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Phylogenetic Diversity and Biocontrol Activities of Rhizosphere Microorganisms Isolated from Different Area in Saudi Arabia kingdom

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ABSTRACT

Rhizosphere is soil area surround the plant root, in which the exchange of nutrients, minerals and water with the root itself occurs. Fungi with antagonism towards plant pathogens play a key role in plant growth and health**.** In this study samples collected from rhizosphere of healthy and infected plants from different area in makka region in Saudi Arabia Kingdom. Nineteen fungal species were isolated from rhizosphere region of *Solanum melongena, Cucurbita peop, Eruca sativa, Brassicaoleracea var botrytis* and *limon* plants, and seven species of pathogenic fungi were isolated from the two-plant's rhizosphere (Solanum lycopersicum and *Ziziphus spina-christi).* The antifungal activity of the fungal crude extracts against isolated pathogenic fungi were assessed in terms on soled media of minimum inhibitory concentrations (MICs), the extracts of MIES-7 - and MHES-12 strains were inhibited the growth of all pathogenic fungi. While, extract of tow strains TABB-5 and MIES 20 had no inhibitory effect on the isolated pathogenic fungi in this study.

Molecular identification of 15 isolated fungi (12 rhizospheric fungal and 3 pathogenic isolates) by 18S ribosomal RNA analysis and PCR reaction. Comparing the sequences of the isolates with the related species in BLAST GenBank revealed that similarity percentages were ranged from 91% in isolate (MIES-15) that identified as (*Penicillium decumbens* strain 13-01) to 98% in isolate (MIES-20) that identified as (*Fusarium oxysporum* strain NSF2).

BLAST alignment of 18S rRNA revealed four families and five genera after comprised with different identified fungal strains, uniquely 15 new identified strains with similarity percentages less than 98%. Ten of the isolated strains from all plant's rhizosphere are belonged to the family Trichodermaceae. Five belonged to genus *Aspergillus*) MACC-1, TABB-5, MIES-7, MHES-12 and MISL-44(and five to genus *penicillium* (TASM-6, TABB-13, MIES15 MIZC-4l and MISL-42).

On the other hand, three isolates are belonged to the family Nectriaceae and genus *Fusarium* (TABB-17, TACP-14, and MIES20). Tow fungal isolates (TASM-4وMACC-18) were found belong to tow famileis (Pleosporaceae and Togniniaceae) and genera (*Alternaria and phaeoacremonium*), spectively. The study recommends using rhizospheric microorganisms in many fields: medical, biological, agricultural.

KEYWORDS: Rhizospheric Microorganism, Phytopathogens, Biocontrol agents, Chemical fungicides, Fungi.

الملخص:

تهدف هذه الدراسة إلى الاستفادة من ميكروبات التربة و استخدامها في المكافحة الحيوية للقضاء على انواع مختلفة من الفطريات الممرضة للمحاصيل النباتية في مناطق مختلفة في المملكة العربية السعودية , وقد تم عزل و تعريف وتوصيف العديد من الفطريات التي تنمو في المنطقة الملاصقة لجذور النبات والتي تسمى بمنطقة الريزوسفير نباتات الباذنجان، القرنبيط، الكوسا، الجرجير والليمون (*Solanum melongena, Cucurbita peop,* *Brassica oleracea var. botrytis, Eruca sativa* and *limon*) المحلي على التوالي ؛ وكما تم دراسة مدى إمكانية استخدام هذه الفطريات أواستخدام أحد نواتجها كمضادات حيوية لفطريات ممرضة تم عزلها من نبات السدر والطماطم (. *Solanum lycopersicum* and *Ziziphus spina-christi*) على التوالي.

ويمكن تلخيص النتائج المتحصل عليها في هذه الدراسة على النحو التالي:

عزل الفطريات السليمة والممرضة: تم عزل عدد 19 عزلة فطرية من منطقة الريزوسفير لأنواع النباتات المختلفة التي تم تجميعها من عدة أماكن في منطقة مكة (عسفان- الكامل- هدا الشام- والهدا) سبعة عزلات ممرضة، وتمت زراعتها على بيئة فطريات عند 28 درجة مئوية وتسميتها وترقيمها حسب مصدرها.

القدرة التضادية للعزلات الفطرية: تم اختبار القدرة التضادية للفطريات السليمة مع الفطريات الممرضة على الاطباق بإستخدام بيئة سابروويد وتحضينها لمدة 48 ساعة عند28 درجة مئوية. تم اختيار السلالات التي اظهرت نتائج ايجابية للتضاد وعددها 10 عزلة (TASM-4, TASM-6, TACP-14, MHES-12, MIES-7, MIES-15, MIES-18-20, TABB-5, TABB-13, TABB-17, MACC-1 and MACC.) لعمل مستخلص فطري وتحديد اقل تركيز مثبط للفطريات الممرضة وهي (MISL MISL42 MIZC-41-44). وقد أظهرت الدراسة أن هناك سلالتين فقط (MIES-12-MHES-7) كانت الأكثر قدرة على تثبيط نمو الفطر الممرض (MISL42 MIZC-41 MISL-44)

التعريف الجزيئي للعزلات الفطرية: لدقة التعريف تم تعريف السلالات وراثيا عن طريق تقنيه تفاعل البلمره المتسلسل لجين S rRNA18 باستخدام بادئ متخصص وأمكن تفريد القطع الناتجة من تفاعل البلمرة المتسلسل كهربياً باستخدام تقنية التفريد الكهربي وباستخدام هلام الاجاروز كوسط للتفريد وقد تم التأكد من مضاعفة المنطقة المرغوبة من الجين والحصول على أطوال جزيئية متطابقة لكل عزلة من العزلات والتي تمثل ١٦٠٠ زوج من القواعد من التتابع النيوكليوتيدي للجين. تم بعد ذلك تنقيه هذه القطع من الهلام وإرسالها لقراءة تتابعها النيوكليوتيدي حيث ظهرت نسبه تشابه تتراوح بين 91% إلى 99% لجين S rRNA18 من خلال مقارنتها ببنك الجينات الدولي على الرابط http://blast.ncbi.nlm.nih.gov.

وجد ان عشرة من السلالات تنتمي الى عائلة: Trichocomaceae, خمسة منها (MACC-1, TABB-5, 44-MIES-7, MHES-12 and MISL) من جنس: *Aspergillus* : (*Aspergillus niger* strain ISSFR-019, *Aspergillus niger* strain ISSFR-019, *Aspergillus sojae* strain JPDA1, *Aspergillus terreus* isolate ATE1 and *Aspergillus versicolor* strain HDJZ-ZWM-16) على التوالي.

وخمس عزلات MISL, MIZC-4l, MIES-15, TABB-13, TASM-6-42,)) تنتمي الى جنس : *Penicillium* (*Penicillium chrysogenum* strain UPSC 2020, *Penicillium decumbens strain L-06*, *Penicillium decumbens* strain ML-017, *Penicillium namyslowskii* and *Penicillium decumbens* strain L-06) , كما وجدت ثلاث عزلات ( ) (TABB-17, TACP-14, and 20-MIES) تنتمي الى عائلة: Nectriaceae وجنس *Fusarium* : (*Fusarium equiseti* strain Salicorn8 and *Fusarium oxysporum strain NSF2*) على التوالي.

كما وجدت عزلتين (18-TASM-4 and MACC) تنتمي الى عائلتين Pleosporaceae و Togniniaceae , وأجناس (*Phaeoacremonium australiense* strain STE-U 5959) و (*Alternaria alternata* strain S-f6 and) على التوالي.

من خلال هذه الدراسة يمكن أن نستنتج أن السلالات الفطرية المعزولة من ريزوسفير النباتات في المناطق المختلفة من المملكة يمكن أن يكون لها دور مهم في مكافحة الفطريات الممرضة. وكما يمكننا أيضا من هذه الدراسة أن ننصح باستخدام السلالات المعزولة والتي أثبت التجارب قدرتها العالية على التضاد باستخدامها على نطاق واسع في المملكة العربية السعودية بغرض استخدامها كمبيد عضوي ضد الفطريات الممرضة. كما تحث الرسالة على دراسة ميكانيكيات تنظيم الجينات في العزلات المتميزة.

1. Introduction

Rhizosphere is soil area surrounding the plant root, in which the exchange of nutrients, minerals and water with the root itself occurs. Many plant photosynthesis products are exchanged with rhizosphere from root. (Taketani et al., 2015).

The rhizosphere contains numerous microorganisms, which may be useful or phytopathogenic; plant activity can change the decomposition of soil organic matterby so-called rhizosphere priming. (Studer et al., 2016). The rhizosphere microorganisms influence plant health, productivity and dynamics by mechanisms like production and modification of plant hormones (Sergeeva et al., 2007).

In the kingdom of Saudi Arabia, the agriculture of many crops (potatoes, lemons, tomatoes) is very promising despite losses caused by pathogenic microorganisms of bacteria and fungi (Al-Saleh, 2011).

1.1 Aim of the work

The aim of the present study was to isolate and identify and analyze phylogenetic diversity of rhizosphere microbial flora inhabiting the rhizosphere of different healthy plants cultivated in different areas in Saudi Arabia as well as isolation of locally phytopathogenic fungi from infected plants.

2. Review of literature

About 40% of photosynthesis products are exchanged with the rhizosphere from root. Rhizosphere is soil area surrounding the plant root which exchange nutrients, minerals and water with the root itself. Very useful microorganisms are present in the rhizosphere area and called rhizobacteria.

In the kingdom of Saudi Arabia, the agriculture of potatoes, lemons, tomatoes and much more is very promising, making their agriculture a major contribution to the Saudi economy. Despite, losses caused by pathogenic microorganisms of bacteria and fungi in this industry is at a global rate (Al-Saleh, 2011).

2.1. Phytopathogens:

Soil-borne plant pathogens such as Rhizoctonia solani (Kuhn), Pythium ultimum (Trow) and Sclerotinia trifoliorum (Eriks) can reduce grass and forage legume establishment. (Kandula et al., 2015) Phytopathogens are microorganisms inhabiting soil specifically rhizosphere region that exert harmful effect on the plant causing plant lesions and diseases (Kaymak et al., 2008).

2.1.1. Phytopathogenic fungi:

Fungi occupy diverse environmental niches and many have evolved to live a pathogenic lifestyle, causing devastating diseases in plants and animals. Pathogens secrete effector proteins that manipulate the host to the pathogen’s advantage. (Sperschneider et al, 2015).

2.2. Rhizospheric Microorganism:

It's common that one microorganism antagonizes another one. Plant pathogenic fungi and bacteria can also be affected by fungal and bacterial antagonists. Such interference naturally occurrebetween beneficial microorganisms and plant pathogens affect the natural buffering of cropping systems, thus preventing or limiting disease development.

This is most obvious in suppressive soils where an antagonistic potential has been built up in the presence of the pathogen population (Weller et al., 2002).

2.2.1. Rhizospheric fungi:

Diversity of microbes in soil is an important factor for soil health determination and is the main drivers in soil suppressiveness. The rhizosphere is a complex environment that is influenced by active microhabitat and plant roots. Composition of the rhizosphere microbiota can influence plant traits such as health, development, stress tolerance, and productivity. Fungi and fungus-like organisms are diverse groups of the Eukaryotes which represent an essential functional component of soil microbial communities.

2.3. Biocontrol agents:

Biological control products depend on microbials are considered a plant protection product in most countries. And so, government regulations for registration and use are applied in the same way as for synthetic chemical plant protection products.

2.3.1. Rhizospheric fungi as biocontrol agents:

Species of fungi were noticed on phyla plane of Datura metel L. The fungi were Colletotrichum sp., Trichoderma viride Pers.and Pseudocercospora sp. All the three fungi along with Trichothecium roseum Link were found on Vigna catjangL. Uedocercospora sp., Trichoderma viride, Trichothecium roseum and Colletotrichum sp. belongs to the class Deuteromycetes. Pseudocercosporasp. Causes indistinct leaf spots with greenish black superficial mycelial growthmostly on ventral surface of leaves. Colletotrichum sp. is a facultative parasite which is responsible for anthracnose of various economically important plants. Trichoderma viride is a well-known biocontrol agent throughout the world. T. roseum is a saprophytic fungus, found as laboratory contaminants. (Shamsi and Naher, 2010).

2.4. Fruitful biocontrol agent produced by Fungi:

Fungi produce mixtures of carbon-based compounds called volatile organic compounds (VOCs). These compounds are small size so they are able to diffuse through the atmosphere and soils. Fungal VOCs is important in the success of some biocontrol species of Trichoderma. VOCs also play important roles for fungi in their natural environments. (Morath et al., 2012).

2.5. Chemical fungicides:

Chemical fungicides are diverse molecules with different targets and mechanisms of action. Low concentrations of these antifungal molecules can damage the cell, they activate one or more stress response pathways allows the cell to respond to the damage but not cause death. Higher concentrations of these molecules overwhelm the fungal stress response system and lead to inhibition of growth or death. Fungicides can cause acute toxicity, and some cause chronic toxicity as well (Goldman, 2008).

2.6. Kingdom Saudi Arabia as a potent source for bioactive microorganisms:

Saudi Arabia is mostly barren except the southwestern highlands which are susceptible to environmental changes. It is a hotspot for biodiversity, but no enough studies for microbial diversity and composition. Generally, total soil organic matter (SOM) is of low percent and nitrogen were detected in some soil samples. 33 different phyla were identified across the samples, including dominant phyla Proteobacteria, Acidobacteria and Actinobacteria. Jackknifed principal coordinates analysis (PCoA) revealed, over all differences in the bacterial community were more related to the quantity of specific OTUs than to their diversity among the studied samples (Yasir et al., 2015).

2.7. Identification of rhizospheric fungi:

Several approaches are used to extract fungal genomic DNA from soil such as commercially available kits which are designed for the extraction of microbial DNA from soil and techniques which are optimized for the extraction of nucleic acids from specific soil types. The purity and quality of the nucleic acid pool extracted is vital for successful PCR amplification of genomic DNA/RNA. Estimation of soil microbial diversity can be influenced by the technique used in extraction, by different protocols providing clear division of biodiversity (Martin-Laurent et al., 2001).

3. Materials and Methods

3.1. Materials:

3.1.1. Soil Samples:

Seven soil samples were collected from four different sources in Makkah and Al- Taif. The sources region (Asfan, Alkamel, Hada Al-sham, Al-Hada) as follows:

- Two samples from Asfan (N21.919584, E39.418362), (N21.912440, E39.403417).
- Three samples from Al-Hada (N21.362175, E40.277059), (N21.361927, E40.277309),
- One sample from Alkamel (N22.258111, E39.790769) and one sample from Hada al sham (N21.781264, E39.699713).

3.1.2. Culture media used in this study:

All types of media used in this work were sterilized by autoclaving at 121˚C for 15-20 min.

3.1.3. Solutions and buffers:

Chemicals and Kits implicated in molecular biology tests (DNA extraction kit, PCR product purification kit, Go Taq ® Green Master Mix, DNA marker, Agarose and lysozyme were obtained from QIA GEN, 70% ethanol and Methanol was purchased from (sigma Aldrich).

3.2. Methods:

3.2.1. Samples collection:

Seven soil samples were collected from four different regions in Makkah and Al-Taif. Soil sample were taken from plant rhizosphere and placed in sterile plastic bags and transported to the microbiology laboratory for isolation the rhizosphere and pathogenic fungi.

3.2.2. Isolation of rhizospheric and pathogenic fungi:

Isolation of pathogenic and floral mold fungi of plant rhizosphere soils of different plants were isolated using serial dilution technique. The bulk of the soil around infected or healthy plant roots was carefully broken away from the roots until only closely adhering to the layers of soil (about 1 mm) remained around the root.

3.2.3. Purification of isolated fungi:

Colonies of mycoflora were selected randomly and were purified using Aradhya et al. (2001) method by transferring part of the edges shoots fungal foreign colony to Petri dishes containing PDA dishes and then incubated at a temperature of (28 ± 2°C) for 3-7days, the purified isolates were maintained by periodical transfer on PDA at 4°C for other studies.

4. Results

4.1. Isolation of rhizospheric and pathogenic fungi:

Nineteen fungi species were isolated in the different plant's rhizosphere and seven species of pathogenic fungi were isolated from the two-plant's rhizosphere in Saudi Arabia.The soil samples were isolated from two different locations Taif and Makkah region in Saudi Arabia.

TASM-4 and TASM-6 strains were isolated from the rhizosphere plant Solanum melongena, while, TACP-14 strain was isolated from the rhizosphere plant Cucurbita peop and TABB-5, TABB-13, TABB-17 and TABB-19 isolated from Brassica oleracea var botrytis. The roots of these plants were collected from Taif area and the temperature was 18°C. Whereas MIES-2, MIES-3, MIES-7, MIES-9, MIES-10, MHES-12, MIES-16, MIES-15 and MIES-20 strains were isolated from the rhizosphere plant Eruca sativa and the limon plant was used to MACC-1, MACC-8 and MACC-18 strains and the roots of these plants were collected from Makkah area and the temperature.

4.2. Determination of MICs of the crude extracts against different pathogenic isolates.

The antifungal activity of the fungal crude extracts against different isolated pathogenic fungi was assessed in terms of minimum inhibitory concentrations (MICs) as previously described. Significant antifungal activity of most crude extract against MIZC-41 isolate pathogenic fungi was shown in (Table 1), the isolats (MACC-1, TASM-6,MIES-7,MHES-12, TABB-13 and MACC-18) were inhibited the growth of pathogenic fungi (MIZC-41) in high concentration of crude extract that growen in PDB,(28.9- 46.26- 66.02- 63.33- 91.71 and 165.7mg/ml) respectively, while isolates (MIES7, TABB13, TACP-14)which growen on tryptic soy broth inhibited the growth of the pathogenic fungi (MIZC-41) in the second concentrations of fungal crude extracts(77.54- 73.81 and 87.55) respectively, but isolates (MIES-7 ,MIES-12, MIES-15 and TABB-17) which growen on malt yeast peptone glucose broth were inhibited the growth of (MIZC-41) in the fourth and fifth dilutions of fungal crude extracts(9.766- 3.492, 24.4 and 75.27) respectively, while the other isolates shows no inhibition activity against the pathogenic fungus (MIZC-41).(Fig:1-4).

Table (1): Determination of MICs of different concentration of fungal crude extracts of rhizospheric isolated fungi against pathogenic fungi MIZC-41.

Different concentrations of crude extract of Isolate MIZC-41								
Fungal Isolates	Media	Dilutions mg/ml						
		1	2	3	4	5	6	7
MACC-1	P*	28.9	14.45	7.225	3.612	1.806	0.903	0.452
MACC-1	M*	33.75	16.87	8.437	4.219	2.109	1.055	0.527
MACC-1	T*	168.9	84.45	42.23	21.11	10.56	5.278	2.639
TASM-4	P	43.03	21.51	10.76	5.378	2.689	1.345	0.672
TASM-4	M	60.3	30.15	15.07	7.537	3.769	1.884	0.942
TASM-4	T	164.1	82.03	41.01	20.51	10.25	5.127	2.563
TABB-5	P	24.6	12.3	6.15	3.075	1.538	0.769	0.384
TABB-5	M	37.4	18.7	9.35	4.675	2.337	1.169	0.584
TABB-5	T	143.7	71.84	35.92	17.96	8.98	4.49	2.245
TASM-6	P	46.26	23.13	11.57	5.783	2.891	1.446	0.723
TASM-6	M	60.36	30.18	15.09	7.545	3.773	1.886	0.943
TASM-6	T	213.2	106.6	53.29	26.65	13.32	6.662	3.331
MIES-7	P	66.02	33.01	16.5	8.252	4.126	2.063	1.032
MIES-7	M	78.13	39.06	19.53	9.766	4.883	2.441	1.221
MIES-7	T	155.1	77.54	38.77	19.39	9.693	4.847	2.423
MHES-12	P	63.33	31.67	15.83	7.917	3.958	1.979	0.99
MHES-12	M	54.87	27.43	13.72	6.858	3.429	1.715	0.857
MHES-12	T	155.8	77.91	38.95	19.48	9.738	4.869	2.435
TABB-13	P	91.71	45.86	22.93	11.46	5.732	2.866	1.433
TABB-13	M	55.72	27.86	13.93	6.966	3.483	1.741	0.871
TABB-13	T	147.6	73.81	36.9	18.45	9.226	4.613	2.307
TACP-14	P	47.07	23.53	11.77	5.883	2.942	1.471	0.735
TACP-14	M	67.55	33.78	16.89	8.444	4.222	2.111	1.055
TACP-14	T	175.1	87.55	43.77	21.89	10.94	5.472	2.736
MIES-15	P	70.66	35.33	17.67	8.833	4.416	2.208	1.104
MIES-15	M	48.8	24.4	12.2	6.1	3.05	1.525	0.762
MIES-15	T	211.4	105.7	52.85	26.43	13.21	6.606	3.303
TABB-17	P	28.13	14.07	7.033	3.517	1.758	0.879	0.44
TABB-17	M	75.27	37.63	18.82	9.408	4.704	2.352	1.176
TABB-17	T	146.3	73.14	36.57	18.28	9.142	4.571	2.286
MACC-18	P	165.7	82.86	41.43	20.71	10.36	5.179	2.589
MACC-18	M	105.7	52.85	26.43	13.21	6.606	3.303	1.652
MACC-18	T	173	86.49	43.25	21.62	10.81	5.406	2.703
MIES-20	P	49.97	24.98	12.49	6.246	3.123	1.561	0.781
MIES-20	M	46.8	23.4	11.7	5.85	2.925	1.463	0.731
MIES-20	T	191.4	95.71	47.85	23.93	11.96	5.982	2.991

*P= potato dextrose broth, *m= malt yeast peptone glucose broth,*T= tryptic soy broth

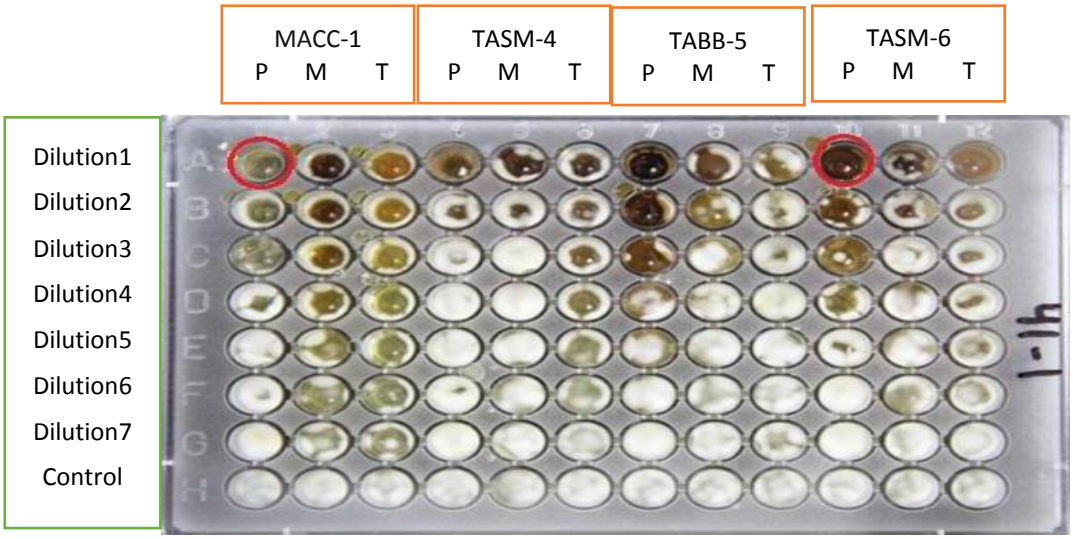

Fig. (1): Effects of fungal extracts of strains MACC-1, TASM-4, TABB-5, TASM-6 against pathogenic fungi MIZC-41. A#1 read highlighted was the MICs for MACC-1 extract in PDA. A#10 read highlighted was the MICs for TASM-6 extract in PDA

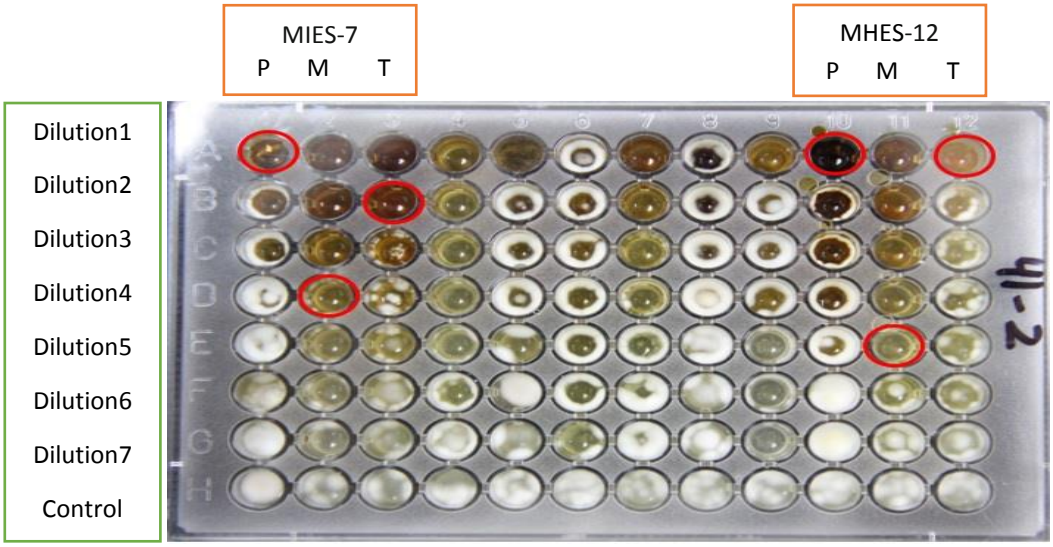

Fig. (2): Effects of fungal extracts of strains MIES-7, MHES-12 against pathogenic fungi MIZC-41. A#1 read highlighted was the mic for MIES-7extract in PDA. D#2 read highlighted was the MICs for MIES-7extract in malt yeast peptone glucose brothB#2 read highlighted was the MICs for MIES-7extract in tryptic soy broth. A#10 read highlighted was the MICs for MHES-12 in PDA.E#1110 read highlighted was the MICs for MHES-12 in malt yeast peptone glucose broth. A#12 read highlighted was the MICs for MHES-12 in TSB.

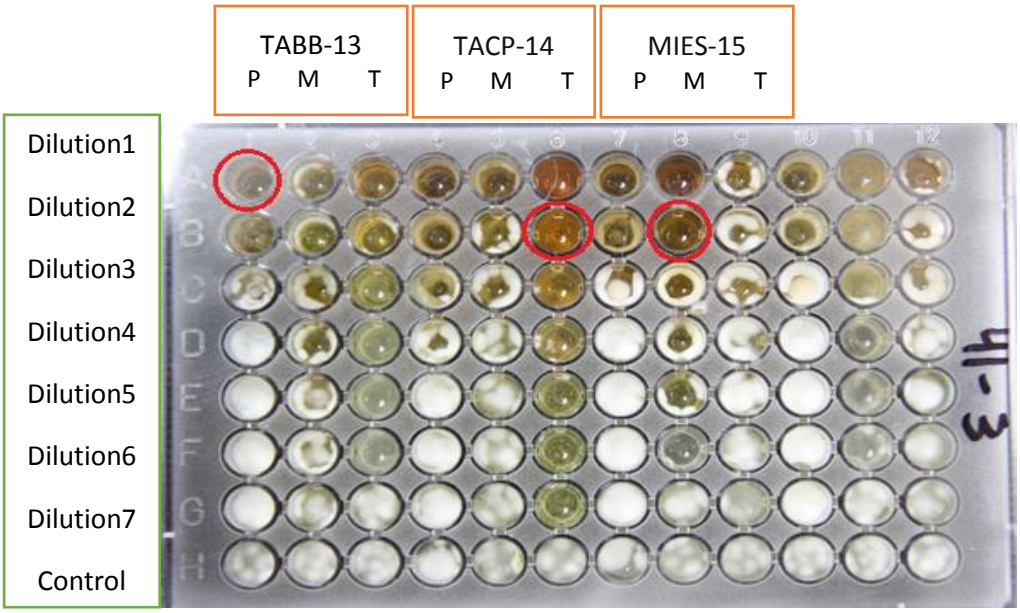

Fig. (3): Effects of fungal extracts of strains TABB-13, TACP-14 and MIES-15 against pathogenic fungi MIZC-41. A#1 read highlighted was the MICs for TABB-13 extract in PDA. B#6 read highlighted was the MICs for TACP-14 extract in TSB. B#8 read highlighted was the MICs for MIES-15 extract in in malt yeast peptone glucose broth.

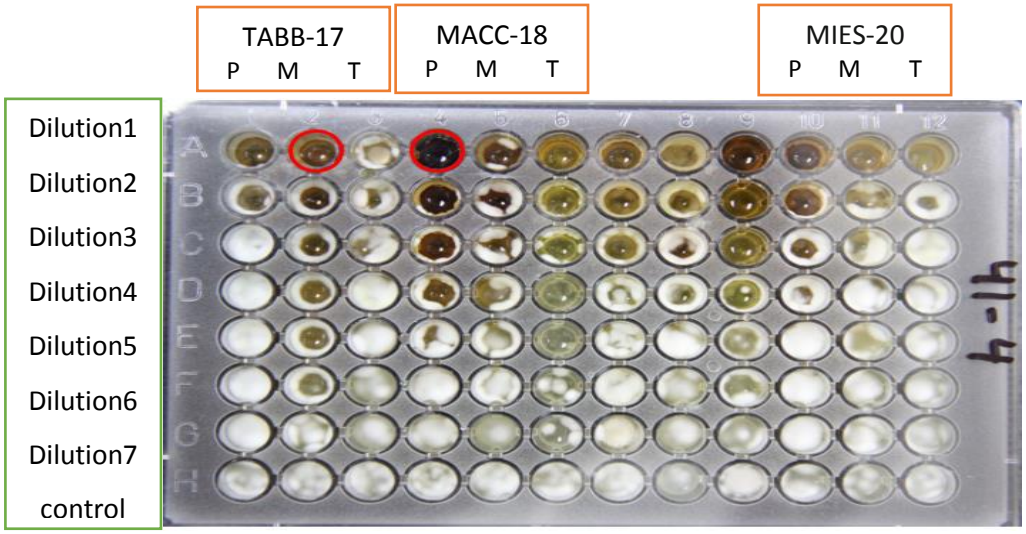

Fig. (4): Effects of fungal extracts of strains TABB-17and MACC-18 against pathogenic fungi MIZC-41. A#2 read highlighted was the MICs for TABB-17extract in malt yeast peptone glucose broth. A#4 read highlighted was the MICs for MACC-18 extract in PDA

4.3. Molecular identification of fungi strains:

Out of the 15 fungal isolates (12 rhizospheric fungal and 3 pathogenic isolates) were identified on the basis of sequence analysis of 18S rRNA. Amplified, sequenced and submitted to genbank. Amplification with primers NSI and NS8 resulted in approximately 1550-1650 bp of fragment. The fragments with different molecular size represented the 18S rRNA gene were detected and sequenced**. (Fig.5).** The obtained sequences were compared with those in the NCBl Nucleotide Sequence Database by using the BLAST algorithm. A BLAST analysis was carried out via blastn search through GenBank (http://www.ncb.inlm.nih.gov) shown that the 15 fungal isolates (12 fungal and 3 pathogenic isolates) belonged to five families and six genera.

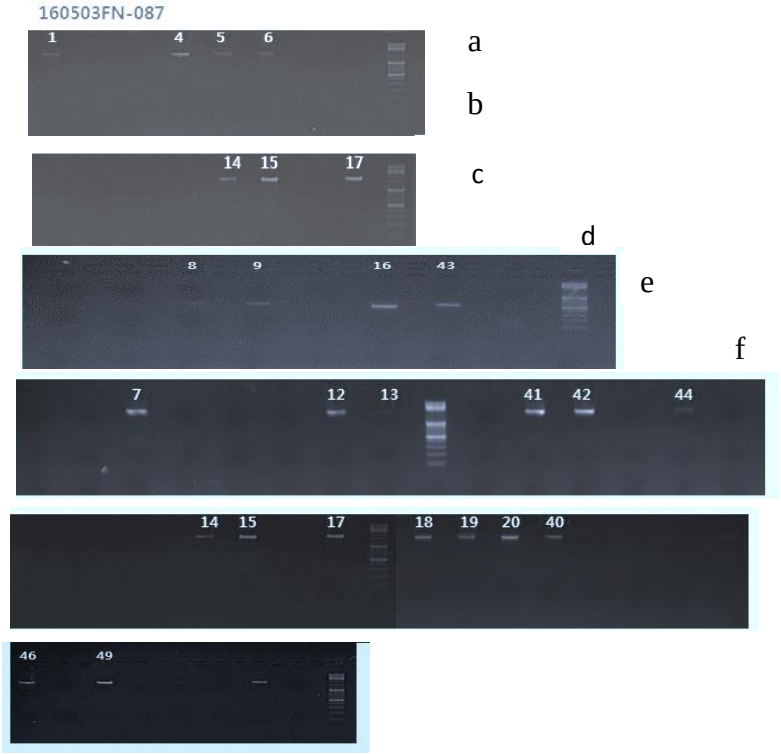

Fig. (5): Agarose gel electrophoresis of full-length sequences of 18S rRNA amplification products from 15 isolated fungal cultures (12 rhizospheric fungal and 3 pathogenic isolates) isolated from different regions and plants, M; DNA molecular weight.

4.3.1. DNA sequencing of 18s rDNA using universal primers for pathogenic fungi isolates:

The DNA sequence was compared to the Gen Bank database in national center in biotechnology information (NCBI) using the BLAST (Basic Local Alignment) program. BLAST search was used to find close relation through sequence identity. **Figure (29)** display the collected data of pathogenic fungal strain MIZC-41 isolated from *Ziziphus spina-christi* collected from Makkah (Asfan). BLAST search using identified MIZC-41sequence (macrogen) showed high identity 95% with *Penicillium namyslowskii* (AB028190.1). Furthermore, a phylogenetic analysis suggested MIZC-41 to be closely related to the identity 95% with *P. namyslowskii* .**Figure (6)** display the collected data of pathogenic fungal strains MISL-42 and MISL-44 isolated from *Solanum lycopersicum* collected from Makkah (Asfan). BLAST search using identified MISL-42 sequence (macrogen) showed high identity 97% with *Penicillium decumbens* strain L-06 (EU273880.1). Furthermore, a phylogenetic analysis suggested MISL-42 to be closely related to the *P. decumbens* strain L-06.

BLAST search using identified MISL-44 sequence (macrogen) showed high identity 96% with *Aspergillus versicolor* strain HDJZ-ZWM-16 (GU227343.1). Furthermore, a phylogenetic analysis suggested MISL-44 to be closely related to the *A. versicolor* strain HDJZ-ZWM-16. (**Table: 2).**

Table (2): Comparison of nucleotides sequence for MIZC-41, MIZC-46, MISL-42 and MISL-44 identity BLAST software were used to identify the isolated fungi sequence.

	Isolation code	accession number	The closest strain of the gene bank	Identity %	Coverge (%)
1	MIZC-41	EF067336.1	Aspergillus unguis strain F3000054	96%	73%
		AB008403.1	Emericella nidulans	96%	73%
		HM161749.1	Penicillium sp. Y12 EG-2010	95%	80%
		AB028190.1	Penicillium namyslowskii	95%	80%
		AB008410.1	Aspergillus ustus	94%	80%
2	MISL-42	EU667998.1	Penicillium decumbens strain 13-01	97%	83%
		EU273880.1	Penicillium decumbens strain L-06	97%	84%
		EF413620.1	Eupenicillium javanicum isolate AFTOL-ID 429	97%	84%
		FJ458446.1	Penicillium decumbens strain ML-017	97%	84%
		KJ680324.1	Fungal sp. h2	97%	84%
3	MISL-44	KP872530.1	Aspergillus sp. Y30-2	96%	89%
		KP872521.1	Aspergillus sp. Y19-2	96%	89%
		GU227343.1	Aspergillus versicolor strain HDJZ-ZWM-16	96%	90%

Fig. (6): Neighbor-joining phylogenetic tree from the analysis of full-length sequences of 18S rDNA from cultured pathogenic fungal strain MIZC-41 (isolated from Ziziphus spina-christi), MISL-42, and MISL-44 (isolated from Solanum lycopersicum) collected from Makkah (Asfan**).**

5. Conclusions and Recommendations

5.1. Conclusions

Maximizing the benefits comes from our native environment to decrease the toxicity of pesticides and chemical fertilizers has been achieved on this study using local isolates of Evaluation of biocontrol activity of extract fungi, which, as an devious pathogen and has antibiotic activity against a range of phytopathogenic fungi. The primary screening of this metabolite is done by fungi from the media. The results of the present study also showed that crude extract from different strains has antifungal activities and can be used as biological fungicide to control pathogenic fungi. Molecular identification of 15 rhizosphric and pathogenic isolates showed that are belonged to four families and five genera: Trichocomaceae, five isolates grouped to genus *(Aspergillus*), and five to genus (*penicilliums)*, three belonged to *Nectriaceae*, genus *(Fusarium.)*, and tow belonged to *(Pleosporaceae* and *Togniniaceae*), genus*(Alternaria* and *Phaeoacremonium*) respectively. It is expected that using extract as therapeutic agents in agriculture in addition to the ability of using the antifungal agenet that isolated in this study from plants rhizospher for producing transgenic plants have fungal resistance.

5.2. Recommendations

1. Increase in the investigation into the possibility of using rhizospheric microorganisms as biological fungicide and insecticide.
2. Use rhizospheric microorganisms in many fields: medical, biological, agricultural as a biological control.
3. The use of chemical fungicide and insecticide should be avoided as much as possible, and their use should be in a manner that is according to the instructions and under medical supervision.

4. Biological control products depend on microbial are considered a plant protection products in most countries.
5. Also, this study recommends using rhizospheric microorganisms in wider industrial fields as biological fungicide and insecticide instead of chemical fungicide and insecticide in the agriculture.
6. Study strains of rhizospheric microorganisms performed to produce the enzymes production.

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