ISOLATING AND TESTING PATHOGENIC FUNGUS FROM SALINE SOILS

AMER JASSEM TOUFAN

Department of Plant Protection, College of Agriculture, Wasit University, Wasit, Iraq

Corresponding author's email: ajassim@uowasit.edu.iq

Abstract

This investigation was carried out with the objective of identifying the types of fungi that are found in saline soils in the Wasit Governorate. There were five primary sites chosen from which to collect random samples of saline soil, and these samples were gathered from the following locations: (Al-Maleh area located between Wasit and Babil governorates, Numaniyah district, Badra district, Al-Hay district, Wasit governorate center) As a consequence of these findings, a large number of fungus belonging to 15 different genera were successfully isolated (Alternaria solani, Fusarium graminearum, Fusarium solani, Penicillium sp, Fusarium oxysporum Penicillium italicum, Alternaria alternata, Pythium sp, Rhizoctoniasolani, Bipolaris sp, Aspergillius flavus, Curvealarea sp, Drechslera sp, Pythium aphanidermatum, ،Macrophomina phaseolina). In pathogenicity testing employing radish seeds, the fungus Alternaria alternata was shown to have the ability to lower the percentage of germinated seeds to 11.1%, compared to the control treatment's 100% germination rate. When compared to the control treatment, in which the percentage of radish seeds that germinated was 100%, the effect that the fungus Fusarium solani had on the germination of radish seeds was the least significant. The germination rate was only 94.4%.

Keywords: Soil fungi, Fungi isolation, Pathological test

Introduction

Many different species of microorganisms call the soil their home. Fungi make up a significant portion of these organisms, and they play an important part in the upkeep and management of the environmental systems that surround man. They do this by continuing the natural cycle of decay by converting dead organic materials, particularly plant remnants, into materials that can be used. This is accomplished through the action of enzymes that are secreted into the surrounding medium, and the process can take a significant amount of time. Today, man takes advantage of the dissolving power of fungus by harnessing it and putting it to work in a variety of industries, including the food industry, the manufacturing of organic acids and alcohols, antibiotics, proteins, and other areas .

The climate, which is comprised of temperature, humidity, pH, and salinitys the abiotic components that is considered to be the most significant. The alteration of only one of these elements is what causes the development of what are known as severe environmental conditions (extreme environments). Because it

ISSN 2072-3857

causes an increase in osmotic pressure and, as a result, leads to a decrease in the cellular water content, high salinity is considered to be one of the most important factors that affect the activity and physiology of the fungal cell. This is because it causes the osmotic pressure to rise. The increased buildup of salts, particularly magnesium and sodium salts, is the root cause of the issue of soil salinity, which can be problematic for agricultural practices. The rapid rate at which water is lost to the atmosphere is to blame for this rise. About seven percent of the land surface is covered by saline soil, which can be identified by a set of physical properties that are incompatible with the development of living things. However, studies have revealed that certain types of microbes, namely fungi, are able to thrive and adapt in environments with these characteristics. This capability is the result of the evolution of a variety of physiological acclimatization processes over time (10). The pathological symptoms of soil-borne pathogens typically appear on the plant's vegetative system after the pathogen has completely destroyed the plant's root system. This makes soil-borne pathogens one of the most dangerous and harmful pathogens to crops, as they are located in places that are inaccessible to humans (8). The fact that it may infect a large number of different plant species, is able to withstand poor weather circumstances, and can remain dormant in the ground and on the remains of infected plants for an extended period of time all contribute to its lethality (9.(

It was mentioned (17) that an increase in soil salinity led to an increase in the stress of the chickpea plant and increased its sensitivity to disease. The levels of soil salinity have an effect on the growth of the plant, causing it to become more susceptible to disease and to experience higher levels of stress. He also mentioned (11), that an increase in the salinity of the soil has an effective effect in reducing the percentage of germination, as well as a decrease in the rate of fresh and dry weight of the vegetative and root system of the tomato plant. This was something that he found particularly interesting. The inability of the plant to absorb water and nutrients was proposed as a possible explanation for this phenomenon. According to the findings of other research, elevating the salinity levels of irrigation water has a detrimental impact on the growth characteristics of plants because it causes substantial alterations in the morphological and physiological make-up of the plant (2.(

In a study that looked at the effect of the overlap between irrigation water salinity levels and infection of watermelon plants with M.phaseolina fungus and root-knot nematodes, it was discovered that plant susceptibility to fungus infection increased with increasing salinity levels, whereas nematodes were not significantly affected by increased salinity levels. The study was conducted on watermelon plants (13). It was also discovered that the infection of watermelons and grapes with the charcoal rot disease was severe in farms that used drip irrigation, and the reason for this was attributed to the fact that the drip irrigation caused increased soil salinity in areas close to the soil surface, which helped the occurrence of the disease. [Citation needed] (4.(

Chemical control is one of the tactics that has been used to combat soil fungi, and it is one of the most extensively utilized approaches due to the ease with which it can be employed and the immediate effect that it has on pathogens.

ISSN 2072-3857

However, the continued use of chemical pesticides led to the development of resistance in fungi, which in turn caused a great deal of trouble in terms of environmental pollution and its influence on the health of both humans and animals. (16.(

It has been directed towards the use of microorganisms and natural plant extracts such as medicinal, aromatic, and wild plants in protecting plant production from diseases caused by fungi, bacteria, viruses, and nematodes that infect various plant hosts,causing huge economic losses. This is because of the negative effects caused by these chemicals over the long term and for the purpose of protecting the environment (3). As a result, the objectives of the research were as follows :

A. Isolation of fungi from random samples of salty soil collected from various places.

B. The process of obtaining pure fungus by purifying those that have already been isolated.

C: Investigating the plant-killing potential of fungi that have been isolated from salty soils in order to find the most virulent and destructive fungal isolates possible

D:The use of some biological resistance agents to combat pathogenic fungi.

E:Studying the effect of plant extracts on the growth of fungi isolated from plant

MATERIALS AND METHODS

Culture media for the isolation and identification of fungi

In this investigation, many types of culture media were utilized in order to isolate, cultivate, and identify fungi, as well as for the purpose of the researchers' own experimentation, as will be demonstrated in the following:

Potato Sucrose Agar (P.S.A(

In order to produce the medium, 200 g of peeled potato tubers that had been cut into little pieces were boiled in 500 cm3 of distilled water in a glass beaker for 20-30 minutes. The capacity of the beaker was measured to be 500 cm3. In order to obtain the filtrate, the mixture that had been boiled for the allotted time was poured into a glass beaker and filtered using a piece of gauze cloth. After dissolving twenty grams of sucrose sugar and seventeen grams of agar in another five hundred milliliters of liquid, add the potato filtrate to the mixture and bring the total amount up to one liter. As required, the medium was transferred into glass flasks, the nozzles of the flasks were plugged with cotton stoppers, and the flasks were sterilized in an autoclave at a temperature of 121 degrees Celsius for twenty minutes under a pressure of 15 pounds per square inch. Following the conclusion of the sterilization process, the flasks were allowed to cool down before being supplemented with chloramphinicol at a concentration of 250 milligrams per liter. This was done prior to the medium becoming solid. The media was then transferred into a Petri dish in accordance with the experiment's specifications, and it was stored in the refrigerator until it was needed.

Potato Sucrose Broth (P.S.B.(

This medium was utilized for the cultivation of fungi and the subsequent analysis of their filtrates (supernatant) or bacteriophage. It was made in the same manner as described in the

ISSN 2072-3857

preceding paragraph, excluding the addition of agar

Isolation and characterization of the fungi that were investigated in this study

The dilution method for isolating soil fungus

Random samples were taken from the three replicates of each treatment, and then they were thoroughly mixed together in a cellophane bag. After that, 1 gram of the sample was taken on the basis of its dry weight, and it was passed through a series of dilutions in tubes each containing 9 ml of sterile distilled water. This was done by transferring 1 ml of the sample from one tube to the other tube after shaking the first tube well, and this continued until a After that, the dishes were moved about so that the suspension could be mixed in with the medium. In order to dilute it, there were three times when it was repeated. At a temperature of 28.2 degrees Celsius, the dishes were placed in the incubator. After a period of four days, the growing fungal colonies were observed, prepared, and purified on the same medium before being examined under a microscope for the purpose of making a diagnosis of them based on the characteristics mentioned by each of (7) and (12). The fungal frequency percentage was calculated by applying the following equation:

The fungi were re-isolated and identified in the middle and end of the season as in the above method.

Isolation of fungi by plant traps

Three replicates of each treatment's soil (each containing 250 g) were used to make dilutions, and these were then used to stuff cucumber fruits, which were then incubated for 5 days to see if the soil's fungal activity would promote fruiting. The colonies of fungi that had grown on cucumber fruit were then transplanted to P.S. The number of fungal colonies on various Petri plates is measured using a culture medium.

Preservation the fungal isolates used in the study

Preservation on medium P.S.A.

Fungi were isolated, cultivated on P.S.A. culture medium, and then purified and identified using the dilutions and traps method. 20 ml slanted test tubes were used to contain the participants. After being sterilized, the medium was allowed to harden. Discs of P.S. 0.5 cm in diameter were used to inoculate them. A 4-day-old fungal colony is growing in isolation on a culture medium. For seven days, these tubes were stored at 28°C in an incubator before being transferred to the refrigerator, where they remained at 4 °C until usage.

Preservation of millet seeds

After being thoroughly washed to remove dust and other impurities from them, the seeds of the local millet (Panicum miliacem) were used for the purpose of preparing fungal vaccines. After being soaked in water for a period of six hours, one hundred grams of the seeds were placed in a glass beaker that had a capacity of two hundred and fifty milliliters. It was then moistened with a small amount of distillate water, and the seeds were sterilized using an autoclave device at a temperature of 121°C and a pressure of 0.5 bar for one hour. The sterilization process was then repeated the following day at the same temperature, pressure, and time as well. The flasks were then inoculated individually by inserting five

ISSN 2072-3857

discs with a diameter of half a centimeter each from the P.S. in each flask. Each flask contained a culture medium on which the fungus was growing at the age of 5 days, and the flasks were incubated for a duration of 10 days at a temperature of 252°C. (6) Consider giving the flasks a good shake once every two days in order to ensure that the fungus is evenly distributed.

Pathogenicity tests of fungi isolated from saline soils

seeds used in the study

In the experiment, local radish seeds were utilized to investigate the pathogenicity of various fungal isolates, which are given in Table 1. After being superficially cleaned with a sodium hypochlorite solution concentration of 2% for two minutes and then washed with sterilized distilled water twice to remove any remnants of the sterilized material, seeds were purchased from local markets in the Wasit Governorate. The viability of these seeds was determined by taking 10 seeds of each type of seed and placing them in a Petri dish with a diameter of 9 cm. Next, a piece of medical cotton that had been saturated with distilled water was placed in the Petri dish to provide the necessary moisture for seed germination. Finally, the dish was incubated at a temperature of 25 + 2 C for a period of 96 hours, and this experiment was carried out. Calculations were done to determine the proportion of seeds that germinated in order to get an idea of the effectiveness of the seeds that were utilized in the study.

Testing the pathogenicity of fungi isolated from saline soil on seed germination and seedling death of radish plants in plastic pots

The infectious potential of each of the 15 fungal isolates was evaluated. This experiment was carried out in plastic pots with a capacity of 300 grams. Ethyl alcohol at a concentration of 70% was used to disinfect the soil before it was used for planting. After being packaged in polyethylene bags and mixed with alcohol, the soil was then sealed in the bags for three days, after which the bags were opened and the dirt was left in the sun for the same amount of time. After that, the soil was put into plastic pots, with a total of two hundred and fifty grams of sterile soil placed inside each pot.

The soil was then wet with sterile distilled water and contaminated with an inoculum of fungal isolates that had been cultured on millet seeds at a rate of 1% (weight/weight). Three days after the soil was contaminated, the pots were planted with six radish seeds per root. This was done in three replications, and a comparator treatment was also included that contained sterilized soil and millet seeds that were free of fungus. After the seeds were planted, the pots were given a drink of water and then covered with nylon bags. After the seeds had germinated, the plastic covering was taken off. After seven days had passed since the seeds had been planted (5), the proportion of seeds that had germinated was determined using the following equation:

After two weeks of planting, the percentage of dead seedlings was calculated according to the following equation:

Isolation and identification of fungi isolated from saline soils

ISSN 2072-3857

The results of the isolation and identification of fungi isolated from saline soils taken from 5 samples from the regions in the Wasit governorate, specifically the center, Al-Maleh, Al-Hay, Badra, and Al-Numaniyah regions, showed a variation in the isolated fungi according to the variation in the samples and their locations. This was the case because the isolated fungi were found in saline soils. Table (1) presents the findings of the investigation into the isolating and diagnosing of 15 distinct species of fungi (Alternariasolani, Fusarium graminearum, Fusarium solani, Penicillium sp, Fusarium oxysporum,Penicilliumitalicum, Alternaria alternata, Pythium sp, Rhizoctonia solani, Bipolaris sp, Aspergillius flavus, Curvealarea sp, Drechslera sp, Pythium aphanidermatum, ،Macrophominaphaseolina.(

Table 1 : Saline soil fungus

No.	Types of fungi	Sample name	Sample No.
1	***Alternaria solani***	**Al-Numaniyah**	**1**
2	***Alternaria alternata***	**Al-Maleh**	**2**
3	***Fusarium graminearum***	**Badra**	**3**
4	***Fusarium oxysporum***	**Al Koot Centre**	**4**
5	***Fusarium solani***	**Badra**	**5**
6	***Penicillium sp***	**AL Hay**	**6**
7	***Penicillium italicum***	**Al Koot Centre**	**7**
8	***Pythium sp***	**Al-Maleh**	**8**
9	***Pythium aphanidermatum***	**Al-Numaniyah**	**9**
10	***Rhizoctonia solani***	**Al-Maleh**	**10**
11	***Bipolaris sp.***	**Al-Maleh**	**11**
12	***Aspergillius flavus***	**Al Koot Centre**	**12**
13	***Curvealarea sp.***	**AL Hay**	**13**
14	***Drechslera sp***	**Badra**	**14**
15	***Macrophomina phaseolina***	**Al-Maleh**	**15**

The results of the measurements, which can be seen in Table No. (2), were obtained by subjecting the sample to a battery of examinations utilizing a PH meter and a device that measures electrical conductivity. Additionally, it was discovered through more in-depth research that fungi thrive in environments with a ratioof10-11millimoles

ISSN 2072-3857

Table 2: Salinity concentration

No	Sample location	Salinity concentration (dS m^{-1})
1	Al Koot Centre	11
2	Al-Numaniyah	10
3	AL Hay	11
4	Badra	9
5	Al-Maleh	15

Pathogenicity tests

Testing the pathogenicity of fungi isolated using radish seeds

In the treatment with the fungus Alternaria alternata, the germination rate was 11.1%, and it ranked first in the effect on seed germination. This was followed by the treatment with the fungus Fusarium oxysporum, which reached 27.7% compared to the control treatment, which was 100%.All of the fungi that were tested caused a significant decrease in the percentage of radish seeds that germinated, and this was the case regardless of the fungus.

When compared to the control treatment, in which the percentage of radish seeds that germinated was 100 percent, the fungus Fusarium solani had the least impact on the germination of radish seeds. The germination rate was 94.4% in this treatment. According to Table No. 3, the disparity that exists between the effects that different fungi have on the proportion of radish seeds that germinate may be attributable to the fact that different fungi have varying degrees of pathogenicity trait. These capabilities are connected to the genetic structures that are present in these fungi. There are also physiological aspects of pathogenicity that lie in the biochemical effects of the microorganisms. These physiological aspects are of fundamental importance in revealing the pathogenicity during the interaction between the pathogen and the plant host, such as the attack mechanism of the pathogen against the defense mechanism of the host. Pathogenicity can also be revealed during the interaction between the pathogen and the plant. The production of decomposition enzymes, poisons, and growth regulators are the three key metabolic activities that contribute to the pathogenicity of bacteria. Pathogenicity can be caused by microorganisms in a number of different ways. The mode of action of these metabolites, either individually or collectively, can have an effect on the pathogenicity of the pathogen (18

.(

Table (3) The effect of fungal isolates isolated from saline soils on the germination of radish seeds.

No	Types of fungi	germination %
1	***Alternaria solani***	**72.2**
2	***Alternaria alternate***	**11.1**
3	***Fusarium graminearum***	**77.7**
4	***Fusarium oxysporum***	**27.7**
5	***Fusarium solani***	**94.4**
6	***Penicillium sp***	**77.7**
7	***Penicillium italicum***	**61.1**
8	***Pythium sp***	**66.6**
9	***Pythium aphanidermatum***	**38.8**
10	***Rhizoctonia solani***	**72.2**
11	***Aspergillius flavus.***	**72.2**
12	***Bipolaris sp***	**77.7**
13	***Curvealarea sp.***	**66.6**
14	***Drechslera sp***	**66.6**
15	***Macrophomina phaseolina***	**55.5**
16	**Control**	**100**
$LSD_{0.01}$ 5.55		

The ability of the fungus Alternaria alternata to cause disease is due to the fact that it secretes many degrading enzymes, such as cellulase, pectinase, and calctodonase. These enzymes work to analyze the cell wall, and more specifically, the middle plate, because of the pectin and cellulose it contains (14.(

In spite of the fact that the fungus Fusarium oxysporum produces mycotoxins and growth regulators in addition to the enzyme lipase, which contributes to the pathogenicity of the fungus, it is distinguished by the fact that it secretes a large number of enzymes that decompose both towards the outside and towards the plant host. Lipase is one of the enzymes that contributes to the pathogenicity of the fungus.

In terms of the findings on the influence of fungal isolates isolated from saline soils on the death of seedlings of radish plants, the findings demonstrated that the fungus Rhizoctonia solani was the most dangerous and pathogenic of the fungal isolates. It did exceptionally well in reducing the percentage of seedling death by (49.9%) in comparison to the comparison treatment, which was (0). On the other hand, the fungus Penicillium italicum was not affected at all in the percentage of seedling death of radish plants. This was followed by the two fungi Aspergillius flavus and Curvealarea sp, as the percentage of seedling death for them was (5.5%) in comparison to the comparison treatment, which was (4.(

ISSN 2072-3857

Table (4) The effect of fungal isolates isolated from saline soils on the seedling death of radish plants

ت	Types of fungi	seedling death %
1	***Alternaria solani***	**24.4**
2	***Alternaria alternata***	**16.6**
3	***Fusarium graminearum***	**35.5**
4	***Fusarium oxysporum***	**38.8**
5	***Fusarium solani***	**28.8**
6	***Penicillium sp***	**6.6**
7	***Penicillium italicum***	**0**
8	***Pythium sp***	**27.7**
9	***Pythium aphanidermatum***	**27.7**
10	***Rhizoctonia solani***	**49.9**
11	***Aspergillius flavus***	**5.5**
12	***Bipolaris sp***.	**28.8**
13	***Curvealarea sp.***	**5.5**
14	***Drechslera sp***	**22.2**
15	***Macrophomina phaseolina***	**11**
16	**Control**	**0**
$LSD_{0.01}$= 11.45		

REFERENCES

.1Bhati PK, Singh SK, Singh R, Sharma A, Dhurai SY (2015). Estimation of heterosis for yield and yield related traits in rice (Oryza sativa L.) SABRAO J. Breed. Genet. 47(4): 467-474

.2Abeysinghe, S.(2009).Effect of combined use of Bacillus subtilis CA32 and Trichoderma harzianum RU01 on biological control of Rhizoctonia solani (Capsicum annuum). Plant pathology 8 : 9-16.

.3Aegerter, B. J., Gordon, T. R. and Davis, R. M. (2000). Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. Plant Disease 84: 224 – 230.

.4Al-Bahadli, Kazem Hussein Kazem. (2009). Isolation and identification of fungi associated with the roots of citrus trees and their effect on rotting disease, deterioration of orange seedlings, and biological resistance to the disease. Master's thesis. College of Agriculture, University of Kufa.

.5Al-Hamdani, Hazem Sabah Rahma. (2006). Evaluation of the efficiency of some fungi in the biological control of Fusarium Oxosporium and the effect of some factors on it. Master's thesis, College of Agriculture, University of Basra.

.6Bolkan, H. A., and D. F. Butler .(1974) . Studies on heterokaryosis and virulence of Rhizoctonia solani . Phytopathology46 : 513-522.

ISSN 2072-3857

.7Dewan, M. M.(1989) . Identify and frequency of occurrence of fungi in root of Wheat and Ryegrass and their effect on take – all and hostgrowth. Ph.D. Thesis. Univ. West Australia, 210pp.

.8Domsch, K. H., W.Gams and T.Anderson (1980). Compendium of Soil Fungi. V. I Academic Press. 859 pp.

.9Garrett , S.D. (1970). Pathogenic root – infecting fungi : Cambrodge Univ. Press, Cambridge . England . 294 pp.

.10Heitefuss, R. and Williams, P.H.(1976). Physiological plant pathology . Springer, Verlay Berlini . Heiclebbery, New York, 890 pp.

.11Kogej, T., Ramos, J., Plemenitas, A. and Gunde-Cimerman, N.(2005). The halophilic fungus Hortaea werneckii and the halotolerant fungus Aureobasidium pullulans maintain low intracellular cation concentrations in hypersaline envirenments. Appl. Environ. Microbiol. 71: 6600-6605.

.12Kutuk, C., Gayci, G. and Heng, L. K., (2005). Effect of increasing salinity and N15 Labelled urea levels on growth, N uptake, and water use efficiency of young tomato plants. Australian J. Soil Research, 42 (3) : 345 – 351.

.13Moubasher , A.H. and Aisha, A.A.(1987) . Soil fungi state of Qatar . Scientific and applied research center university of Qatar.

.14Nischwitz, C., Olsen, M. and Rasmassen, S. (2002). Influence of salinity and root – knot nematode as stress factors in charcoal rot of melon. Vegetable Report, University of Arizona College of Agriculture and life sciences. Eau/pubil/ crops as 1292, 1–2.

.15Ravjit, K. K., M.J. Barbetti and M.W. Sweetingham.(1999). Characterization and pathogenicity of Rhizoctonia species on canola. Plant Disease. 83 (8): 714-727.

.16Schutyser, M. A. I.(2003). Mixed solid-state fermentation: Numerical modeling and experimental validation. Ph. D. Thesis, Wageningen University. The Netherlands. pp: 7-

.17Shafique,H.A.; Sultana , V.; Ara, J.; Haque,S. and Athar,M.(2015). Role of antagonistic microorganissem and organic amendment in stimulating to defense system of okra against root rooting fungi .Polish.J.Micro.64(2) pp 157162.

.18Siddiqui, I. A. and Husain, S. L. (1991). Interaction of meloidogyne incongnita race-3 and Macrophoming phaseolina in a root-rot disease complex of chickpea. Neumatologia Modifernca, 19 : 237- 239.

.19Walker , J. C. (1969) plant pathology, third edition TATA. MC grow Hill publishing company L. td, Bomby Newdelhi–India.

ISSN 2072-3857