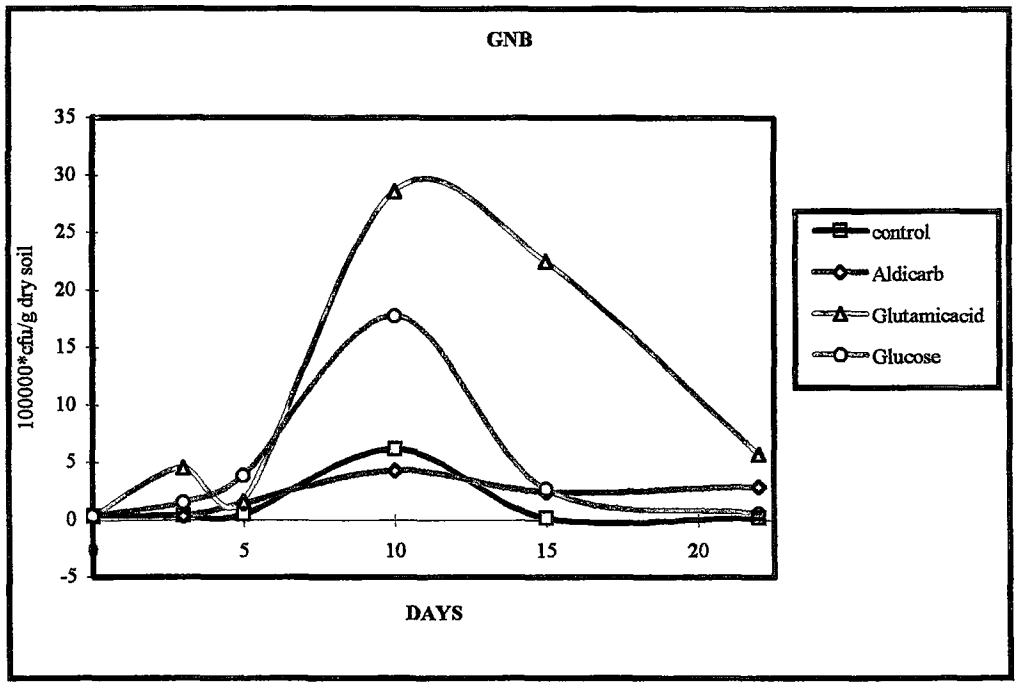


Figure 3.3.34. Growth responses to different carbon sources of gram negative bacterial populations associated with Bezostaja cultivar grown in GNB medium

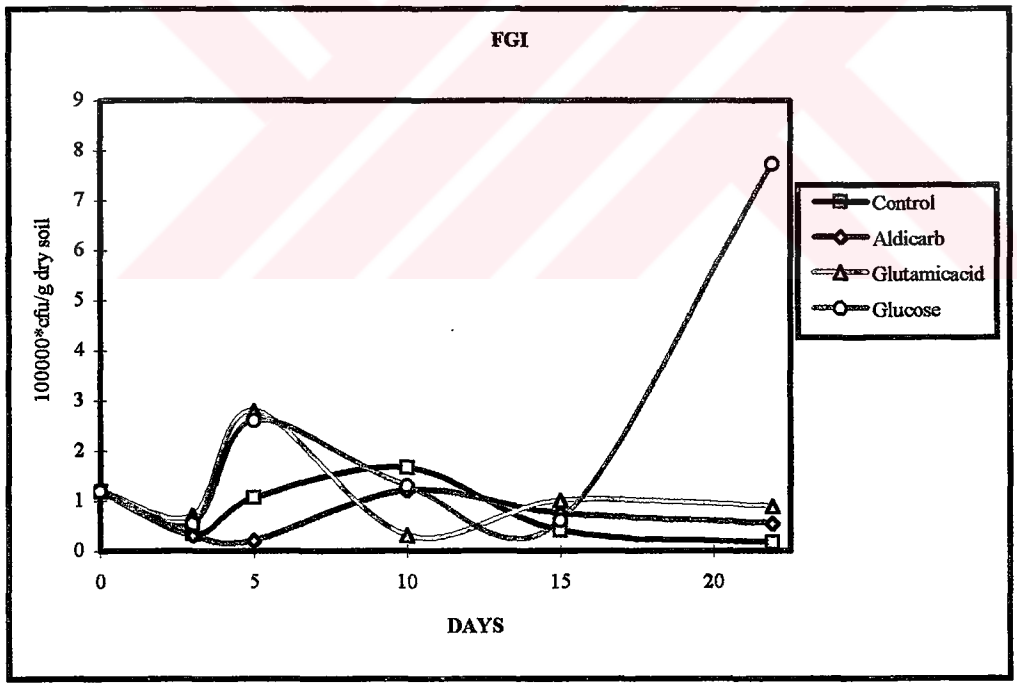


Figure 3.3.35. Growth responses to different carbon sources of fungal populations associated with Bezostaja wheat cultivar grown in FGI medium

3.4. Growth Responses of Microbial Populations in associated with Seven Wheat Cultivars to Different Types of Amino Acids

In the previous part of the study, it was observed that most of the time soil microbe populations responded glutamic acid amendments maximally. Based on this observation the responses to other amino acids were also studied. The amino acids included in the study were glycine (neutral, non-polar amino acid), serine (neutral, polar amino acid), and arginine (basic, polar amino acid).

The bacterial population associated with Bolal cultured on BMS medium readily utilized serine. This was an early response peaking at day 3. The cycle appeared to be completed by day 5. Glycine and arginine amendments caused slight increases at day 3. The results indicated that serine was better in stimulating soil microbe populations compared to glutamic acid (Figure 3.4.1.).

Similarly the bacterial populations cultured on BNA medium exhibited the highest growth response to serine. The second highest a later response was given to glutamic acid. Arginine was utilized better than glycine (Figure 3.4.2.).

Actinomycete populations appearently exhibited a similar response to serine. In addition a phasic response was obtained with arginine. The response to arginine peaked at day 10 and the cycle appeared to be completed at day 15. Glycine caused a small stimulation compared to other amino acids (Figure 3.4.3.).

Interestingly, the Gram negative bacterial populations were not stimulated by serine. Instead glutamic acid was preferred. Glycine and arginine were not used by the populations either (Figure 3.4.4.).

The fungal populations responded glutamic acid amendment better. Interestingly, however, a late stimulation was observed with glycine. Populations responding to serine proliferated at day 15. There was a cycle of moisture effect which was obvious at day 10 (Figure 3.4.5.).

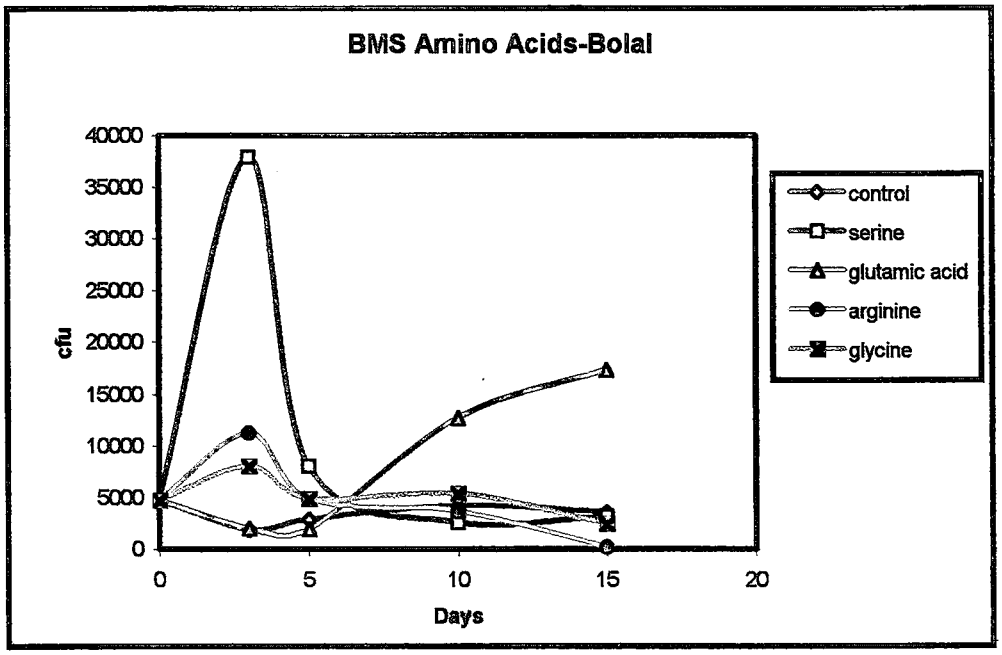


Figure 3.4.1. Growth responses of bacterial populations associated with Bolal wheat cultivar grown in BMS medium to different types of amino acids

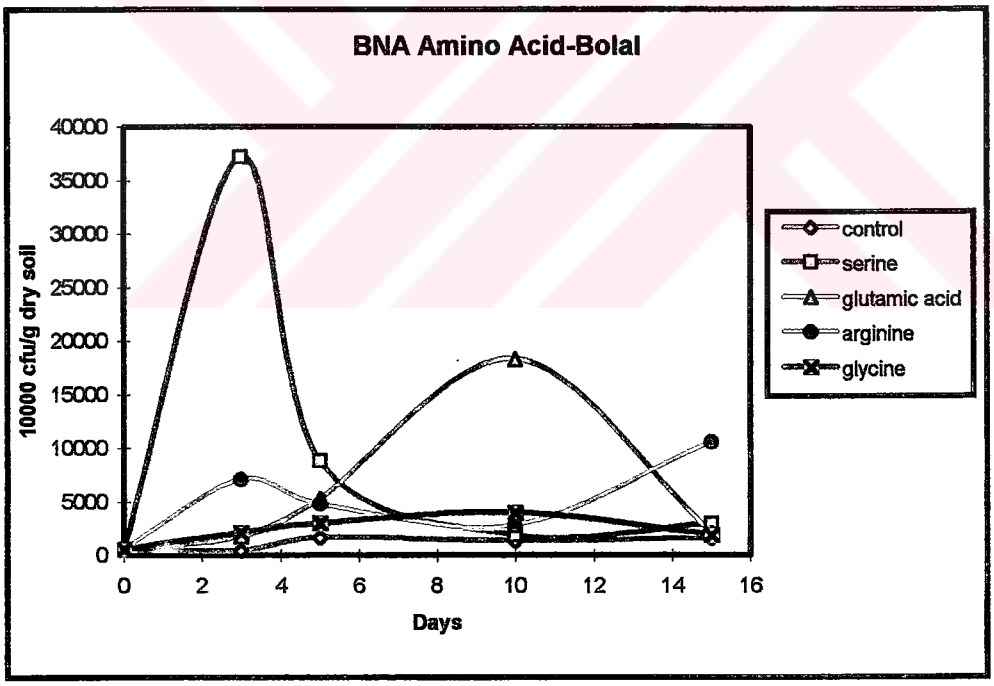


Figure 3.4.2. Growth responses of bacterial populations associated with Bolal wheat cultivar grown in BNA medium to different types of amino acids

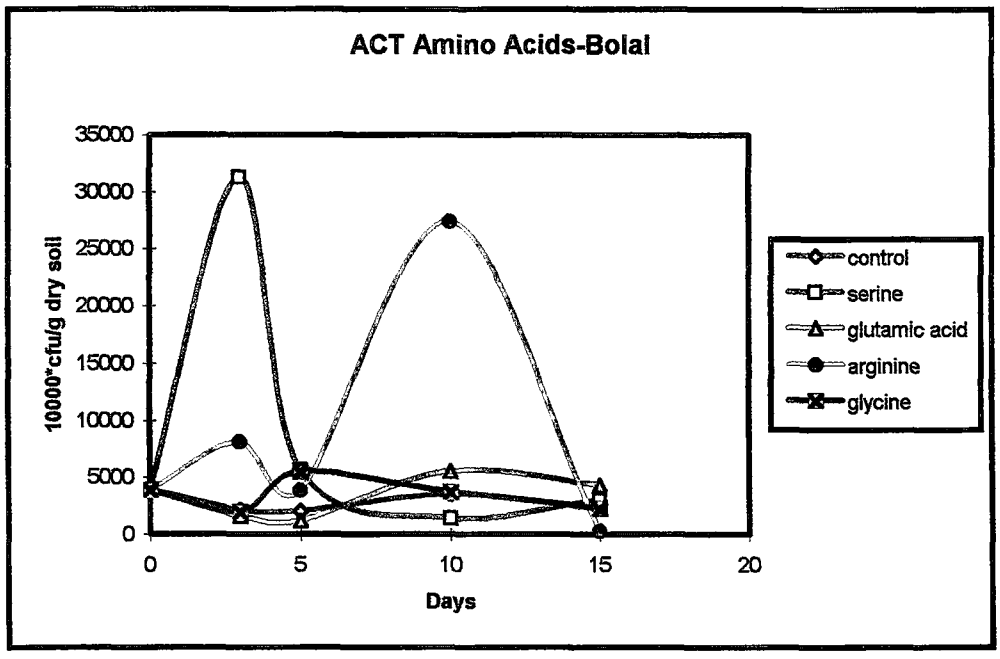


Figure 3.4.3. Growth responses of actinomycete populations associated with Bolal wheat cultivar grown in ACT medium to different types of amino acids

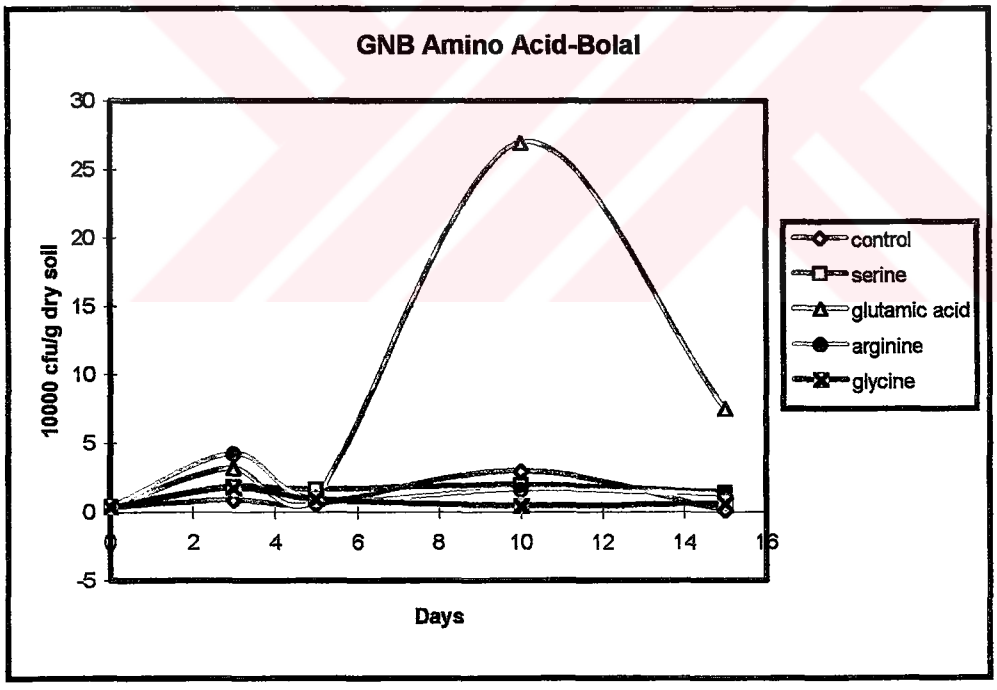


Figure 3.4.4. Growth responses of gram negative bacterial populations associated with Bolal wheat cultivar grown in GNB medium to different types of amino acids

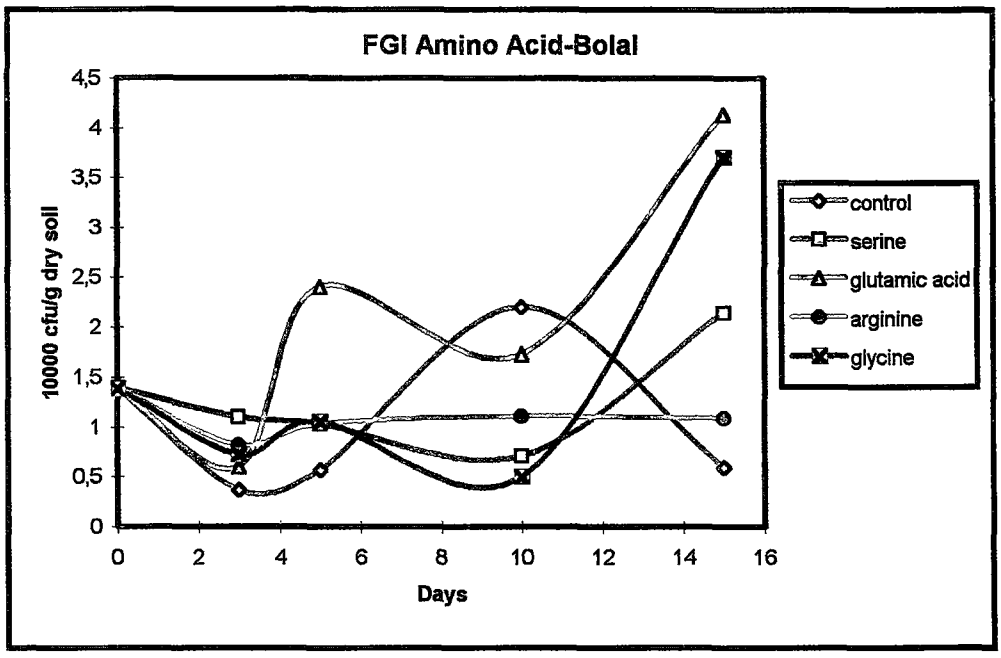


Figure 3.4.5. Growth responses of fungal populations associated with Bolal wheat cultivar grown in FGI medium to different types of amino acids

3.5 Statistical Analysis of Data

Statistical analysis of the data indicated that there was a significant cultivar effect on groups of soil microbe populations investigated in this study. In addition time and substrate amendments had significant ($p < 0.001$) effect on soil microbial populations. The results are summarized as tables in appendix I.

CHAPTER 4

DISCUSSION

The alterations of the numbers of microorganisms belonging to broad categories were compared in terms of the influences of seven different wheat cultivars. The cultivars were growing in the same field side by side as rows. There were no differences in altitute between cultivar rows and they all had the same treatment. Supposedly, the only parameter contributing to the quantitative variations in microbe populations was the cultivars themselves (Ozan *et al.*, 1998).

In BMS medium, which is composed of defined mineral salts, mainly the growth of general K strategist bacteria were favored. K strategists are known to depend upon physiological adaptations in using environmental resources or carrying capacity of the environment and reproduce more slowly than R strategists. However, BNA which is a rich medium containing complex carbohydrates, proteins, vitamins and amino acids, promoted the growth of R strategist bacteria associated with wheat cultivars investigated in this study. They tend to proliferate in situations that are not resource limited (Atlas and Bartha, 1992) This kind of environment tends to cause cells to grow more rapidly and their colonies appear to be larger than those in the mineral salts medium (Mandelstam *et al.*, 1982).

In rich growth media, the bacterial cells attain large sizes. Also in this study the colonies grown in mineral salts medium (BMS) appeared smaller than colonies grown on rich medium (BNA) (Figure 3.1.1; Figure 3.1.2).

Bergey's Manual divides the actinomycetes into seven sections, primarily based on properties such as cell wall type, conidia (asexual, thin walled spores)

arrangement and the presence or absence of a sporangium (Prescott *et al.*, 1993). Actinomycete populations in samples taken from soils of seven different wheat cultivars were cultured on selective ACT medium. Due to the presence of different colony morphologies observed during the experiments, the actinomycete population in the soil samples might have represented groups described in Bergey's Manual (Figure 3.1.3).

In GNB media, the growth of gram negative bacteria were stimulated. Crystal violet was the selective agent favoring the growth of gram negative bacteria while inhibiting gram positive populations (Figure 3.1.4). The Gram negative bacterial colonies were large since GNB was essentially a rich medium.

FGI medium was used for enumeration of soil fungi growing under the influence of cultivars tested. Of the soil organisms, the fungi as a group are the organotrophs primarily responsible for the decomposition of organic residues. They exhibit lower propagule numbers per unit of soil than bacteria (Paul and Clark, 1989). In our studies inclusion of the chloramphenicol inhibited the bacterial growth and gave opportunity to fungi colonise the medium (Figure 3.1.5).

The counts of microbial populations cultured from the soils under the influence of seven different wheat cultivars were compared. The results of this study indicated that Bolal wheat cultivar stimulated the growth of bacterial populations cultured on BMS, BNA & ACT media more efficiently than other wheat cultivars. It also supported the fungal populations to a considerable extent. Kıraç wheat cultivar supported the growth of soil fungi much more powerfully than other cultivars did. However, it did not promote the growth of soil bacterial populations investigated in this study. The lowest number of actinomycete populations was recorded with Kıraç (Figure 3.2.2). The cultivars may exhibit variations in capacities of releasing metabolites into the soil. It has been known that some plant species have tendencies to lose metabolites much more than others. However, some other plant species are thought to evolve mechanisms

which provide the loss of small amounts of water & metabolites. Kıraç cultivar might have similar characteristics with latter ones in terms of releasing capacity of metabolites and water. The result of this study implied that Kıraç cultivar was not suitable for the growth of bacterial populations. Continuous cultivation of this cultivar in long term might affect negatively the bacterial potential in the soil. Microbial biomass N and microbial biomass C are very sensitive to changes in soil management and diminish with the years under cropping (Studdert *et al.*, 1997). This may cause the wheat field to become infertile and can upset the balance of distribution of the soil microorganisms in the soil. Crop rotation and other traditional agricultural practices have been shown to be more appropriate for sustainable agriculture practices (Nieto-Cabrera *et al.*, 1997). If Kıraç were to be considered for cultivation, it would be better to rotate it with other cultivars or species.

The different counts of microbial populations in the seven sampling areas could be due to the differences in root exudations. Plants lose nutrients from their roots into the soil. The nutrients including primary and secondary metabolites change the microbial composition in the soil. Plant species show variations in terms of quality and quantity of metabolites released into the soil. Even these variations can be seen among the cultivars of the same species (Atlas and Bartha, 1992).

Wheat cultivars have generally been obtained through classical breeding methods and they represent new genetic combinations. Different genetic compositions may have an impact on quantity & quality of root exudates. It may in turn affect the microbial community structure in soil under the influence of different cultivars.

The results of this study hinted that there were differences among wheat cultivars in terms of amount and quality of root metabolites. Organic materials released from roots include amino acids, keto acids, vitamins, sugars, tannins, alkaloids, phosphatides, phenolics, and variety of other substances. Although a

few of these materials inhibit the growth of microorganisms, most of them stimulate the growth (Atlas and Bartha, 1992). The results implied that Kıraç might not exude the root metabolites which are necessary for the maximal growth of the bacterial populations in the soil (Figure 3.2.1., Figure 3.2.2., Figure 3.2.3., and Figure 3.2.4.). Nevertheless, they were suitable for the maximal growth of the fungal populations. Fungi can grow on substances with a very low moisture content, generally too low to support the growth of bacteria and usually grow better in an environment with a pH of 5.0, which may be too acidic for the growth of most common bacterial populations. Increases in fungal populations and decreases in bacterial populations occur together with a decrease in the soil pH in the rhizosphere environment which can be caused by the metabolites released from the roots of plant (Nakas and Hagedorn, 1990). Besides these, fungi require less nitrogen than bacteria for equivalent amount of growth (Tortora *et al.*, 1991). Our data suggested that this might be the case for Kıraç.

The ability of bacteria to grow in a wide variety of environmental conditions is a reflection of their metabolic versatility. All bacteria have the same fundamental requirements for growth energy sources, reducing power, carbon, nitrogen and other elements. There is considerable variation in the sources that can be utilized for these purposes. Energy for bacterial growth may be obtained from light or from oxidation-reduction reactions involving inorganic compounds for example H_2S, Fe^{+2} and H_2. The sources of carbon may be carbon dioxide or a large number of organic compounds.

The range of compounds that can be used for growth is characteristic of any given organism. The products of microbial metabolism are often excreted into the growth environment. Although many bacteria can utilize relatively simple compounds as sources of carbon or nitrogen they may also require minute amounts of specific growth factors which they themselves can not synthesize (Mandelstam *et al.*, 1982).

Most of the time the numbers representing microbial populations were greater in cultivar rows compared to lanes between the rows. However, an opposite trend was observed for Gram negative populations. The counts of Gram negative bacteria were lower in the cultivar rows than lanes between the rows. This may indicate the presence of growth inhibitory metabolites in root exudates of the cultivars investigated.

Growth yields of fermenting bacteria have been studied intensively. When growing in nutritionally rich media, bacteria may use carbohydrate fermentation only to generate energy and employ other substances like amino acids as raw material for biosynthesis (Prescott *et al.*, 1993). The growth responses of microbial communities in association with seven different wheat cultivars to different carbon sources were investigated in this study in time dependent fashion. Glucose, glutamic acid and aldicarb were applied to the soil samples.

Microorganisms employ several metabolic pathways to catabolize glucose and other sugars. The ways in which microorganisms degrade sugars to pyruvate and similar intermediates are introduced by focusing on only three routes: (1) glycolysis, (2) the pentose phosphate pathway, and (3) the Entner-Doudoroff pathway. The Embden-Meyerhof or glycolytic pathway is thought to be the most common pathway for glucose degradation to pyruvate. It was encountered in most of the groups of microorganisms and functions in the presence or absence of O_2.

The second pathway, the pentose phosphate or hexose monophosphate pathway may be used concurrently with glycolytic pathway or the Entner-Doudoroff sequence. It can operate aerobically or anaerobically and is important especially in biosynthesis.

Sugars with six carbon atoms, called hexoses are the most important electron donors for many microorganisms and are also important structural components of microbial cell walls, capsules, slimes, and storage products (Brock *et al.*, 1984). Even though, glucose may be expected to be a readily utilizable

carbon source, when compared to glutamic acid, its stimulation was not impressive. Perhaps this is due to the fact that just as glucose is a preferred carbon source by microorganisms in the sense of permitting faster growth than other sugars such as lactose, but a particular nitrogen source which may be an amino acid, would be preferred over others by a given bacterium (Figure 3.3.6) (Mandelstam *et al.*, 1982). However, glucose was the most efficient mainly on the growth of fungal populations under the influence of almost all wheat cultivars except for Gerek and Gün (Figure 3.3.20). Besides fungal populations, gram negative bacteria associated with Kıraç, Gün, and Lancer; were able to use glucose effectively for growth. In addition, bacterial populations representing the soil under the influence of cultivar Bolal cultured on BMS, BNA and ACT media used glucose effectively.

Bolal wheat cultivar did not promote the growth of gram negative bacterial population which can utilize glucose efficiently (Figure 3.3.19). This inability may be due to composition of root exudates released from Bolal. Glucose resulted in response peaks on the growth curve of gram negative bacteria on early days of the whole period. Glucose was readily consumed and the number of gram negative bacteria increased but then the numbers decreased because of the following possible reasons. With sufficient amount of sugar, a higher yield or a continuous growth might have been obtained but at some concentration of glucose another component in the medium would be limiting, e.g. nitrogen, magnesium or sulphur. Moreover, the pH value of the medium might, as a result of the formation of acid products of glucose catabolism, fall to a value at which growth was no longer supported (Mandelstam *et al.*, 1982).

Glucose was utilized most efficiently by fungal populations present in soil under the cultivation of Kırkpınar, Kıraç, Bolal, Bezostaja and Lancer. The reasons may be due to most fungi are more resistant to osmotic pressure than bacteria are; most fungi are therefore able to grow in high sugar or salt concentration (Tortora *et al.*, 1994). Additionally, the fungal genera which are mainly found in the soil samples might be sugar fungi "the zygomycetes" which

can ferment diverse carbohydrate substrates (Paul and Clark, 1989). In this study it was observed that the responses of fungal population to glucose amendments were long-lasting. In addition, the substrate utilization cycles were not completed during the entire experimental period. The reason of this long-lasting growth response of fungi may be due to the formation of acidic by-products as a result of bacterial glucose breakdown. Fungi usually grow better in environments with acidic ranges. Therefore, fungi acquire selective advantage in the presence of glucose.

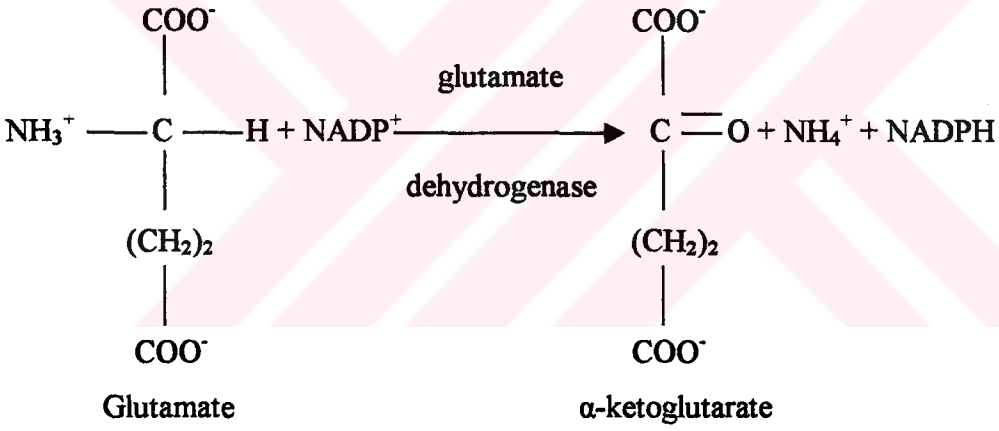
The highest colony counts were recorded for bacterial populations cultured on BMS, BNA and ACT media after glucose additions to soil samples representing Bolal (Figure 3.3.16., Figure 3.3.17., Figure 3.3.18.). This may suggest that the exudates of Bolal supports the glucose metabolizing bacteria.

Among the substrates used in the study, glutamic acid was the most effective substrate in terms of microbial growth (Figure 3.3.11.) It is an acidic, polar amino acid.

The microbial cell is composed of a multitude of organic compounds that together are responsible for structure and physiological activity. These include amino acids, vitamins, purines and pyrimidines. The simplest organic substances that exist free or are bound in polymers are synthesized by the cell, but certain organisms cannot produce one or more of these essential metabolites. Hence, for vegetative growth to proceed, an external source must be provided. These exogenously supplied substances are the growth factors that in low concentrations promote growth (Prescott *et al.*, 1993). The nutritional properties of the bacterial community of soil and root surroundings have been well defined. A large percentage of the native bacteria of soil, the rhizosphere and the root surface exhibits an absolute requirement for amino acids and vitamins. Plant roots excrete an assortment of amino acids. This explains the abundant presence of amino acid auxotrophs in the rhizosphere (Nakas and Hagedorn, 1990).

The first step in amino acid utilization is deamination because before amino acids can be catabolized, they must be converted to substances that can enter into Krebs cycle. The amino group of an amino acid is removed. This is often accomplished by transamination type reactions. The amino group is transferred from an amino acid to an α-keto acid acceptor. The organic acid resulting from deamination can be converted to pyruvate, acetyl CoA, or a TCA cycle intermediate and eventually oxidized in the TCA cycle to release energy. It can also be used as a source of carbon for the synthesis of cell constituents. Excess nitrogen from deamination may be released as ammonium ion. This process may cause the environment to become alkaline (Prescott *et al.*, 1993).

Glutamate has a five carbon skeleton. Transamination or deamination of glutamate produces an intermediate called "α-ketoglutarate" (Lehninger *et al.*, 1993) which enters the TCA cycle to produce energy essential for microbial growth.



The growth of bacteria can be considered either in terms of what is happening in the individual cells or in terms of the growth characteristics of large cell populations. At the level of individual cell behavior, the initiation and duration of events such as chromosome replication and seperation, the synthesis and insertion of new cell wall material and the signals that co-ordinate chromosome replication with cell division. The study of population behavior is concerned with concepts such as the kinetics of growth, the factors that affect the mean generation time, the environmental limits to growth (Mandelstam *et al.*,

1982). The results indicated that glutamic acid utilization and stimulation were common among the microbial groups associated with the wheat cultivars investigated.

It is well established that microorganisms are the major or frequently the only means of degradation and detoxification of several pesticides in the environment (Kumar *et al.*, 1996). From the microbiological standpoint, the majority of soil pollutants are considered as sources of energy or growth inhibitory substances. In this study, it was noted that aldicarb was utilized as a carbon source by some soil microbial populations associated with Gün, Kıraç, Kırkpınar, and Lancer cultivars (Figure 3.3.4., Figure 3.3.14., Figure 3.3.24., Figure 3.3.29.). Especially, gram negative bacterial populations were successful in utilization of this toxic pesticide. It is well known that gram negative bacteria exhibit a great versatility in their ability to utilize various substrates (Mandelstam *et al.*, 1982). That is to say, gram negative bacteria when compared to other microbial populations have more efficient utilization mechanisms for pesticides for their growth. The results obtained in this study were in agreement with the results of Halıcıgil which were concerned with the characterization of the most efficient soil bacterial isolates that can biotransform aldicarb. The characterization studies indicated aldicarb degrading bacteria were aerobic, gram negative, slightly curved rods that occurred singly or in pairs (Halıcıgil, 1995).

A most remarkable nutritional characteristic of microorganisms is their extraordinary flexibility with respect to carbon sources. There is no naturally occuring organic molecule that can not be used by some organisms. Actinomycetes can degrade amyl alcohol, paraffin, and even rubber. Some bacteria seem to be able to employ almost anything as a carbon source, for example, *Pseudomonas cepacia* which is a gram negative bacterial species, can use over 100 different carbon compounds. It was reported that gram negative populations are quite effective in utilization of phenolic compounds released mostly from the roots of leguminous plant species (Ozan *et al.*, 1997).

Unfortunately, many man-made substances like plastics and DDT are degraded slowly or not degraded at all. The soil microbial community also can change its characteristics upon exposure to complex organic molecules. Rhizosphere populations of nonpathogenic and asymbiotic organisms may be either increased or decreased, depending on the pesticide employed and its rate of application. After the community's repeated exposure to a given chemical, faster rates of degradation may occur. A microbial community can become so efficient at rapid herbicide decomposition that herbicide effectiveness is diminished (Prescott *et al.*, 1993).

The wheat cultivars which promoted the growth of gram negative bacteria utilizing aldicarb as a carbon source were mainly Kıraç, Kırkpınar, Gün and Lancer. This shows that the root exudates released from these four different wheat cultivars provided the growth and selection of bacteria with diverse metabolic capabilities.

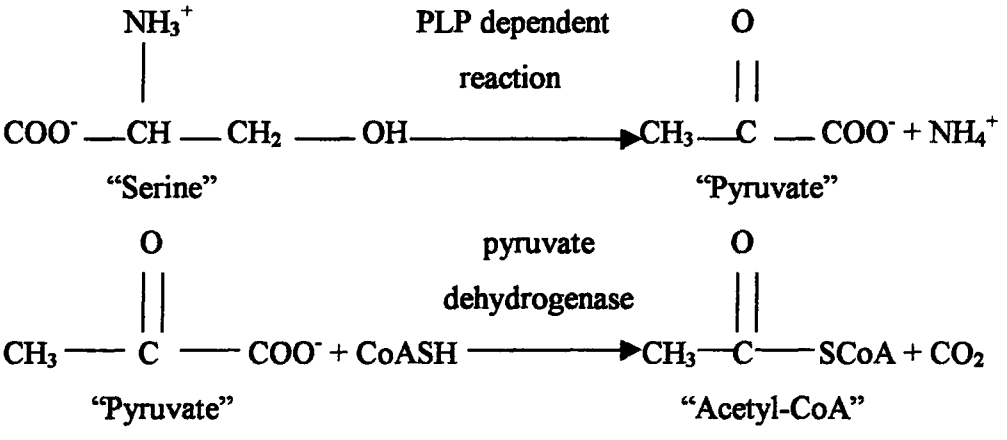
There are a few instances in which catabolic pathways have evolved that are general for xenobiotics. In our study an anacclimated soil was used. The soil was not preexposed to aldicarb application. One of the most common feature for the evolution of catabolic pathways is the repeated application of the same or structurally related pesticides. This event yields a selective build up for microbial populations capable of degrading the pesticides at much faster rates. Enhanced microbial degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of adapted microorganisms. In this study, it was shown that even in anacclimated soil, it is possible to find xenobiotic degraders.

In the third part of the study, the growth responses of microbial populations to different types of amino acids were investigated. The experiments were carried out by using soil sample associated with Bolal. The amino acids employed were arginine, glycine and serine other than glutamic acid used in previous part of the study.

The activity of the catabolic pathways can vary greatly from one amino acid to the next, depending upon the balance between requirements for biosynthetic processes and the amounts of a given amino acid available. The 20 catabolic pathways converge to form only five products, all of which can enter the citric acid cycle. From here the carbons can be diverted to gluconeogenesis or ketogenesis, or they can be completely oxidized to CO_2 and H_2O. All or part of the carbon skeletons of ten of the amino acids are ultimately broken down into α-ketoglutarate, four into succinyl-CoA, two into fumarate, and two into oxaloacetate (Lehninger *et al.*, 1993).

When the effectivenesses of the amino acids were compared in terms of stimulating the growth of microbial populations, in this study, the data indicated that serine was the most readily utilized amino acid by bacterial populations cultured on BNA, BMS, and ACT media (Figure 3.4.1., Figure 3.4.2., Figure 3.4.3.). Also immediately it caused the greatest peaks of growth response. This may indicate that besides utilized as a carbon source serine was a crucial growth factor. Ideally, the amount of microbial growth is directly proportional to the quantity of growth factors present. If the growth factor concentrations double, the final extent of bacterial growth also doubles (Prescott *et al.*, 1993).

Serine is a neutral, polar amino acid. It is converted to pyruvate by serine dehydratase. Both the β-hydroxyl and the α-amino groups of serine are removed in PLP (Pyridoxal phosphate)-dependent reaction



Arginine was used most efficiently by actinomycete populations. The response of actinomycetes to arginine was delayed compared to serine (Figure 3.4.3.). Arginine is a basic amino acid. It contains five adjacent carbons and a sixth carbon attached through a nitrogen atom. Arginine enters the TCA cycle after being firstly converted to glutamate which then is converted to α-ketoglutarate that is an intermediate substance in the TCA cycle.

In general, glycine was not a preferred amino acid as a carbon source for the growth of microorganisms compared to the other amino acids tested in the study. Glycine is converted to pyruvate that follows the conversion to AcCoA which enters the TCA cycle. Glycine has the simplest amino acid structure and nonpolar. This nonpolar nature may render this amino acid to be hardly reactive for microbial reactions.

Serine and glycine follow the same reactions to be used in catabolic pathways to generate energy. However, in this study, it appeared that serine exerted its growth stimulating effect maximally on more of the bacterial populations cultured on BNA, BMS and ACT media whereas glycine did not have a good growth promotion effect on those bacterial populations. This may indicate that glycine was not utilized as a growth factor which is needed in protein synthesis. Instead it was catabolised to generate energy. But it was inefficient to enter TCA cycle to produce energy due to the shortage or lack of intermediates to generate OAA. Therefore, the TCA cycle may not operate efficiently.

In this study, seven different wheat cultivars were studied and general categories of soil microorganisms were investigated. According to the results of this study the wheat cultivars affected the soil microbe populations differentially. This implied that root metabolites released from the seven wheat cultivars investigated had differences in terms of quantity and quality. These differences appeared to cause alterations of composition in terms of numbers of soil microorganisms. The results of the study presented here pointed out that in the

selection of wheat cultivars to be applied in sustainable agriculture practices, the wheat-microorganism interactions should be included as one of the criteria.

CHAPTER 5

CONCLUSION

- Soil microbe populations were altered under the influence of different wheat cultivars-Eg. Bolal cultivar supported more of the bacterial populations, Kıraç, however, exerted a positive effect on stimulating soil fungi.

- Most of the time the bacterial populations responded maximally to glutamic acid compared to glucose, aldicarb and moisture effect.

- Aldicarb, which is a carbamate pesticide was utilized as a carbon source most efficiently by gram negative bacterial populations among the other populations tested in the study.

- Even though aldicarb was used as a carbon source by gram negative bacterial populations, it did not cause pronounced growth responses.

- Among the four amino acids tested, serine was found to be most effective on the Actinomycete populations as well as the populations of bacteria cultured in minimal (BMS) and rich (BNA) media; Glutamic acid exerted its stimulating effect on Gram negative bacteria and soil fungi.

REFERENCES

Ahmed, H. U., Mundt, C. C., Hoffer, M. E., and Coakley, S. M., 1996."Selective influence of wheat cultivars on pathogenicity of *mycosphaerella graminicola* (anamorph *Septoria tritici*)", *Phytopathology* , 86, 454.

Aldwell, C. R., 1997. "Hydrogeology and sustainable agriculture", *Environmental Geology*, 32(2), 93.

Atlas, R. M., and Bartha, R., 1992. " Microbial ecology: Fundemantals and applications", *Third Edition, The Benjamin/Cummings Publishing Company, Inc. U.S.A.* pp. 8-9,69-83,131, 176, 275, 301-303, 383, 400.

Barea, J. M., Calvet, C., Estaun, V., and Camprubi, A., 1996. " Biological control as a key component in sustainable agriculture" , *Plant and Soil* 185: pp. 171-172.

Bartos, P., Stuchlikova, E., and Hanusova, R., 1996 . "Adaptation of wheat rusts to the wheat cultivars in former Czsechoslovakia", *Euphytica* , 92, pp. 95-97

Borneman, J., Skroch, P. W., O'sullivan, K. M., Palus, J.A., Rumjanek, N. G., Jansen, J. L., Nienhus, J., and Triplett, E. W., 1996. "Molecular microbial diversity of an agricultural soil in Wisconsin", *Applied and Environmental Microbiology*, 62(6), 1935.

Brock, T. D., Smith, D. W., and Madigan, M. T., 1984. "Biology of microorganisms", *Fourth Edition, Prentice-Hall Int. Inc. New Jersey . U.S.A.* pp. 171.

Cabrera, C. N., Francis, C., Caicedo, C., Gutierrez, P. F., and Rivera, M., 1997. "Response of four andean crops to rotation and fertilization", *Mountain Research and Development*, 17(3),273.

Chanway, C. P., 1997. "Inoculation of tree roots with plant growth promoting soil bacteria: An emerging technology for reforestation", *Forest Science*, 43(1),99.

Charpalamadugu, S., and Chaudhry, G. R., 1991. " Hydrolysis of carbaryl by a *Pseudomonas* sp. and construction of a microbial consortium that completely metabolizes carbaryl", *Applied and Environmental Microbiology*, 57, 744.

Chaudhry, G. R., and Ali, A., 1988. " Bacterial metabolism of carbofuran" , *Applied and Environmental Microbiology*, 54,1414.

Einhelling, F. A., 1996. "Interactions involving allelopathy in cropping systems" , *Agronomy Journal* , 88(6), 86.

Felske, A., Wolterrink, A., van Lis, R., Akkermans, A.D.L., and de Vos, W.M., 1998. "Specific Reactions of a Soil Bacteria Community to Grassland

Succession as monitored by 16s rRNA Levels", *8th International Symposium on Microbial Ecology,Halifax, CANADA.* 16.150.

Fokkema, N. J., 1996. "Biological control of fungi plant diseases" , *Entomophaga,* 41(3/4), 333.

Friedman, I., 1994. "Permafrost as microbial habitat" , *Permafrost Environment,* pp. 21-26.

Golinski, P., Kostecki, M., Losacka, I., Wisniewska, J., and Kaczmarek, Z., 1996. "Moniliformin accumulation and other effects of *Fusarium avenaceum* (Fr.) Sacc. on kernels of winter cultivars", *J. Phytopathology*, 144,495.

Goulding, C., Gillen, C. J., and Bolton, E., 1988. *J. Applied Bacteriology*, 65, 1-5.

Gutsche, V., and Rossberg, D., 1997. "Synops 1.1: A model to assess and to compare the environmental risk potential to activate ingredients in plant protection products", *Agriculture, Ecosystems & Environment* , 64, 181.

Halıcıgil, C., 1995, *Ms. Thesis* "Aldicarb Biodegradation by Soil Bacteria"

Hartmann, A., Aßmus, B., Kirchhof, G., and Schloter, M., 1997. "Direct approaches for studying soil microbes", *Modern Soil Microbiology by Marcel Dekker, INC.*

Hassal, K. A., 1990. " The biochemistry and uses of pesticides: Structure, metabolism, mode of action and uses in crop protection", *McMillan Press Ltd. London*, 125.

Havlickova, H., Cvicrova, M., and Eder, J., 1996. "Phenolic acids in wheat cultivars in relation to plant suitability for and response to cereal aphids", *Journal of Plant disease and Protection*. 103(5) ,535.

Hetrick, B. A. D., Wilson, G. W. T., and Todd, T.C., 1995. "Mycorrhizal response in wheat cultivars: relationship to phosphorus", *Can. J. Bot*. 24,19.

Heuer, H., Zock, A., and Smalla, K., 1998. "Monitoring of Rhizosphere Communities by DGGE-Fingerprints and Their Detailed Analysis by V6-Probing and Sequencing", *8th International Symposium on Microbial Ecolog, Halifax, CANADA*..16, 176.

Hogan, D. A., Buckley, D. H., Nakatsu, T. M., and Hausinger, R. P., 1997. "Distribution of the *tfd*A gene in soil bacteria that do not degrade 2,4-dichlorophenoxyacetic acid (2,4-D)", *Microbial Ecology*. 34,90.

Hokkanen, H.M.T., and Lynch, J.M., 1995. "Biological control- benefits and risks", *Cambridge University Press, Cambridge, UK*.. 304.

Horst, W. J., Abdou, M., and Wiester, F., 1996. "Differences between wheat cultivars in acquisition and utilization of phosphorus", *Z. Pflanzenernähr. Bodenk.* 159, 155.

Hosein, S. G., Milette, D., Butler, B. J., and Greer, C. W., 1997. "Catabolic gene probe analysis of an aquifer microbial community degrading creosote-related polycyclic aromatic and heterocyclic compounds", *Microbial Ecology*, 34, 81.

Hu. S., and Van Bruggen, A. H. C., 1997. "Microbial dynamics associated with multiphasic decomposition of ^{14}C-labelled in soil", *Microbial Ecology*, 33,134.

Kelly, T. C., Lu, Y., and Teasdale, J., 1996. "Economic-environmental tradeoffs among alternative crop rotations", *Agriculture, Ecosystems & Environmental*, 60,17.

Kiss, J., Penszka, K., Tóth, F., and Kádár, F., 1997. "Evaluation of fields and field margins in nature production capacity with special regard to plant protection", *Agriculture, Ecosystems & Environmental*, 63,227.

Kolmer, J. A., 1996. "Genetics of resistance to wheat leaf rust", *Annu. Rev. Phytpathol.* 34,435.

Kumar, S., Mukerji, K. G., and Lal, R., 1996. "Molecular aspects of pesticide degradation by microorganisms", *Critical Reviews in Microbiology*.22(1), 1.

Lang, E., Eller, G., and Zadrazil, F., 1997. "Lignocellulose decomposition and production of ligninolytic enzymes during interaction of white rot fungi with soil microorganisms", *Microbial Ecology*. 34, 1.

Lauret, R., Eville, F. M., Berthier, F., Verges, M. C., Postma, P., Ehrlich, S. D., and Zagorec, M., 1996. "Carbohydrate utilization in *Lactobacillus sake*", *Applied and Environmental Microbiology*. 62, 1922.

Lehninger, A. L., Nelson, D. L., and Cox, M. M., 1993. "Principles of biochemistry", *Second Edition, Worh Publishers, New York, U.S.A.* pp.115-116, 522-526, 532-536.

Leštan, D., and Lamar, R. T., 1996. "Development of fungal inocula for bioaugmentation of contaminated soils", *Applied and Environmental Microbiology*. 62,2045.,

Lew, H., Chelkowski, J., Wakulinski, W., and Edinger, W., 1993. " Moniliformin in wheat and triticale grain" *Mycotoxin Research*, 9, 65.

Liu, J. Q.., and Kolmer, J. A., 1995. "İnheritance of leaf rust resistance in resistance in wheat cultivars grandin and CDC teal ", *Plant disease*. 81, 505.

Ma, H., Singh, R. P., and Abdalla, O., 1997. "Resistance to stripe rust in five durum wheat culture", *Plant Disease*. 81, 27.

Mallik, M. A. B.,1997. "Isolates of soil actinomycetes with potential for phytotoxin production", *Journal of Chemical Ecology*. 23(12), 2683.

Mallik, M. A. B., and Watson III, C., 1997. "Stimulation of Growth and nodulation of soybean and other plants by crop residues", *Allelopathy Journal.* 5(1), 13.

Mandelstam, J., McQuillen, K., and Dawes, I., 1982. "Biochemistry of bacterial growth", *Third Edition, Blackwell Scientific Publications, Edinburg, Great Britain.* pp. 16, 47, 106, 345, 393-395.

Mavingui, P., Berge, O., and Heulin, T., 1990. "Symbiosis", 9: 215-221

May, R., Völksch, B., and Kampmann, G., 1997. "Antagonistic activities of epiphytic bacteria from soybean against *Pseudomanas syringea* Pv. *Glycenia* in vitro and in planta", *Microbial Ecology*. 34, 118.

Mcsorley, R., and Gallahe, R. N., 1997. "Effect of compost and maize cultivars on plant-parasitic nematodes", *Supplement to the Journal of Nemotology*. 29(4S), 731.

Microsoft Office Excel 97 Version

Moore, M. T., Hugget, D. B., Gillespie, Jr. W. B., Rodgers, Jr. J. H., and Cooper, C. M., 1998. "Comperative toxicity of chlordane, chlorpyrifos, and aldicarb to four aquatic testing organisms ", *Arch. Environ. Contam. Toxicol.* ,34,152.

Nakas, J. P., and Hagedorn, C., 1990." Biotechnology of plant-microbe interactions", *McGraw-Hill Publishing Co.U.S.A.* pp.206-209.

Natsch, A., Keel, C., Troxer, M., Albertini, N. V., and Défago, G., 1996. "Importance of preferential flow and soil management in vertical transport of biocontrol strain of *pseudomanas fluorescens* structured field soil", *Applied and Environmental Microbiology*. 62(1), pp. 33.

Nieto-Cabrera, C., Francis, C., Caicedo, C., Gutierrez, P.F., and Rivera, M., 1997. "Response of Four Andean Crops to Rotation and Fertilization", *Mountain Research and Development.* 17(3), pp. 273-282.

O'leske, D. L., Robel, R. J., and Kemp, K. E., 1997. "Sweepnet-collected invertebrate biomass from high- and low-input agricultural fields in Kansas", *Wildlife Society Bulletin.* 25(1),133.

Ou, L.T., Edvardsson, S.V., Rao, P.S.C., and Wheeler, W.B., 1986. "Aerobic and

Cultivars",*8th International Symposium on Microbial Ecology, Halifax, Canada.* 19,259.

Page, S., and Burns, R.G., 1991. "Soil Biol. Biochem." 23, 1025.

Paul, E. A., and Clark, F. E., 1989. "Soil microbiology and biochemistry", *Academic Press Inc., London.*, pp. 46, 55, 79, 81-88, 133.

Prescott, L. M., Harley, J. P., and Klein, D. A., 1993. "Microbiology", *Second Edition, Wm. C. Brown Communications, Inc., U.S.A.* pp. 97-99, 118-119, 47, 160-161, 180-181, 507-509, 860.

Racke, D., and Coats, J. R., 1988. " Enhanced Degradation and the comperative fate of carbamate insecticides in soil" , *J. Agric. Food Chem.*, 36, 1067.

Read, D. C., 1987. " Greatly accelerated degradation of aldicarb in re-treated field soil, and in water", *Journal of Economics Entomology*, 80(1), 156.

Rice, E. L., 1998."Biological controls of Weeds and plant diseases: Advances in applied Allelopathy", *Journal of Chemical Ecology*.24(3), 597.

Schmidt, J. P., Buol, S.W., and Kamprath, E. J., 1997. "Soil phosphorus dynamics during 17 years of continous cultivation: A method to estimate long-term P availability", *Geoderma*.78,59.

Shi, T., Reeves, R. H., Gilichinsky, D. A., and Friedmann, E. I., 1997. "Characterization of viable bacteria from siberian permafrost by 16s rDNA sequencing", *Microbial Ecology*. 33,169.

Shilling, D.G., Liebl, R.A., and Worsham, A.D., 1985. "The suppression of certain broadleaf weeds and the isolation and identification of phytotoxins", In *The Chemistry of Allelopathy.* ACS Symposium Series No. 268: 243-271.

Sokal, R. R., and Rohlf, F. J., 1981. "Biostatistics", Second Edition, W.H. Freeman and Company San Francisco U.S.A.

Staddon, W. J., Duchense, L. C., and Trevors, J. T., 1997. " Microbial diversity and community structure of post disturbance forest soil as determined by sole-carbon-source utilization patterns", *Microbial Ecoloy*. 34,125.

Stoltzfus, J. R., So, R., Malarvithi, P. P., Ladha, J. K., and de Bruijn, F. J., 1997. "Isolation of endophytic bacteria from rice and assessment of their potential for suplying rice with biologically fixed nitrogen", *Plant and Soil*.194, 25.

Studdert, G. A., Echeverría, H. E., and Casanovas, E. M., 1997. " Crop-pusture rotation for sustaining the quality and productivity of a typic Argiudoll", *Soil Sci. Soc. Am. J.* 61,1466.

Suett, D. L., and Jukes, A. A., 1988. "Accelerated degradation of aldicarb and its deoxidation products in previously treated soils". *Crop Protection*, 7, 1467.

Takumi, S., and Shimada, T., 1997 "Variation in transformation frequencies among six common wheat cultivars through particle bombardment of scutellar tissues", *Genes & Genetic Systems*.72(2), 63.

Takumi, S., and Shimada, T., 1997. "Genetic transformation of durum wheat (*Triticum durum* Desf.) through article bombardment scutellar tissues", *Plant Biotechnology*.14(3), 151.

Tortora, G. J., Funke, R. B., and Case, C. L., 1994. "Microbiology: An Introduction ", *Fifth Edition, The Benjamin/Cummings Publishing Company, Inc.* pp. 125-127, 159, 284-285, 299-300, 665-673.

Union Carbide, Agricultural Products Company, Inc. 1983. " Temik aldicarb pesticide: A scientific assesment" , *Union Carbide Research*, Triangle Park, NC, pp 71.

Vadez, V., Beck, D. P., Lasso, J. H., and Drevon, JJ., 1997. "Utilization on the acetylene reduction assay to screen for tolerance of symbiotic N_2 fixation to limiting P nutrition in common bean", *Physiologia Plantarum*. 99,222.

van Deer Voet, E., Kleijn, R., and Udo de Haes, H. A., 1996. "Nitrogen pollution in the European Union- an economy-environment confrontation", *Environmental Conservation*. 23(3), 198.

Van Elsas, J.D., Gelsomino, A.A., Postma, J.J., and Duarte, G.G.F., 1998. "Microbial Populations in Soil and Rockwool/Plant Systems Assessed by Molecular Methods", *8th International Symposium on Microbial Ecology, Halifax, CANADA*. .16,334.

Waage, J., 1996. "Yes, but does it work in the field? The challenge of technology transfer in biological control", *Entomophaga*. 41(3/4), 315.

Watts, J.E.M., and Wellington, E.M.H., 1998. "Analysis of Bacterial Communities in Soil Using Molecular and Metabolic Techniques", *8th International Symposium on Microbial Ecology,Halifax, CANADA*. 16,344.

Xu, L., Li, Q. , Jiang, C., 1996. "Diversity of soil actinomycetes in Yunnan , China" *Applied and Environmental Microbiology*, 62(1), 244.

APPENDIX I

STATISTICAL ANALYSIS OF DATA

ANOVA: Two-factor with replication /BMS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1465071,3	4	366268	114,252	1,3E-57	2,40389
Columns	3105556,2	27	115021	35,879	3,8E-75	1,5257
Interaction	17763818	108	164480	51,3071	3E-138	1,29105
Within	897621,33	280	3205,79			
Total	23232067	419				

ANOVA: Two-factor with replication/BNA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	413104	4	103276	25,8406	3,1E-18	2,40389
Columns	4226989	27	156555	39,1715	3,1E-79	1,5257
Interaction	9297894	108	86091,6	21,5409	6,9E-91	1,29105
Within	1119064	280	3996,66			
Total	1,5E+07	419				

ANOVA/Two-Factor With Replication/ACT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	569329	4	142332	63,9479	2,4E-38	2,40389
Columns	3174850	27	117587	52,8302	9,8E-94	1,5257
Interaction	7811390	108	72327,7	32,4958	8E-113	1,29105
Within	623211	280	2225,75			
Total	1,2E+07	419				

ANOVA: Two-factor with replication/GNB

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2229709	4	557427	347,064	4E-107	2,40389
Columns	2834748	27	104991	65,369	1E-104	1,5257
Interaction	6362530	108	58912,3	36,6798	2E-119	1,29105
Within	449715	280	1606,12			
Total	1,2E+07	419				

ANOVA: Two-factor with replication/FGI

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	243261	4	60815,2	162,376	1,1E-71	2,40389
Columns	886334	27	32827,2	87,6482	5E-120	1,5257
Interaction	3428428	108	31744,7	84,758	3E-167	1,29105
Within	104869	280	374,533			
Total	4662892	419				

ANOVA: Two-factor with replication/BMS Bolal

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	112190	3	37396,7	6,91283	0,00102	2,90112
Columns	850441	3	283480	52,4017	1,9E-12	2,90112
Interaction	1530485	9	170054	31,4347	2E-13	2,18876
Within	173112	32	5409,75			
Total	2666228	47				

ANOVA: Two-factor with replication/BNA
Bolal

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	257195	3	85731,7	91,6589	8,5E-16	2,90112
Columns	601819	3	200606	214,476	2,9E-21	2,90112
Interaction	1348719	9	149858	160,218	5E-24	2,18876
Within	29930,7	32	935,333			
Total	2237664	47				

ANOVA: Two-factor with replication/ACT/Bolal

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	35331	3	11777	12,1611	1,8E-05	2,90112
Columns	457961	3	152654	157,632	3E-19	2,90112
Interaction	779630	9	86625,6	89,4507	4,1E-20	2,18876
Within	30989,3	32	968,417			
Total	1303912	47				

ANOVA: Two-factor with replication/GNB/Bolal

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	104779	3	34926,3	143,092	1,28488E-18	2,90112
Columns	100021	3	33340,3	136,594	2,55992E-18	2,90112
Interaction	260205	9	28911,7	118,45	5,45505E-22	2,18876
Within	7810,67	32	244,083			
Total	472816	47				

ANOVA: Two-factor with replication/FGI/Bolal

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	116439	3	38813,1	25,2484	1,4E-08	2,90112
Columns	109909	3	36636,2	23,8323	2,7E-08	2,90112
Interaction	121344	9	13482,7	8,77064	1,7E-06	2,18876
Within	49192	32	1537,25			
Total	396884	47				