



KINGDOM OF SAUDI ARABIA KING SAUD UNIVERSITY COLLEGE OF SCIENCE BOTANY AND MICROBIOLOGY DEPARTMENT

Isolation and identification of fungai and count bacteria in soil

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Introduction

Soil fungi

Soil fungus is the beginning of a soil food network that helps other living organisms in the soil and supports healthy soil functions. They are cells that resemble microscopic plants. They may be single cells or grow in long, stringy structures. They do not depend on certain types of plants, As they are slow to grow, these fungi prefer acidic environment, perennial plants, slow recycling time, high stability forms of organic residues containing high values of carbon and nitrogen, and direct nutrient sources directly from the plant.

Benefits of soil fungus

Some 460 million years ago, some species of soil fungus have developed a symbiotic relationship with plants. These fungi offer several benefits to soil:

Get nutrients:

Soil fungus helps plants acquire various nutrients such as: nitrogen, zinc, calcium, iron, potassium, copper,

phosphorus, and magnesium.

Protection against disease:

Fungi protects the roots from pathogens, and also provides them with antibiotics.

Formation of fertile soil

Soil fungi help form soil that allows the movement of water, air, microbes, nutrients and organic matter through them.

Drought resistance:

The fungus activates the cells responsible for the absorption and transport of water, which contributes to moisturizing the plant, and in times of drought, these fungi to maintain the physiological activity in the plant cell such as photosynthesis.

Types of Soil fungi

Soil fungus is divided into three groups depending on how they obtain energy:

Analyzers: are fungus that converts dead organic matter into innate biomass, small particles, and carbon dioxide. Takaful is the fungus that provides plant nutrients in exchange for carbon.

Insecticide: A fungus that causes illness or death of plants.

Mycorrhizal Fungi in Agriculture

Mycorrhiza is a symbiotic association between fungi and plant roots and is unlike either fungi or roots alone. Most trees and agricultural crops depend on or benefit substantially from mycorrhizae. The exceptions are many members of the Cruciferae family (e.g., broccoli, mustard), and the Chenopodiaceae family (e.g. lambsquarters, spinach, beets), which do not form mycorrhizal associations. The level of dependency on mycorrhizae varies greatly among varieties of some crops, including wheat and corn.

Land management practices affect the formation of mycorrhizae. The number of mycorrhizal fungi in soil will decline in fallowed fields or in those planted to crops that do not form mycorrhizae. Frequent tillage may reduce mycorrhizal associations, and broad spectrum fungicides are toxic to mycorrhizal fungi. Very high levels of nitrogen or phosphorus fertilizer may reduce inoculation of roots. Some inoculums of mycorrhizal fungi are commercially available and can be added to the soil at planting time.

Aim and objectives

The aim of this research is to identify some types of fungi found in three types of soil (soil under cultivation - soil by the road - soil near the farm) and at different depths

(0-5 cm,5-10 cm,10-15 cm)

- counting the bacteria in different soils

- Identification of pH of soil samples on which microbes have grown

Materials and methods

Materials :-

- 1- Media culture
- Corn meal Agar
- Malt extract Agar
- Yeast extract Agar
- Potato dextrose Agar
- Czapecdox Agar
- Nautrient Agar
- 2- Distilled water to prepare media
- 3- Balance to weigh media and soil
- 4- Graduated cylinder
- 5- Autoclave to stralization media and tools
- 6- Alcohol to sterilization for bench
- 7- Incubatorfor fungi (25°C) for bacteria (37°C)
- 8- flasks 9-petri dishes 10- needle 11-loop 12- test tube
- 13-Microscope 14-methylen blue 15-pipet 16- PH meter
- **17-Microscope slide**

- Collection of samples

- Soil samples were collected from three different places (soil under cultivation – soil on the road – soil near the farm) and with different depths (0-5 cm, 5-10 cm, 10-15 cm). So we had 9 soil samples, we collected each sample with a plastic bag.



Fungi examination:-

- We prepare the media and pour it into the petri dishes
- Then sprinkle a small amount of soil on fungal media
- put them in the fungus incubator for 1 week
- After a week the fungus has grown and we took a pure colony and then planted it in the Petri dishes and the same media on which it grew
- After a week of incubation we examined the pure colonies on the microscope

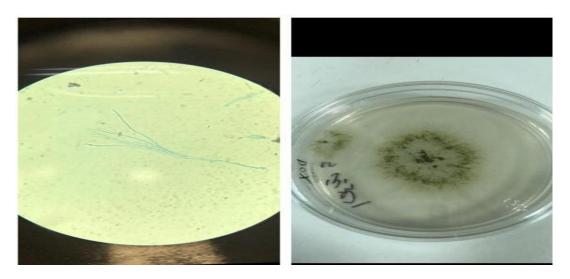


Count bacteria in samples:-

- Put 9 ml of distilled water into six tubes so that the dilution is one of a million
- Weigh one specimen from the sample and put it in the first tube. Then we take one milliliter From the first tube and put it in the second tube. We do this method up to the sixth tube and The dilution is one in one million
- Take one milli of each dilution and put each dilution in a Petri dish containing the nautrient agar media
- We incubate samples at 37 ° C for 2 days
- After two days of incubation we count the colonies

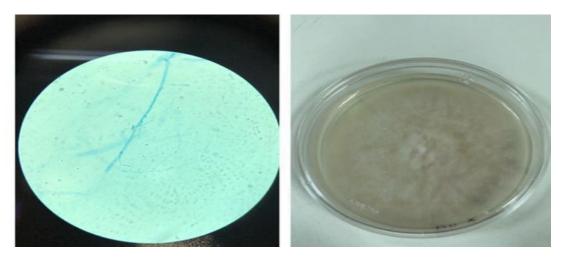
Results and Discussion

These are some of the fungi that we have defined :-

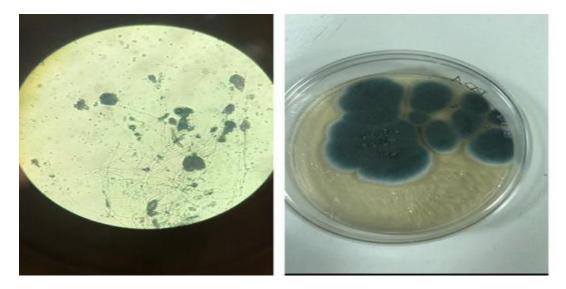


The media: Czapecdox Agar - soil on the road - 0-5cm

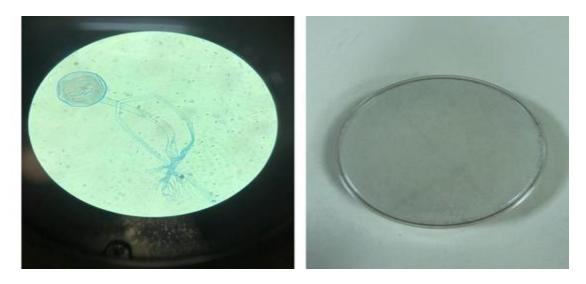
<u>Aspergillus</u> sp



The media: Corn meal Agar - soil on the road – 0-5cm <u>Fusarium</u> sp.



The media: Potato dextrose Agar- soil on the road– 0-5cm . .<u>Penicillium</u> sp

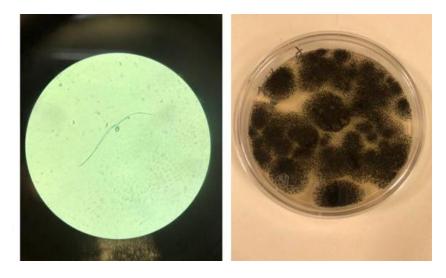


The media: Malt extract Agar - soil on the road – 0-5cm

<u>Rhizopus</u> sp



The media: : Czapecdox Agar - soil on the road– 10-15cm <u>Mucor</u> sp.



The media: **Potato dextrose Agar**- soil near the farm– 5-10cm

<u>Aspergillus</u> niger

Road soil was the most diverse of fungus

And either counting bacteria It was as follows:-

Dilutions	15-10	10-5	5-0
1/10	over	over	over
1/100	390	328	354
1/1000	105	84	95
1/10000	84	72	68
1/100000	63	43	15
1/1000000	46	31	4

soil under cultivation

soil near the farm

Dilutions	15-10	10-5	5-0
1/10	over	over	over
1/100	398	339	363
1/1000	108	94	159
1/10000	84	77	101
1/100000	46	52	42
1/1000000	22	34	30

soil on the road

Dilutions	15-10	10-5	5-0
1/10	274	297	308
1/100	117	121	129
1/1000	88	77	71
1/10000	53	41	34
1/100000	23	17	11
1/1000000	20	7	4

The lowest number of bacteria was in the soil of the road

Soil PH:-

- soil under cultivation

0-5cm (7,55)

5-10cm(7,31)

10-15cm(7,13)

- soil near the farm:-

0-5cm(7,11)

5-10cm(6,88)

10,15cm(6,69)

- soil by the road:-

0-5cm(6,87)

5-10cm(6,62)

10,15cm(6,39)

Conclusion

The fungi were more diverse in the soil of the road, and bacteria were more numerous in soil under cultivation.

Road soil was the most acidic and the most basic were.

References

• [1]

Colmer T D and Vosenek L A C J 2009 Flooding tolerance: suites of plant traits in variable environments Functional Plant Biology **36** 665-681 Crossref

• [2]

Arnell N and Liu C 2001 Climatic Change 2001: hydrology and water resources. Report from the Intergovernmental Panel on Climate Change. Available at <u>http://www.ipcc.ch/</u>

• [3]

Vartapetian B and Jackson M 1997 Plant adaptation to anaerobic stress Annals of Botany 3 3-20 Crossref

• [4]

Gustavo G S 2012 *Botany* ed J. Mworia (InTech) Flooding stress on plants: anatomical, morphological and physiological responses

• [5]

Drew M C 1991 Plant Life under Oxygen Deprivation ed MB Jackson, DD Davies and H Lambers (The Hague: Academic Publishing) Oxygen deficiency in the root environment and plant mineral nutrition 301-316

• [6]

Kreuzwieser J, Fu[°]rniss S and Rennenberg H 2002 The effects of flooding on the N metabolism of flood tolerant and sensi-tive tree species *Plant*, *Cell and Environment* 25 1039-1050 Crossref

• [7]

Ellis J R 1998 Post flood syndrome and vesicular-arbuscularmycorrhizal fungi Journal of Production Agriculture 11 200-204 Crossref

• [8]

Lipiec J and Stepniewski W 1995 Effects of soil compaction and tillage systems on uptake and losses of nutrients Soil Tillage Research 35 37-52 Crossref

• [9]

Orgiazzi A, Lumini E, Nilsson R H, Girlanda M and Vizzini A 2012 Unravelling soil fungal communities from different Mediterranean land-use backgrounds *PLoS One* 7 e34847 <u>Crossref</u>

• [10]

Jenkins A 2005 Soil fungi. In: Soil biology basics, information series, NSW Department of Primary Industries. Url: <u>http://www.dpi.nsw.gov.au/______data/assets/pdf__file/0020/41645/Soil__fungi.p</u> <u>df</u> • [11]

Suhag M 2016 Potential of biofertilizers to replace chemical fertilizers International Advanced Research Journal in Science, Engineering and Technology 3 163-167

• [12]

Berruti A, Lumini E, Balestrini R and Bianciotto V 2016 Arbuscularmycorrhizal fungi as natural biofertilizers: Let's benefit from past successes Frontiers in Microbiology 6 1559 Crossref

• [13]

Unger I M, Kennedy A C and Muzika R M 2009 Flooding effects on soil microbial communities Applied Soil Ecology 42 1-8 Crossref

• [14]

Waksman S A 1922 A method for counting the number of fungi in the soil Journal of Bacteriology 7 339-341

• [15]

Dobranic J K and Zak J C 1999 A microtiter plate procedure for evaluating fungal functional diversity *Mycologia* **91** 756-765 Crossref

• [16]

Wu S C, Ca Z H, Li Z G, Cheung K C and Wong M H 2005 Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial *Geoderma* 125 155-166 <u>Crossref</u>

• [17]

Rashid M I, Mujawar L H, Shahzad T, Almeelbi T and Ismail I M 2016 Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils Microbiological Research 183 26-41 <u>Crossref</u>

• [18]

Dube B and Smart G C Jr 1987 Biological Control of Meloidogyne incognita by Paecilomyceslilacinus and Pasteuriapenetrans Journal of Nematology **19** 222-227

• [19]

Kiewnick S and Sikora R A 2006 Biological control of the root-knot nematode Meloidogyne incognita by Paecilomyceslilacinus strain 251 Biological Control 38 179-187 Crossref

• [20]

Wagh V H and Pramanik A 2014 Evaluation of Paecilomyceslilacinus for management of root knot nematode Meloidogyne Incognita in tomato Supplement on Plant Pathology 9 407-411

• [21]

Rendig V V and Taylor H M 1989 Principles of soil-plant interrelationships (New York: McGraw-Hill Publishing Company)

• [22]

Cosgrove D J 1967 Soil biochemistry 1 ed A D McLaren and G H Peterson (New York: Marcel Dekker) Metabolism of organic phosphates in soil 216-228

• [23]

Whitelaw M A 1999 Growth promotions of plants inoculated with phosphatesolubilizing fungi Advances in Agronomy 69 99-151 Crossref

• [24]

Chhonkar P K and SubbaRao NS 1967 Phosphate solubilization by fungi associated with legume root nodules Canadian Journal of Microbiology 13 749-753

<u>Crossref</u>

• [25]

Narsian V and Patel H H 2000 Aspergillusaculeatus as a rock phosphate solubilizer Soil Biology and Biochemistry 32 559-565 Crossref

• [26]

Narsian V and Patel H H 2002 Aspergillusaculeatus as an organic phosphate mineralizer Indian Journal of Agricultural Sciences 72 177-179

• [27]

Cerezine P C, Nahas E and Banzatto D A 1988 Soluble phosphate accumulation by Aspergillusniger from fluorapatite Applied Microbiology and Biotechnology 29 501-505 Crossref

• [28]

Caravaca F, Alguacil M M, Azcon R, Parlade J, Torres P and Roldan A 2005 Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate and Aspergillusniger Microbial Ecology 49 73-82 Crossref

• [29]

Kang S C, PandeyPiyush, KhillonRajat and Maheshwari D K 2008 Process of rock phosphate solubilization by Aspergillussp PS 104 in soil amended medium Journal of Environmental Biology **29** 743-746

• [30]

Singh S M, Yadav L, Singh S K, Singh P, Singh P N and Ravindra R 2011 Phosphate solubilizing ability of Arctic Aspergillusniger strains Polar Research 30 7283 Crossref

• [31]

Muentz 1890 Surladécomposition des rocheset la formation de la terrearable CR Academic Science 110 1370-1372

• [32]

Prajapati K B and Modi H A 2012 Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil CIBTech Journal of Microbiology 1 8-14

• [33]

Prajapati K B, Sharma M C and Modi H A 2013 Growth promoting effect of potassium solubilizing microorganisms on Abelmoscusesculantus International Journal of Agricultural Science 3 181-188

• [34]

Fan Y, Luan Y and Yu K 2008 Arbuscularmycorrhizae formed by Penicilliumpinophilum improve the growth, nutrient uptake and photosynthesis of strawberry with two inoculum-types Biotechnology Letters **30** 1489-1494 <u>Crossref</u>

• [35]

Boring L R, Swank W T, Waide J B and Henderson G S 1988 Sources, fates, and impacts of nitrogen inputs to terrestrial ecosystems: review and synthesis Biogeochemistry 6 119-159 <u>Crossref</u>

• [36]

Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y and Takeuchi Y 2007 Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscularmycorrhizal fungi and root parasites *Planta* 227 125-132 Crossref

• [37]

Van Der Heijden M G A, Bardgett R D and Van Straalen N M 2008 The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems *Ecology Letters***11** 296-310 Crossref

• [38]

Hodge A and Fitter A H 2010 Substantial nitrogen acquisition by arbuscularmycorrhizal fungi from organic material has implications for N cycling Proceedings of the National Academy of Sciences U.S.A. 107 13754-13759 Crossref

• [39]

Sinsabaugh R L 2010 Phenol oxidase, peroxidase and organic matter dynamics of soil Soil Biology and Biochemistry 42 391-404 Crossref

• [40]

Schimel J P and Bennett J 2004 Nitrogen mineralization: challenges of a changing paradigm *Ecology* 85 591-602 Crossref

• [41]

Olutiola P O and Nwaogwugwu R I 1982 Growth, sporulation and production of maltase and proteolytic enzymes in Aspergillusaculeatus Transactions of the British Mycological Society 78105-113 Crossref

• [42]

de Castro RuannJanserSoares and Harumi Sato Helia 2014 Protease from Aspergillusoryzae: biochemical characterization and application as a potential biocatalyst for production of protein hydrolysates with antioxidant activities Journal of Food Processing Crossref

• [43]

Farnell E, Rousseau K, Thornton D J, Bowyer P and Herrick S E 2012 Expression and secretion of Aspergillusfumigatus proteases are regulated in response to different protein substrates *Fungal Biology* **116** 1003-1012