The fungicidal effect of Rosa Damascena Mill extracts against fungal infections invading Plants in Al Taif

Mohammed Hassan Ali Dosh[1], Saleh Hamdan Alamri[2], Saleh Bakheet Al-Ghamdi[3], Salma A. Al-Zahrani[4], Sahar A. El-Sayed[5], Mohammad Melebari[6], Khaled S. Gazi[7], Haitham Abdulrahman S. Alharthi[8], Salman Faleh Al- Mutairi[9], Mohammed A. Thabet[10], Bandar Fahad Almiman[11]

1 Researcher in Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
2 Biology Instructor, Makkah, Saudi Arabia,
3 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
4 Researcher in Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
5 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
6 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
7 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
8 Researcher in Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia,
9 Researcher in Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia
10 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia, (ORCiD: https://orcid.org/0000-0001-8644-7855)
11 Biology Department, Faculty of Science, Al-Baha University, Al Bahah, 65779, Saudi Arabia, (ORCiD: https://orcid.org/0000-0002-6605-8462)

Abstract. Plant pathogens of fungus have become of the most crucially harmful objects that may affect overall plants growth rate and crops production worldwide. This study involved the identification of six species and six genera was achieved from the ten fungal plant pathogen isolates that gathered from different sites and regions in Taif region, Saudi Arabia. The identification of these organisms was conducted using molecular markers; among the identified species, 30% were *Mucor circinelloides*, 20% were *Penicillium crustosum*, and 20% were *Alternaria alternata*. The remaining 30% was comprised of *Cladosporium tenuissimum*, *Phoma macrostoma*, and *Fusarium equiseti*. A considerable proportion of these genera are widely acknowledged for their capacity to generate mycotoxins. The linear growth regression derived by the new plant extract is higher than fungicide, it has been found that the growth inhibition percentage driven by fungicide was between (20 to 85%), whereas for plant extract was between (80 to 100%) across all isolates. This data facilitate the contributing to the exploration of a more secure alternative to fungicides. Further, it will facilitate the development of regulatory approaches aimed at alleviating the detrimental consequences of fungal infections.

Keywords: Rosa Damascena Mill- Fungal Pathogens- Plants- Taif- Saudi Arabia.

1. Introduction

The Taif region's primary source of revenue has historically been agriculture. Farmers used both modern and traditional irrigation techniques even in pre-Islamic times, diverting rainwater that was falling downward to agricultural terraces or bringing rainwater from dams

to irrigate dry wadis. In the past, the tribes of Taif produced wheat, barley, and a variety of fruits, including dates, limes, apricots, oranges, olives, figs, peaches, and pomegranates. The Taif region's native vegetation, however, paid a high price for agricultural expansion. Numerous wild species have vanished because of the extensive tracts of virgin territory that have been converted to agricultural land over time [18].

Plant diseases pose a significant challenge in the fields of agriculture and food security, as they are estimated to account for as much as 40% of annual crop losses worldwide [32] and [13]. Fungi rank second in the frequency of plant maladies owing to their severe detrimental impacts on plants. For example, they are accountable for a 50% annual reduction in maize productivity [19]. Infection of crops with mycotoxins and direct reduction in crop production are both capabilities of fungi. Mycotoxins, which are substances produced by fungi, are hazardous to the health of both humans and animals. They are carcinogenic, as stated by the International Agency for Research [25]. Furthermore, according to the Food and Agriculture Organization (FAO) [19], mycotoxins can contaminate as much as fifty per cent of the world's annual crops.

The need to develop tactics that control and fight these infections has intensified as a result of the approaching withdrawal of pesticides and fungicides from some nations due to their negative impact on humans, animals, and other beneficial insects or fungi [16] and [13]. To protect crops from invasive phytopathogenic species, some nations, including Sweden, Canada, and Indonesia, have developed strategies to replace the use of pesticides and fungicides with environmentally friendly alternatives, such as plant or microorganism extracts, biological controls, and environmental controls [26] and [27].

Rosa damascena Mill (family Rosaceae), also known as Damask rose, holds a significant and emblematic status in Saudi Arabia, with widespread uses in gastronomy and traditional medicine [33]. Decoction and artisanal jam or jelly derived from rose petals have been employed as a diuretic and gentle laxative for constipation. Rose water is commonly utilized to improve the taste of many confections, such as Turkish delight, rice pudding, and yogurt [33]. In addition to its culinary use, it is recognized as an antibacterial agent, antifungal against fungi that invade plants [17] and [18]. Therefore, this research aims to assess the fungicidal effect of Rosa Damascena Mill extracts against fungal infections invading plants in Taif region, Saudi Arabia.

2. Materials and methods

2.1 Sample Collection Sites and Sampling

This study selected 10 geographical sites for sample collection and from different plant parts within Al-Hada city which is administratively affiliated with the Taif Governorate.

2.2 Isolation and purification of the isolated fungal strains

Following a thorough cleansing with tap water, the harvested crops were subsequently subjected to a two-minute disinfection period utilizing 1% sodium hypochlorite (Sigma Aldrich, USA) and the plant surfaces were rinsed with distilled water after the disinfection. Following the proper preparation of the crops, spores or mycelium were transferred to a Potato dextrose agar (PDA) (Oxoid, Germany) medium which was prepared according to the manufacturer instructions for cultivation using a sterilized loop to puncture the surface of the infected tissue. After two times of subculture in PDA for 7 days for each subculture at 30°C. To obtain a culture of a pure isolate, a 0.5 mm inoculum was re-inoculated onto a fresh Potaro dextrose agar (PDA) medium for subculturing, which was done five days after the initial

cultivation on the PDA medium at 25°C [1],[3].and [18]. Subsequently, stock cultures of newly acquired fungal isolates were established and preserved in the Al-Baha University stock culture bank.

2.3 Fungicide preparation

Four grams of fungicide (TOLEX 500WP) in powder form were added into 1 L of PDA medium after autoclaving. Later this medium was poured into 9 mm Petri dishes and kept for the next experiment.

2.4 Plant extract preparation (Rosa Damascena Mill)

The first step; drying plant tissue and then grinding it to be in powder form. After that, weigh 50 grams of the plant and dissolve it in 200 ml of 80%ml methanol, a concentration of 20% distilled water and put it in a closed bottle for a week. After that, we take the extract or the bottle and filter it through A paper filter to get rid of plant residues. After that, we put the pure extract in a glass dish or an aluminum dish, and after two days it dries, we have added 1:10 volume to volume, for example for each 1 gram of plant extract 9 ml of DMSO was add, to have a raw plant extract of 100% concentration. The PDA medium was modified by plant extract at 10% level via adding 10 ml pre-prepared plant extract into each 100 ml of PDA medium prior to solidifying.

2.5 Growth rate assessment at three different media conditions

To establish fungal cultures on 9 cm PDA plates, 0.5 mm agar plugs were inoculated with material extracted from vigorously developing sections of 5-day-old cultures. Five replicates of each isolate were subsequently cultivated for six days under three distinct media conditions: the first replicate served as the negative control and was not treated with fungicide, the second replicate was treated with fungicide, and the third replicate was treated with plant extract. To determine the rate of growth, radial expansion was monitored for six days [5].

2.6 DNA extraction kit-based method

The mycelium material was transferred from the liquid culture Potato dextrose broth (PDB) into a 2 ml tube before being drained off the medium and centrifuged to form a particle. Grinding commenced after an equivalent volume of sand and Chelex was added to the mycelium mass, the DNA extraction was carried out according to the manufacturer instruction kit (DNA extraction purified kit, Thermofischer, Germany) (Ultimately, the column was disposed of, while the tube was frozen for subsequent utilization [5].

2.7 Phylogenetic tree analysis and molecular identification

PCR reactions of 20 and 50 microliters were prepared using the forward and reverse primers (ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC) following the specified PCR conditions. PCR product of the amplified samples was placed onto the agarose gel for visualization using agarose gel electrophoresis as described by [4], [29] and [35].

The products of the amplicons were subsequently purified using the technique of the Qiagen QIAquick PCR Purification Kit. Afterwards, the PCR products were sent to Macrogen Inc., an external laboratory that specializes in sequencing, for sequencing. The returned sequencing results were represented as trace data. Subsequently, the FASTA format was employed for species identification in BLAST analysis on either UNITE or NCBI, as described by [6] and [28].

For phylogenetic analysis, only sequences from gene banks that had a similarity of more than 90% were downloaded and used as reference sequences [23]. The MUSCLE alignment tool in Geneious Prime was used to align several sequences. The Generous tree builder approach was employed to construct the phylogenetic tree utilizing the distance tree and neighbor joining methods. The genetic distance of the tree was calculated using the Bootstrap supporting value range of 70% to 100% for the consensus phylogenetic tree, employing the Tamura-Nei model. The General Time-Reversible evolutionary model with 500 bootstrap replications was chosen to obtain the most accurate consensus phylogenetic tree [4] and [22].

3. Results

3.1 Collection and Molecular Identification of isolated fungal strains

From an each of the 10 samples collection sites Multiple fungal samples were taken from different hosts and plant parts, to confirm the predominant fungal pathogen in each of the 10 geographical sites and to avoid of transient species. Only the confirmed casual disease for each site was carried over in this study. The 10 new isolates were confirmed as fungi though DNA extraction and the PCR amplification using ITS marker, based on gel electrophoresis image (Figure 1).
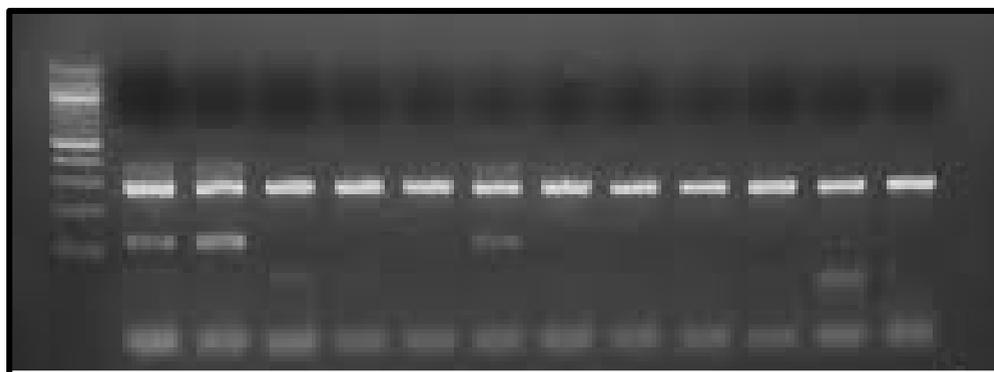
Figure 1. This image represented the PCR products along with the DNA ladder on agarose gel electrophoresis.

The generated FASTA sequence for each isolate was successfully used for species identification and drawing a phylogenetic tree by matching the new sequence isolate with high similarity corresponding species above 90% in GenBank National Center for Biotechnology Information (NCBI) (Tables 1, 2 and 3). After that, the phylogenetic tree was drawn to identify the new isolates, by grouping the new and reference isolates in one species group (Figure 2).

Table 1. The background information for the new isolates collected in this research.

N	Isolate code	Species	Host	Plant part
1	MD01	*Cladosporium tenuissimum*	*Vitis vinifera*	Stem
2	MA012	*Mucor circinelloides*	*Psidium guajava*	Leaf
3	MB02	*Penicillium crustosum*	*Vitis vinifera*	Fruits

4	MA020	*Alternaria alternata*	*Prunus armeniaca*	Leaf
5	MD08	*Phoma macrostoma*	*Ficus*	Leaf
6	MA013	*Mucor circinelloides*	*Brassica oleracea var*	Leaf
7	MA07	*Mucor circinelloides*	*Ficus*	Leaf
8	MA021	*Alternaria alternata*	*Prunus armeniaca*	Leaf
9	MA011	*Penicillium crustosum*	*Psidium guajava*	Leaf
10	MA06	*Fusarium equiseti*	*Brassica oleracea var*	Leaf

Table 2. Details of the reference sequence isolates downloaded from NCBI gene bank.

N	Accession number	Species	Host	Origin
1	MH712244.1	*Cladosporium tenuissimum*	*Cerops tagal*	Hainan – China
2	MH427070.1	*Penicillium crustosum*	*Citrus × sinensis*	Beijing - China
3	LC543661.1	*Mucor circinelloides*	*Glycine max*	Hiroshima - Japan
4	MW555341.1	*Mucor circinelloides*	*Beta vulgaris*	Harbin - China
5	AJ878535.1	*Mucor circinelloides*	*Fagus sylvatica*	Harpenden - United Kingdom
6	KY549055.1	*Fusarium equiseti*	*Capsicum annuum*	Punjab - Pakistan
7	DQ474115.1	*Phoma macrostoma*	*Cirsium arvense*	Saskatoon - Canada
8	MH992147.1	*Alternaria alternata*	*Citrus × sinensis*	Erzurum - Turkey
9	MZ664272.1	*Alternaria alternata*	*Camellia*	Hunan - China

Table 3. Represent the percentage similarity across new and reference isolates.

|	MD01(new	MH712244	MB02(new	MH427070	MA06(new	KY549055	MA07(new	LC543661	MD08(new	DQ474115	MA011(ne	MA012(ne	MW55534	MA013(ne	AJ878535.	MA020(ne	MH992147	MA021(ne	MZ664272
MD01(new_sample)		82.163	58.904	56.388	65.804	64.404	41.325	37.34	57.81	58.007	58.319	41.158	42.744	31.716	39.32	57.018	57.517	58.246	57.968
MH712244	82.163		52.705	53.299	60.075	60.075	36.392	41.86	52.855	52.45	53.147	36.677	36.825	28.351	43.292	51.585	51.404	51.408	51.773
MB02(new	58.904	52.705		94.211	57.143	56.336	43.673	40.536	58.763	58.966	69.172	44.257	44.907	30.93	39.269	57.661	54.219	57.661	57.833
MH427070	56.388	53.299	94.211		55.651	60	43.323	44.66	57.539	60.832	93.497	44.635	43.701	41.159	41.519	56.882	58.209	57.048	56.551
MA06(new	65.804	60.075	57.143	55.651		93.333	43.918	38.611	57.829	55.732	58.276	43.103	43.286	32.464	39.675	57.192	55.959	57.192	55.882
KY549055.	64.404	60.075	56.336	60	93.333		41.915	41.503	55.221	58.053	56.089	41.824	41.824	42.593	41.531	54.733	56.799	55.422	54.639
MA07(new	41.325	36.392	43.673	43.323	43.918	41.915		78.855	44.833	43.375	33.527	92.258	91.721	56.595	66.401	50.635	46.396	49.761	48.882
LC543661.	37.34	41.86	40.536	44.66	38.611	41.503	78.855		40.422	42.02	30.533	80.284	80.922	48.763	76.439	42.59	40.889	42.79	42.163
MD08(new	57.81	52.855	58.763	57.539	57.829	55.221	44.833	40.422		94.061	43.632	44.094	45.31	33.413	41.244	74.955	70.307	75.592	75.046
DQ474115	58.007	52.45	58.966	60.832	55.732	58.053	43.375	42.02	94.061		59.24	43.192	44.322	33.254	43.514	73.418	75.949	74.684	73.779
MA011(ne	58.319	53.147	69.172	93.497	58.276	56.089	33.527	30.533	43.632	59.24		34.375	33.76	25.397	29.405	42.543	44.945	43.032	57.429
MA012(ne	41.158	36.677	44.257	44.635	43.103	41.824	92.258	80.284	44.094	43.192	34.375		93.569	55.795	64.937	49.214	46.349	49.289	48.418
MW55534	42.744	36.825	44.907	43.701	43.286	41.824	91.721	80.922	45.31	44.322	33.76	93.569		55.012	65.348	49.365	47.297	50.718	51.04
MA013(ne	31.716	28.351	30.93	41.159	32.464	42.593	56.595	48.763	33.413	33.254	25.397	55.795	55.012		59.184	35.986	34.471	35.995	35.919
AJ878535.	39.32	43.292	39.269	41.519	39.675	41.531	66.401	76.439	41.244	43.514	29.405	64.937	65.348	59.184		42.362	40.658	42.563	41.93
MA020(ne	57.018	51.585	57.661	56.882	57.192	54.733	50.635	42.59	74.955	73.418	42.543	49.214	49.365	35.986	42.362		89.384	96.709	95.772
MH992147	57.517	51.404	54.219	58.209	55.959	56.799	46.396	40.889	70.307	75.949	44.945	46.349	47.297	34.471	40.658	89.384		90.753	96.154
MA021(ne	58.246	51.408	57.661	57.048	57.192	55.422	49.761	42.79	75.592	74.684	43.032	49.289	50.718	35.995	42.563	96.709	90.753		96.875
MZ664272	57.968	51.773	57.833	56.551	55.882	54.639	48.882	42.163	75.046	73.779	57.429	48.418	51.04	35.919	41.93	95.772	96.154	96.875	
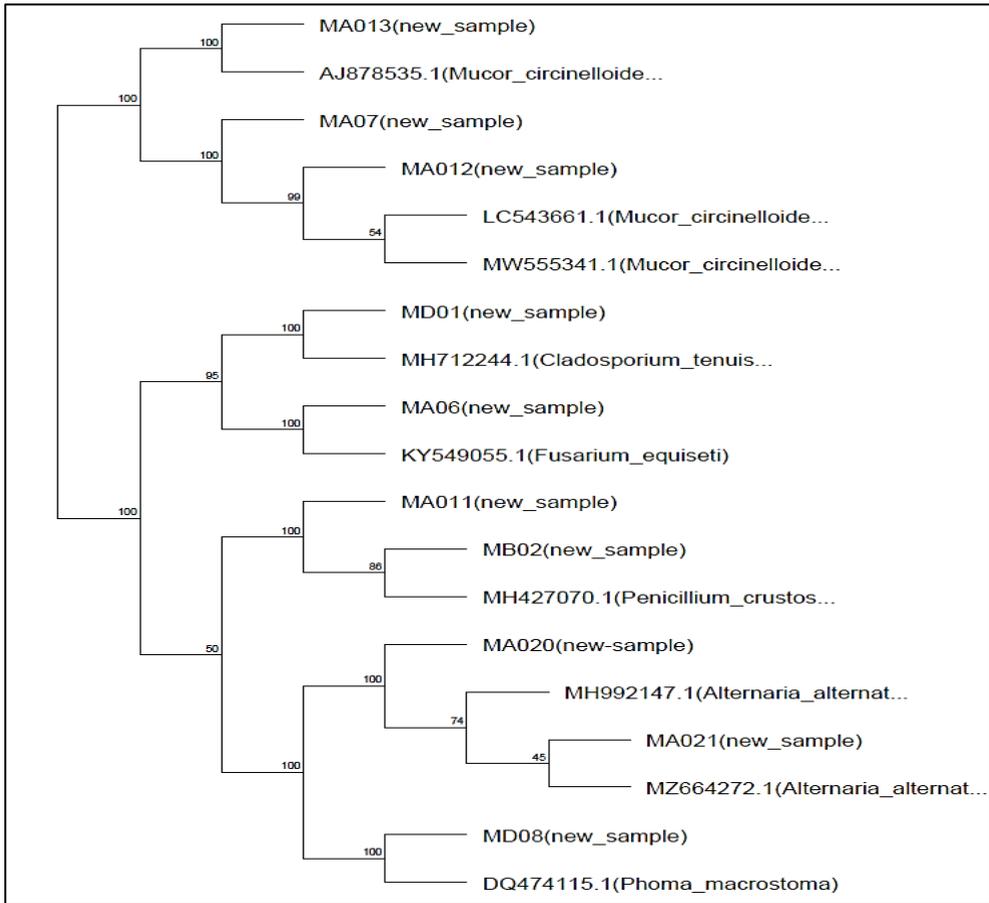
Figure 2. The phylogenetic tree was constructed utilizing ITS genetic markers and a consensus tree-based approach based on neighbor-joining. The 10 de novo isolates have been assigned the isolates identifier, while the reference sequences have been assigned the NCBI accession number (MEGA 11).

3.2 Evaluation of the inhibition capability for plant extract and fungicide against fungal growth

After six days of fungi growth, these ten new isolates are representative of six species and genera, showed that the isolates encoded (MA012, MA013 and MA021) represent *Mucor circinelloides* species that showed a growth rate between 4.8-4.4 cm at PDA medium, while the growth rate was recorded at 0.5-1.6 cm for fungicide medium and for the plant extract medium the growth rate was between 0.0-0.3 cm. Whereas the two isolate codes (MB02 and MA011) belonging to *Penicillium crustosum* species showed a growth rate between 4.9-5.1 cm in the PDA medium, while the growth rate was recorded at 2.1-4.0 cm for the fungicide medium and for the plant extract medium the growth rate was between 0.8-1.0 cm. Whereas the two isolates code (MA020 and MA021) belongs to *Alternaria alternata* species showed a growth rate between 4.2-4.9 cm in the PDA medium, while the growth rate was recorded at 0.8-1.1 cm for the fungicide medium and for the plant extract medium the growth rate was between 0.1-0.16 cm. The isolate code (MD01) belongs to *Cladosporium tenuissimum* species and showed a growth rate of 3.1 cm in the PDA medium, while the growth rate was

recorded at 0.8 cm for the fungicide medium and for the plant extract medium the growth rate was 0.1 cm. The isolated code (MD08) belonging to the *Phoma macrostoma* species showed a growth rate of 3 cm in the PDA medium, while the growth rate was recorded at 0.8 cm for the fungicide medium and the plant extract medium the growth rate was inhibited. On the other hand, isolate code (MA06) belonging to *Fusarium equiseti* species showed a growth rate of 4.3 cm in the PDA medium, in contrast, the growth rate was reduced to 0.7 cm for the fungicide medium and for the plant extract medium the growth rate was 0.6 cm (Figure 3). The MIC values and fungicidal results with percentage in plant extracts are shown in Figure 4.
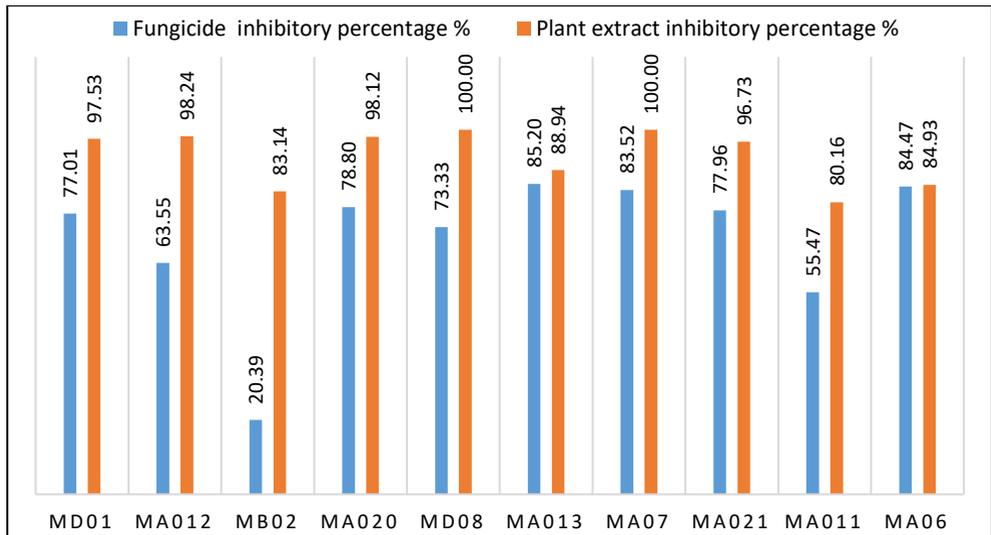
Figure 3. The Minimum Fungicide inhibitory concentration (MFC)% for fungicide and plant extract.
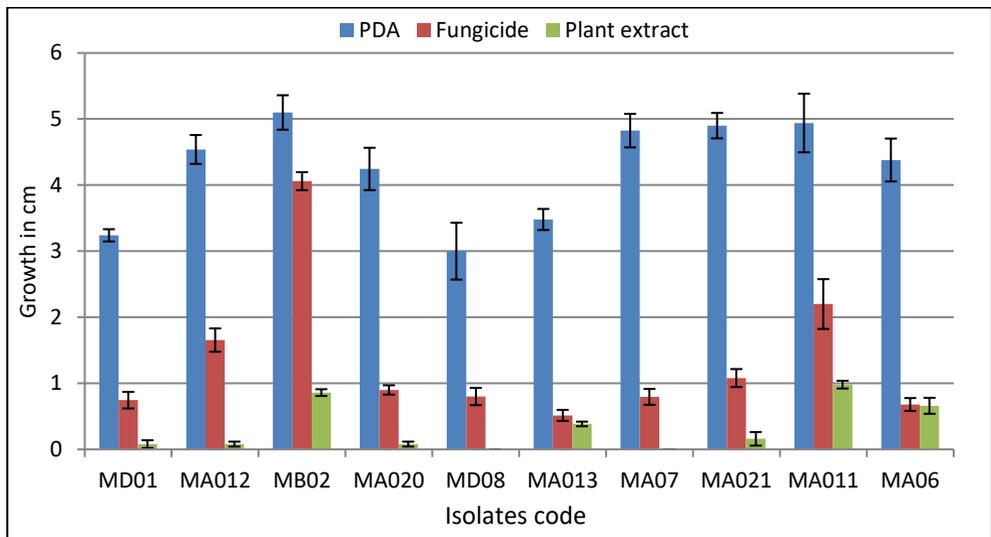
Figure 4. The growth rate at three variances; the blue bar represents the negative control condition (PDA); the red bar represents the growth at the medium treated with fungicide: while the green bar represents the growth at the medium treated with plant extract.

4. Discussion

The utilization of molecular traits and phylogenetic relationships derived from the ITS genetic markers enabled the identification of six species and six genera. The utilization of ITS markers has significantly enhanced the ability of researchers to identify and differentiate isolates from distinct species. Nevertheless, the process may prove to be less effective when attempting to distinguish isolates from closely related species.

The previous claim that ITS markers are more helpful for species identification and interspecies distinction is supported by the data. According to [3], [10], [21], [34] and S. Rolfsii's mycelia in vitro, ITS markers for Aspergillus and Alternaria allow for precise species differentiation [16].

Because biocontrol agents are highly selective, biodegradable, non-toxic to plants, ecologically friendly, and often employed in disease management, biological control is the best alternative technique to restrict the activity of pathogens [31]. As a result, substitutes for these artificial pesticides are being used, such as vegetable oils and plant extracts, such as M. oleifera extract [13].

According to this study, using plant extracts seemed to inhibit the pathogens' linear growth, which caused the foliar diseases or root rot that were investigated. All infections' linear expansion was generally and at varying degrees suppressed by the Rosa Damascena Mill extract.

For example, the results of this study demonstrated that plant extracts were more effective than fungicides at inhibiting certain plant activities (Figure 5).

For the majority of isolates, the fungal growth rate on the PDA medium treated with plant extract ranged from 0 to 0.5 cm, while the growth rate on the PDA medium treated with fungicide ranged from 0.5 to 4 cm.
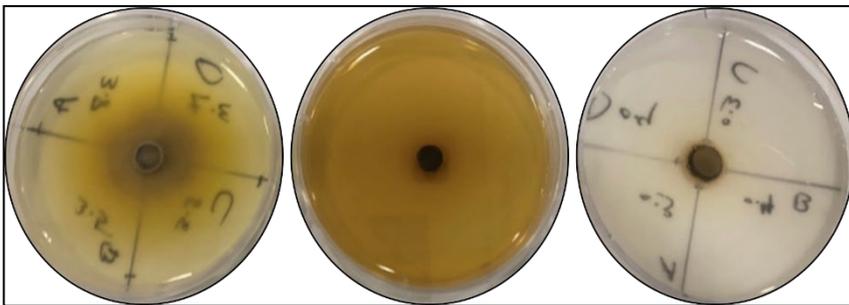
Figure 5. Compare the inhibition ability of the plant extract toward the negative and positive control; the first was the negative control (PDA); the second was treated with plant extract and the third was treated with fungicide.

The findings aligned with the findings of [12] and [21]. Several oils were found to inhibit the growth of multiple pathogens, including *F. moniliform, Helminthosporium oryzar, Alternaria citri, Sclerotium Batticaloa, Aspergillus niger*, and *Penicillium italicum*. Clove juice also hinders the formation of *Macrophomina Phaseolina sclerotia* [9]. Furthermore, the growth of the parasite Aspergillus parasiticus and the synthesis of aflatoxin are inhibited by the essential oils of thyme, cumin, clove, and rosemary [8]. *Penicillium, Alternaria* and *Fusarium* species can therefore be recognized as the most abundant mycotoxigenic genera [3] and [14].

In general, both fungicide and plant extract have a positive influence on fungal growth reduction, but the plant extract showed a higher reduction compared to fungicide (Table 4). For example, the percentage of inhibitory activity of plant extract on Mucor circinelloides species was between (88.9-100%) while in fungicide was about (63.5-85.2%). Also, the

growth reduction percentage for *Penicillium crustosum* and *Alternaria alternata* species was between 83-98% using plant extract and for fungicide, it was between 20.3-78%. While for the remaining isolates *Cladosporium tenuissimum, Phoma macrostoma* and *Fusarium equiseti,* the plant extract scored an inhibitory percentage between (84.5-100%), whereas for fungicide it was around (73.9-84.4%) (Table 3). However, fungal species have developed resistance to these herbicides, although synthetic pesticides have several harmful effects on the immune system and the environment. It has been shown to destroy beneficial insects and cause a range of diseases in both humans and animals, which increases the need to find new replacements for fungicides that are more efficient and friendly environment [10] and [24].

Table 4. Represent the summary of the inhibition activity results for both fungicide and plant extract against all fungal species isolates identified in this work.

Isolates code	Fungicide inhibitory percentage%	Plant extract inhibitory percentage%
MD01	77.00617	97.53086
MA012	63.54626	98.23789
MB02	20.39216	83.13725
MA020	78.79859	98.11543
MD08	73.33333	100
MA013	85.20115	88.93678
MA07	83.52332	100
MA021	77.95918	96.73469
MA011	55.46559	80.16194
MA06	84.47489	84.93151

5. Conclusion and Future Perspectives

The present study has brought to light the fact that *Mucor circinelloides* species are the predominant fungi that have an impact on fruit and vegetable produce. Three of ten isolates, also *Penicillium crustosum* and *Alternaria alternata* species appeared 40% of the total isolates number. Whereas the remaining single isolates from each of the following species: *Cladosporium tenuissimum, Phoma macrostoma* and *Fusarium equiseti* represent 30% of a total number of pathogens. Using ITS markers and the data stored in GenBank, a phylogenetic tree was constructed, which confirmed the species of the isolates and fitted them to the appropriate species branch in the tree. Further research is required to ascertain the optimal conditions under which mycotoxigenic organisms can produce mycotoxins. Such research may consider the influence of pH, temperature, and water activity on the virulence of phytopathogenic fungi, among other variables. Prospective lines of inquiry may revolve around the determination of the acceptable level of toxicity for the various varieties of mycotoxins that are generated. To validate the effectiveness of existing plant extracts as fungicide substitutes, research could be conducted in conjunction with these studies to expand their application to include additional isolates and species of fungi.

References

1. Abd-Elsalam, K.A., Roshdy, S., Amin, O.E., Rabani, M., 2010. First morphogenetic identification of the fungal pathogen Colletotrichum musae (Phyllachoraceae) from imported bananas in Saudi Arabia, Genet. Mol. Res. 9, 2335-2342.

2.   Akbar, F. and Alam, S. (2019). Influence of water activity and time duration on fusarium mycotoxins production in maize grains during post-harvest storage. Sarhad Journal of Agriculture, 35: 1326–35.

3.   Sankar, N.R. and Babu, G.P. (2012) First Report of Fusarium Proliferatum Causing Rot of Garlic Bulbs (Allium sativum) in India. Plant Disease, 96, 290.

4.   Alborch, L.; Bragulat, M.R.; Abarca, M.L. and Cabañes, F.J. (2011). Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus carbonarius* on maize kernels. Int. J. Food Microbiol., 147: 53-7.

5.   Almiman, B. (2023a). Effects of temperature and water activity on 25 de novo strains of pathogenic plant fungi in Al-Baha and Baljurashi cities in Saudi Arabia. Journal of Umm Al-Qura University for Applied Sciences, pp. 1-12.

6.   Almiman, B. (2024). Effects of temperature and water activity on 25 de novo strains of pathogenic plant fungi in Al-Baha and Baljurashi cities in Saudi Arabia. Journal of Umm Al-Qura University for Applied Sciences, 10: 301-12.

7.   Almiman, B.F. (2023b). Identifying phytopathogenic fungi in Albaha province, Saudi Arabia through their molecular and morphological features: An overview. Saudi Journal of Biological Sciences, 30: 103572.

8.   Sima, W.X., Tan, W., Yang, Q., (2011) Long AC Arc Movement Model for Parallel Gap Lightning Protection Device with Consideration of Thermal Buoyancy and Magnetic Force. Proceedings of the CSEE, 31, 138-145.

9.   Radwan, D.E.M., Lu, G., Fayez, K.A., and Mahmoud, S.Y. (2008). Protective action of salicylic acid against bean yellow mosaic virus infection in Vicia faba leaves. Journal of Plant Physiology 165(8): 845-857.

10.   Faraj BA, Gottlieb GR, Camp VM, et al. (1989) Development of a sensitive radioassay of histamine for in vitro allergy testing. J Nucl Med ;25:56-63.

11.   Armitage, A.D.; Barbara, D.J.; Harrison, R.J.; Lane, C.R.; Sreenivasaprasad, S.; Woodhall, J.W. and Clarkson, J.P. (2015). Discrete lineages within *Alternaria alternata* species group: Identification using new highly variable loci and support from morphological characters. Fungal biology, 119: 994-1006.

12.   Seo, B. Martin, Emily; Hoffmeister, Mathias; Jun, Mansing; Koellner, Thomas; Reineking, Björn; Shope, Christopher L; Tenhunen, John(2014): Deriving a per-field land use and land cover map in an agricultural mosaic catchment. *Earth System Science Data*, 6(2), 339-352.

13.   Betihaa , Z.M. Kheiralla b , A.S. Mansour c , A.N. Emam, Samy B. El-Henawya , Eslam A. Mohameda and N.A. Negm(2022). A Review on Different Plants Extract Mediated Silver Nanoparticles: Preparation, Antimicrobials, and Antioxidant Egypt. J. Chem. Vol. 65, No. 5 pp. 575 - 589 (2022).

14.   Bebber, D.P.; Ramotowski, M.A.T. and Gurr, S.J. (2013). Crop pests and pathogens move polewards in a warming world. Nature Climate Change, 3: 985-988.

15.   Bennett, J. and Klich, M. (2003). Chotoxins. C. lin. Microbiol. Rev., 16: 497-516.

16.   Abd El-Hai and Abeer A. Ali, 2019. Formulation of Trichoderma, Saccharomyces and Rhizobium Metabolites Against Damping-off and Root Rot Pathogens in Peanut Plant. Asian Journal of Biological Sciences, 12: 114-121.

17.   Dean, R.; Van Kan, J.A.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R. and Ellis, J. (2012). The Top 10 fungal pathogens in molecular Plant Pathology. Mol. Plant Patho.,13: 414-430.

18.   El-Sayed, S.A. (2022). Collaborative potentialities of Trichoderma spp. and Saccharomyces cerevisiae against damping-off and root rot diseases of Faba bean. Egyptian Journal of Phytopathology, 50: 65–78.

19. Fadl, M.A.; Farrag, H.F. and Al-Sherif, E.A. (2015). Floristic composition and vegetation analysis of wild legumes in Taif district, Saudi Arabia. Int. Res. J. Agric. Sci. Soil Sci., 5: 74-80.

20. Fandohan, P.; Hell, K.; Marasas, W. and Wingfield, M. (2003). Infection of maize by Fusarium species and contamination with fumonisin in Africa. African Journal of Biotechnology, 2: 570-9.

21. Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M. and Zaks, D.P. (2011). Solutions for a cultivated planet. Nature, 478 (7369): 337-342.

22. Gasperini, A.M.; Medina, A. and Magan, N. (2022). Comparison of growth and aflatoxin B1 production profiles of Aspergillus flavus strains on conventional and isogenic GM-maize-based nutritional matrices. Fungal Biology, 126: 82-90.

23. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Meintjes, P. and Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28: 1647-9.

24. Mbareche, H.; Veillette, M. and Bilodeau, G.J. (2021). In Silico Study Suggesting the Bias of Primers Choice in the Molecular Identification of Fungal Aerosols. Journal of Fungi, 7: 99.

25. Moyo, B.; Oyedemi, S.; Masika, P.J. and Muchenje, V. (2012). Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. Meat science, 91 (4): 441-447.

26. Nelson, P.E.; Desjardins, A.E. and Plattner, R.D. (1993). Fumonisins, mycotoxins produced by Fusarium species: biology, chemistry, and significance. Annu. Rev.

27. Pal, K.K. and Gardener, B.M. (2006). Biological control of plant pathogens. The plant health instructor, 2: 1117-42.

28. Pimentel, D.; Hepperly, P.; Hanson, J.; Douds, D. and Seidel, R. (2005). Environmental, energetic, and economic comparisons of organic and conventional farming systems. Bioscience, 55: 573-82.

29. Raja, H.A.; Miller, A.N.; Pearce, C.J. and Oberlies, N.H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. J. Sarrwat mountains at taif, KSA. AJPS, 6: 134-45.

30. Saleh, A.A.; Sharafaddin, A.H.; El-Komy, M.H.; Ibrahim, Y.E.; Hamad, Y.K. and Molan, Y.Y. (2017). Fusarium species associated with date palm in Saudi Arabia. Eur. J. Plant Pathol., 148: 367-77.

31. Sehsah, M.D.; El-Kot, G.A.; El-Nogoumy, B.A.; Alorabi, M.; El-Shehawi, A.M.; Salama, N.H. and El-Tahan, A.M. (2022). Efficacy of *Bacillus subtilis*, Moringa oleifera seeds extract and potassium bicarbonate on Cercospora leaf spot on sugar beet. Saudi journal of biological sciences, 29 (4): 2219-2229.

32. Sena, A.; Chaibub, A.A.; Côrtes, M.V.; Silva, G.B.; Silva-Lobo, V.L.; Prabhu, A.S. and Araújo, L.G. (2013). Increased enzymatic activity in rice leaf blast suppression by crude extract of *Epicoccum* sp. Tropical Plant Pathology, 38: 387-397.

33. Takan, J.P.; Chipili, J.; Muthumeenakshi, S.; Talbot, N.J.; Manyasa, E.O.; Bandyopadhyay, R. and Sreenivasaprasad, S. (2012). Magnaporthe oryzae populations adapted to finger millet and rice exhibit distinctive patterns of genetic diversity, sexuality and host interaction. Molecular biotechnology, 50: 145-158.

34. Vahdani, M.; Shoeibi, S. and Sharifan, A. (2022). Antifungal Susceptibility of Aspergillus flavus, *Aspergillus ochraceus*, and *Fusarium graminearum* to

Ganoderma lucidum Extract. Jundishapur Journal of Natural Pharmaceutical Products 17.

35. Woudenberg, J.; Groenewald, J.; Binder, M. and Crous, P. (2013). Alternaria redefined. Stud. Mycol., 75: 171-212.

36. Yang, R.; Su, J.; Shang, J.; Wu, Y.; Li, Y.; Bao, D. and Yao, Y. (2018). Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PloS one 13, e0206428.