THE ROLE OF BACTERIA IN WATER CONSUMPTION REDUCTION ON GERMINATION GROWTH OF WHEAT

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ABSTRACT

Soil is one of the most important and various habitats with species enrichment in earth and also it has one of the most diverse live creatures. Almost, any branch of classification of live creatures above soil is observed in soil and it has full species variety.

After consecutive culture in specific media and initial biochemical tests, the bacteria were isolated and identified. After the selection of best bacteria on germination growth of wheat seed, Chamran diversity was investigated.

The isolated bacterium of earthworm belongs to bacillus, Aeromonas, Flavobacterium and Arthrobacter and the highest growth was dedicated to bacillus and lowest one to Arthrobacter compared to control sample as water and germination growth in bacterium sample was high.

Earthworms in their guts strengthen or weaken the growth of important bacteria in terms of agriculture. These bacteria establish symbiotic relationship with mutual benefits with earthworm gut passage. The water consumption in the media with bacteria had rapid germination growth compared to water media.

Keywords: Earthworm, Bacillus, Wheat seed, Water consumption reduction

INTRODUCTION

Based on water resources and consumption per capita , Iran is one of the countries as located in the group of countries with physical water shortage. This group includes the countries as encountered with physical shortage of water in 2025. It means that even with the highest output in water consumption, they don't have adequate water. About 25% of people in the world are included in this group. According to Falcon mark, Iran is under water crisis condition. In 2001, 2011, about 69% of total renewable water are applied annually and based on UN index, Iran is under serious water crisis. According to international institute of water management, Iran is exposed to serious water crisis [3]. According to the mentioned indices, Iran should increase 112% to water resources and this value is impossible based on water resources.

Soil is one of the most important habitats with species enrichment in earth and consisting of one of the best compositions of various live creatures. Almost, each branch of classification of recognized live creatures above soil is observed in soil and it has an enrichment of species variety [6].

Water supply as an ecosystem service via water influence in soil pores is effective on plant productions. Soil invertebrates play important role via biological and drilling movement. The variety, form and their size allow that water is stored in great range of soil water potential. The activity of snails, earthworms and other earth creatures increase water penetration into soil. Earthworms in their guts strengthen or weaken the growth of important bacteria in terms of agriculture. These bacteria establish symbiotic relationship with mutual benefits with earthworm gut passage. Some measurements as deforesting, pollution and urbanization are the major reasons of deforesting and other plants with Fauna, macrofauna and megafauna [9].

Improvement of agriculture soils by earthworms is done by two ways: Via adding worm directly to soil or adding their excretion as it is biohumus. Humus of earthworms is called vermicompost and it is the excretion of worms being fed of urban waste, cow excretion, domestic sludge or any other organic matter.

One of the important acts of earthworms is they eat anaerobic bacteria and fungus as their presence is unsuitable and also they eat the seed of weeds. They crush the organic matters into very fine particles and increase the activities of useful microbes to increase fertilizer. As these materials are excreted from the worm body, a covering

can be useful in gradual release of vermicompost food [5].

The scientists have discovered that the bacteria grown up by earthworms are Azotobacter and can fix azoth and make phosphorus available. Also, they can generate different vitamin and plant hormones as excreted. Vermicompost is equal to the best soil humus in terms of quality as generated by natural factors. Quality of vermicompost depends upon the type of food the worms eat. Although vermicompost have little difference, in their decomposition, high amount of calcium, potassium, sodium and solution phosphoric acid are observed. Great amount of azoth in vermicompost is like urea, uric acid and humicacid and its residual is like Ammonium and nitrate. This fertilizer consists of great amount of plant hormones. Vermicompost has great effect on physical, chemical and biological features of soil as it has rich food elements (e.g. Azoth) as giving them gradually to plant. This issue is of great importance in terms of soil fertility. Also, it stores water in the particles many times more than its weight and finally provides grading and food storage of plants [8]. Jolly et al., found that there was a physical interaction between the filamentous bacteria and the mucus of the intestine of earthworm Octalasionlacteum and Lumbricusterrestris. They were found

to be attached by the hook structures in the walls. Hence, it was concluded, that bacteria adapted to live within the intestine of earthworms. Thus, various studies have been conducted on main bacteria within the intestine of earthworms [6].

Hyung Jung (2004) investigated Aerobic intestinal bacterial community structure of earthworm, Eiseniafetida, based on 16S rDNA analysis. Ninety-one different colonies grown on Brain Heart Infusion medium were randomly isolated under aerobic condition. Based on partial sequence analysis of PCR-amplified 16S rDNA for strains, earthworm intestinal aerobic bacteria (EIAB) were divided into 12 groups, and each group was further divided into subgroups. Groups included 6% Aeromonas, 3% Agromyces, 31% Bacillus, 1% Bosea, 6% Gordonia, 6% Klebsiella, 7% Microbacterium, 2% Nocardia, 10% Pseudomonas, 19% Rhodococcus, 2% Tsukamurella, and 7% Streptomyces, with Bacillus being dominant group [5].

Later, Valle Molinares et al (2007)identified 7 species of bacteria from the genus Bacillusm within the intestine of Onychochaetaborincana. All these species are typical soil bacteria. In addition, it was found that the microbial weight of the intestinal region decreased from the anterior to posterior section. It was

observed that some bacteria increased in the posterior section of the intestines, because this portion presents conditions for their development [4].

Isolation of bacteria in earthworm gut

Sampling was made of agriculture soil around Fars research and science University with earthworms and also they were taken. Later, the specimen were transferred by sterile method to Lab. After washing earthworm, they were transferred into dissection room. Then, they worms were anesthetized by chloroform and by a sterile scissor, an incision was made longitudinally in stomach, then the content of stomach was transferred into a sterile dish, then the specimen were transferred for bacteria isolation to microbiology lab.

Sampling and bacteria count

After transferring the samples (earthworm) to lab, bacteria counting was made by Total viable plate count. In this method by specimen in the gut of earthworms, dillution 10 to 10^{-9} was provided and surface plat was performed in nutrient agar, blood agar and MacConkey . After 24-48 hours, they were incubated at 30℃. The number of colonies were isolated in culture media.

Sterile dissecting

The gut sections were washed in sterile distilled water to free their contents before being suspended in other bottles containing clean

Identification of isolated bacteria

Appropriate strains of bacteria in earthworm gut were identified via microbial and biochemical tests:

Gram Staining, Carbohydrate Fermentation, H2S Production Test, IMVIC Test, Urease Test, Catalase Test, Oxidase Test, Litmus Milk Reaction, Starch Hydrolysis, Gelatin Hydrolysis Test, Spor Staining, Motility Test (Danareski et al., 1986).

RESULTS

Isolation of the best bacteria in earthworm gut:

The bacteria in earthworm gut were purified on nutrient agar medium and biochemical tests were performed for each of them and the results were defined.

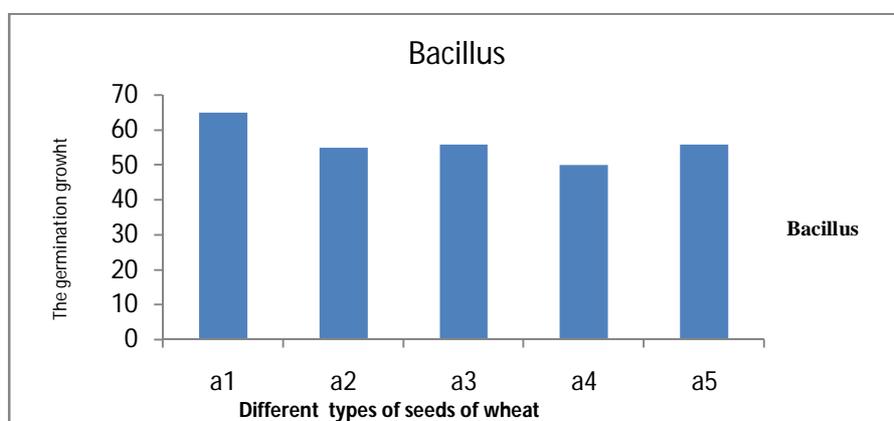The effect of existing bacteria in earthworm gut on growth of wheat

The highest effect of bacteria on what is dedicated to bacillus, Aeromonas, Flavobacterium and Arthrobacter.
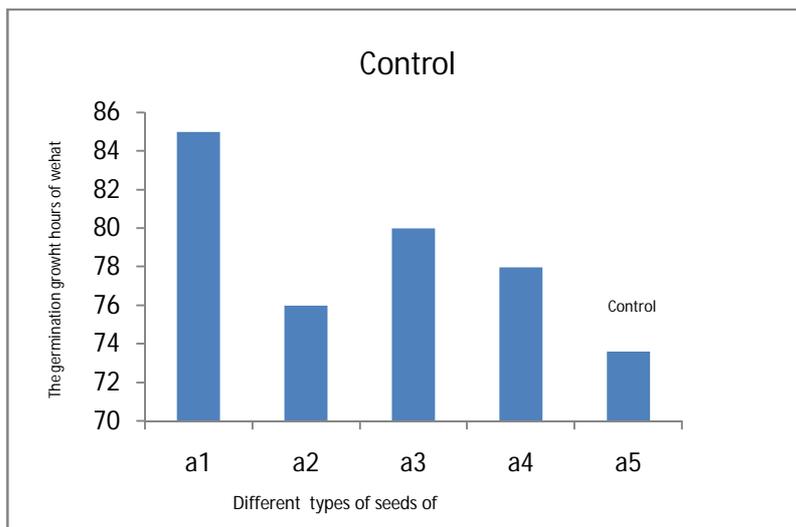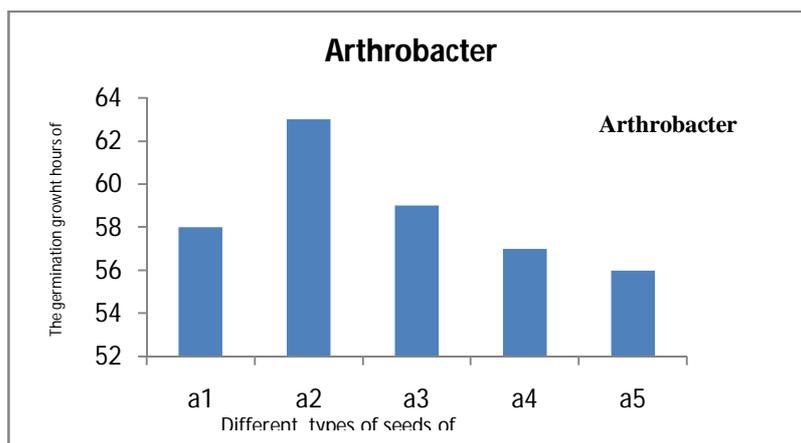
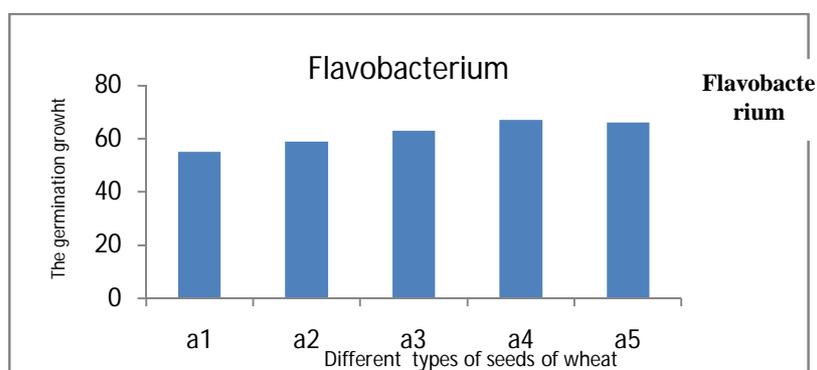**The germination growth of wheat (without bacterium)**
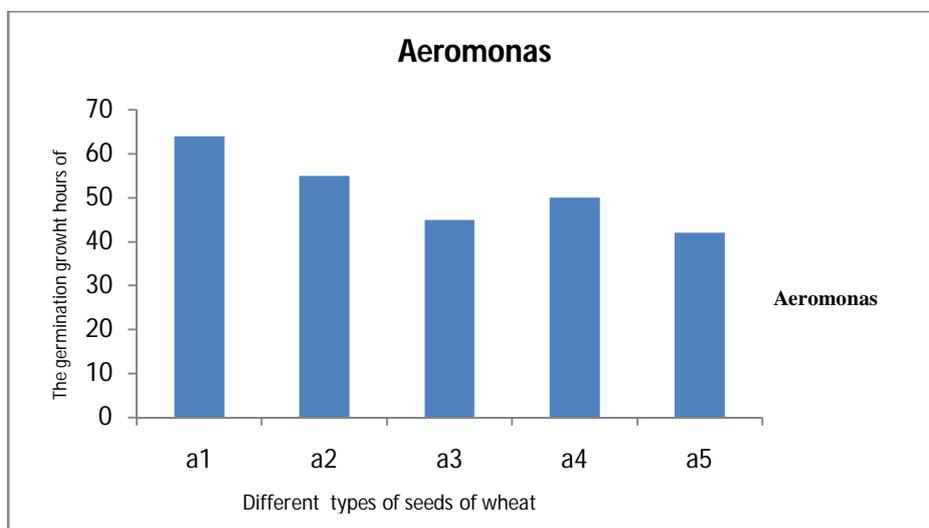
The germination growht of wheat, Chamran cultivar (by adding Aeromonas)

The germination growht of wheat, Chamran cultivar (by adding Bacillus)

Arthrobacter

The germination growht of wheat, Chamran cultivar (by adding Arthrobacter)

Flavobacterium

The germination growht of wheat, Chamran cultivar (by adding Flavobacterium)

DISCUSSION AND CONCLUSION

Earthworms in their guts strengthen or weaken the growth of important bacteria in terms of agriculture. These bacteria establish symbiotic relationship with mutual benefits with earthworm gut passage. The variety of these microorganisms is based on vital conditions and some factors as temperature, humidity, apparent density, PH and organic matter of their food [1]. Earthworms have wide bacteria diversity in their gut but due to non-cultivation features, they are not studied. Soil is a good environment to develop microorganisms of Prokaryotic and eukaryotic and viruses and bacteriophages are present [9].

The bacterial species reported within the intestines of the earthworms belong to the genuses bacillus, Aeromonas, Pseudomonas, Flavobacterium, Nocardia , Gordonia, Vibrio, Closflidium, Proteus, Serrano, Mycobacterium,Klebsiella, , Azotobacter and Arthrobacter. These bacteria inhabit the soil and develop considerably when there are easily degradable organic soil nutrients. The bacterial community inside the digestive tract of earthworms is associated to at least four physiological groups: plant growth promoters, free living nitrogen fixers,

biocides and phosphate solubilizers. The diversity of bacterial communities within the digestive tracts of earthworms depends on climate, soil type and organic matter.

Hyung Jung (2004) investigated Aerobic intestinal bacterial community structure of earthworm, Eiseniafetida, based on 16S rDNA analysis. Ninety-one different colonies grown on Brain Heart Infusion medium were randomly isolated under aerobic condition. Based on partial sequence analysis of PCR-amplified 16S rDNA for strains, earthworm intestinal aerobic bacteria (EIAB) were divided into 12 groups, and each group was further divided into subgroups. Groups included 6% Aeromonas, 3% Agromyces, 31% Bacillus, 1% Bosea, 6% Gordonia, 6% Klebsiella, 7% Microbacterium, 2% Nocardia, 10% Pseudomonas, 19% Rhodococcus, 2% Tsukamurella, and 7% Streptomyces, with Bacillus being dominant group.

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because this portion presents conditions for their development [7.

In the present study, four gorups of bacteira as Bacillus subtilis, Azotobacter and Arthrobacter, Flavobacterium and Aeromonas were identified.

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