MYCOFLORA ASSOCIATED WITH BANANA (MUSA PARADISIACA) GROWN IN JIZAN , SAUDI ARABIA.

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ABSTRACT: Banana (Musa paradisiaca) is commonly grown in Jizan province. This study is an attempt to find the mycoflora associated with this giant herb from three different locations of Jizan province. Potato Dextrose agar media was used for culturing and isolation of mycoflora associated with different symptomatic plant parts like the phylloplane, leaf sheath inflorescence, pseudostem, flower and fruit bunch. The prepared slides are identified by microscopic and macroscopic characteristics. A total of twenty fungal genera belonging to the four classes of fungi were isolated from the three regions Ad Darb, Baysh and Jizan regions of the province. The fungal genera isolated during the study are Pythium, Syncephalastrum, Perenospora, Rhizoctonia, Uredo musae, Sclerotium, Aspergillus, Botrytis, Epicoccum, Fusarium, Chaetomium, Cladosporium, Cochliobolus, Colletotrichum, Curvularia, Mycosphaerella, Nigrospora, Penicillium, Phoma and Verticillium. Herbarium from the symptomatic diseased plant parts is also prepared and preserved. This study reports Black Sigatoka disease caused by Mycosphaerella from all the three locations of study whereas. Anthracnose caused by Colletotrichum which is a major disease of Banana is absent in Baysh and Jizan regions. Uredo musae the causal phytopathogen is restricted only to Ad Darb region. Panama disease or Banana Wilt caused by Fusarium and Cigar end rot caused by Verticillium are the other diseases reported from the three regions of the province during this study. This study will help in effective monitoring and control of the phytopathogens of this valuable and nutritious fruit bearing plant.

Key words: Banana, Mycoflora, Jizan, Saudi Arabia.

INTRODUCTION:

Banana (*Musa paradisiaca*) belongs to the family "*Musaceae*". Banana plant is a giant herb. The fruit is a good source of energy and has high fiber, vitamins, minerals and is an excellent source of potassium. It is good for Heart, kidney cancer, ulcers and depression etc.

Major parts of Saudi Arabia depend on Bananas imported from different countries. Saudi Arabia is one of the largest fruit consuming markets, huge amounts of fruits are imported from different parts of the world. Banana prices in Saudi Arabia have increased by 40% after a halt in the supply from the Philippines and Central America (Ecuador and Nicaragua) as a result of the considerable growth of China's imports. The price per box has increased from 30 to 70 riyals (from 7.15 to 16.67 Euro). It is worth noting that the Saudi market consumes between 40-45 million cartons annually worth 500 million riyals (119.10 million Euro[1].

Bananas (*Musa* sp.) are imported in Saudi Arabia from tropical countries and are considered as most popular fruits [2]. In any case, official sources have stated that the situation of banana prices in the Saudi market has not affected other fruits, as the country imports a wide range of more than 200 products from many countries, including South Africa, Egypt, Chile, Lebanon, India, Pakistan, France or the U.S. [3].

Bananas are commercially grown in some parts of the Kingdom .Banana plantations are very common in Jizan province . Saudi Arabia is a major exporter of fruits and vegetables to its neighbors. Among its most productive crops are watermelon, grapes, citrus fruits, onions, squash and tomatoes. At Jizan in the country's well-watered southwest, Al-Hikmah Research the producing tropical fruits including pineapples, paw-paws, bananas, mangoes and guavas [4].

Attempts to grow and export good varieties of banana which are disease resistant have been taking place since a long time .A study made during his visit to the Kingdom last December revealed that areas in Jizan province are ideal for banana cultivation. These areas have climatic conditions similar to those of Tabasco which is a banana producing state," Sanchez said adding that another study has been planned to determine the temperature, wind, humidity and water condition before the program goes ahead [5]. The Banana plantations are prone to a number of fungal diseases which must be prevented and controlled in order to save from the economic losses due to them. Fungal diseases of banana can be classed into diseases of the foliage, diseases of the root, corm and pseudostem and diseases of the fruit, including those at post harvest, such as anthracnose. Of these, the most important are the fungal disease of the foliage caused by black sigatoka (Mycosphaerella fijiensis) and the wilt disease of the pseudostem caused by the four races of Panama disease (Fusarium oxysporum fm sp cubense). A full review of fungal diseases (of which there are more than 40) which affect bananas and plantains has been given in Jones (2000) [7].

These results suggest that F. thapsinum, which has not been reported yet in Saudi Arabia, could be introduced into the country along with imported bananas and may cause diseases on other plant species [7]. Two isolates of Fusarium recovered from banana fruits were identified as Fusarium thapsinum. A slowly growing white colony that turned grey-violet in pigmentation on the agar was quite variable on PDA [7].

Colletotrichum musae is the causal agent of anthracnose in banana fruits; infection by this fungal pathogen results in severe post-harvest losses [7]. Antracnose disease has also been reported from Saudi Arabia. For the studies and preliminary screening,

eighty actinomycetes isolates were selected as antagonists against Colletotrichum musae, causal agents of anthracnose of banana [8].

Bananas of the world are now at serious risk from Panama disease as the fungicide-resistant pathogen crossed continents and breached quarantine efforts to spread across South Asia, Africa, the Middle East, and Australia [9] .Anthracnose refers to a group of fungal diseases characterised bv development of dark, sunken spots or lesions, often with a raised rim, on affected foliage, stems and fruit. Although C. musae is the most common species associated with anthracnose of banana, gleosporoides has also been reported to be associated with banana anthracnose [10].

Sample collection from the Sampling site:

Surveys are made to the banana plantations from three sampling sites of Jizan , Ad Darb and Baysh regions. The diseased samples from different parts of the banana plant are collected in sterile polythene packs using sterile instruments like scissor , needle and knife. The diseased parts of the banana plant like the phylloplane , leaf sheath , inflorescence ,pseudostem , flower and fruit bunch are collected from Baysh , Ad Darb and Jizan regions Figure 1. Pictures and videos are also taken from all the sampling sites Figures.2, 3 , 4.



Figure 1.Map of Jizan province showing the three sampling sites (Baysh, Ad Darb and Jizan).



Figure 2. Pictures of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Baysh region of Jizan Province).



Figure 3. Pictures of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Ad Darb region of Jizan Province).



Figure 4. Pictures of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Jizan region of the Province).

Materials and Methods:

The glassware is washed after rinsing with $K_2Cr_2O_7$ and then sterilized in hot air oven at $160^{\circ}C$ for 24 hours. Potato Dextrose Media is employed to culture the mycopathogens. 0.5cm square pieces of the diseased plant parts (phylloplane , leaf sheath , inflorescence , pseudostem , flower and fruit bunch from the three regions of Jizan under study are inoculated directly on the petriplates . A qualitative and quantitative assessment of mycoflora is carried out by the fungal cultures on the petri plates and the slides are identified by microscopic and macroscopic characteristics .

Slide Preparation and Identification: Lactophenol and cotton blue in lactophenol are used as mounting and staining media for preparing semi permanent slides which are sealed .Research microscope with adequate high power is used through out the study. Photomicrographs are taken by using the microscope with camera. Identification was carried out by using standard manuals and keys .

Microscopic and Macroscopic observation:

Research microscope with adequate high power has been used through out the study.

Colony characters on media. Morphological characters like nature of mycelium, its colour ,sexual and asexual structures and their characters , conidiophore nature

and spore nature are used in identification. Pictures of the petriplates and photomicrographs are also taken during the study.

Herbarium Preparation: The samples from the diseased plant parts are collected and placed on tissue papers to sun dry . The symptoms are studied by macroscopic and microscopic characteristics on the infected plant parts. The infection of fungal pathogens was identified using different approaches such as looking at the appearance of decayed fruit, including the color sign of the pathogen spore or fruiting bodies, and location of infection sites. Then the samples are spread on white paper and dried under shade for two days and then pasted with a cellotape. The plant parts with thicker plant body are spread on paper and covered with paper and pressed for iron drying before applying cellotape for long term preservation .The papers are placed in plastic covers and arranged in a file with serial numbers, date, place of collection along with the name of the collector . Information on the name of the disease , the classification of the pathogenic genera are also provided .The Herbarium is laboratory of the College of saved in the Botany Science and Arts at Ad Darb, Jizan University Figures 5, 6, 7, 8.



Figure 5. Herbarium of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Baysh region of Jizan Province).



Figure 6. Herbarium of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Ad Darb region of Jizan).



Figure 7. Herbarium of the Different Parts of Banana (*Musa paradisiaca*) infected by Mycoflora from the Sampling Site (Banana plantation at Jizan region of the Province)



Figure 8. Preserved Herbarium

RESULTS AND DISCUSSION:

A total of twenty fungal genera belonging to the four classes of fungi were isolated from the three regions Ad Darb , Baysh and Jizan regions of the province .The fungal genera isolated during the study are *Pythium ,Syncephalastrum , Perenospora ,Rhizoctonia ,Uredo musae ,Sclerotium , Aspergillus, Botrytis, Epicoccum, Fusarium , Chaetomium , Cladosporium ,Cochliobolus , Colletotrichum, Curvularia , Mycosphaerella ,Nigrospora, Penicillium , Phoma and Verticillium .*

The results obtained from leaf surfaces (phyllosphere and phylloplane) were basically similar on the two types of media and the most common fungi were Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Giberella, Memnoniella, Mycosphaerella,

Setosphaeria and Stachybotrys [11].

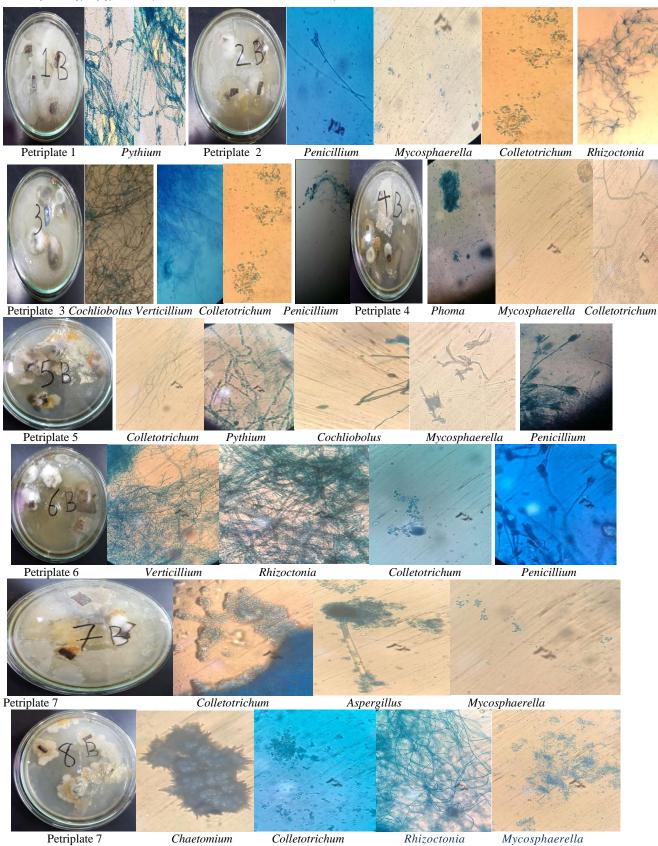
The samples from different parts of the Banana plant like the inflorescence, leaf, pseudostem, leaf sheath, flowers, fruits and fruit bunch were studied from Baysh region of Jizan province. The infected parts showed fungi belonging to Oomycotina Basidiomycotina and Ascomvcotina. Pythium Chaetomium, Rhizoctonia , Aspergillus, Botrytis , Cochliobolus , Colletotrichum , Curvularia , Fusarium Mycosphaerella , Penicillium , Phoma Verticillium are the thirteen genera isolated during the present study from the Baysh region Figure 9, Table 1. Colletotrichum is the predominant genera and is the causal organism of Anthracnose disease which is a major disease of the plant under study from this region.

The samples from different parts of the Banana plant like the inflorescence, leaf, pseudostem, leaf sheath, flowers, fruits and fruit bunch were studied from Ad Darb region of Jizan province. The infected parts showed fungi belonging to *Oomycotina* ,Zygomycotoina, Basidiomycotina and Ascomycotina. Pythium , Syncephalastrum ,Rhizoctonia ,Uredo musae , Aspergillus, Epicoccum, Fusarium , Mycosphaerella , Nigrospora ,Penicillium , Phoma and Verticillium are the twelve genera isolated during the present study from Ad Darb region Figure 10 ,Table Mycosphaerella is the predominant genera and is the causal organism of Black Sigatoka disease which is a severe disease of the plant under study.

The samples from different parts of the Banana plant like the inflorescence, leaf, pseudostem, leaf sheath, flowers, fruits and fruit bunch were studied from Jizan region of Jizan province. The infected parts showed fungi belonging to Oomycotina ,Basidiomycotina and Ascomycotina. Pythium , Perenospora ,Rhizoctonia ,Sclerotium , Aspergillus, Botrytis, Fusarium Chaetomium Cladosporium ,Cochliobolus Colletotrichum, Fusarium Mycosphaerella Penicillium , Phoma and Verticillium are the fifteen genera isolated during the present study from Jizan region Figure 11, Table 3. Mycosphaerella is the predominant genera and is the causal organism of Black Sigatoka disease which is a major disease of the plant under study followed by Colletotrichum which is the causal organism of anthracnose disease.

Eight fungal genera which are isolated from all the three locations during the study are ,Rhizoctonia, Aspergillus, ,Fusarium , Mycosphaerella Penicillium , Phoma and Verticillium Mycosphaerella is the causal organism for the Sigatoka diseases and Verticillium is the causal organism of Cigar end rot . Four fungal genera which are isolated from Baysh and Jizan regions only are Botrytis . Chaetomium , Cochliobolus and Colletotrichum. Curvularia was isolated only from Baysh. Four fungal Syncephalastrum , Uredo musae , Epicoccum and Nigrospora are isolated only from Ad Darb region only. Banana rust caused by Uredo musae is isolated from Ad Darb region only. Three fungal genera Perenospora , Sclerotium and Cladosporium restricted only to Jizan region Figure 12.

Mycosphaerella is the predominant genera and is the causal organism of Black Sigatoka disease which is a severe disease of the plant under study and is reported from all the three regions under study. Colletotrichum is the predominant genera and is the causal organism of Anthracnose disease which is a major disease of the plant under study from Baysh region only. Banana rust caused by Uredo musae is isolated from Ad Darb region only.



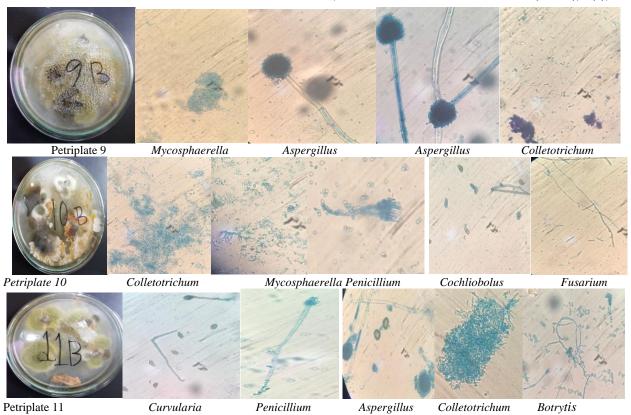
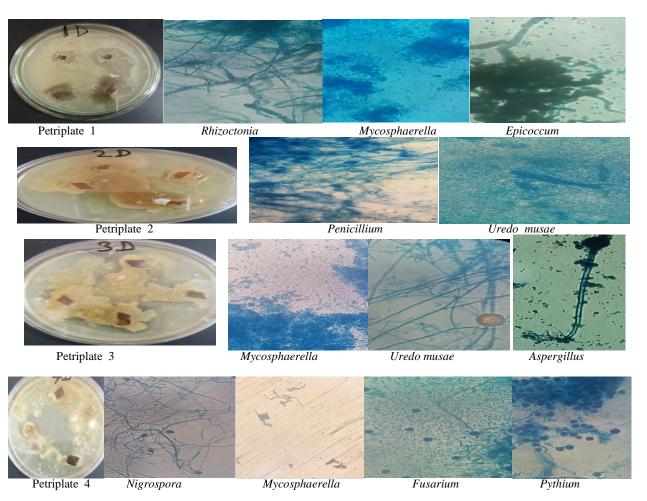


Figure 9. Photomicrographs of the Mycoflora from the different parts of the Banana plant from Baysh region of Jizan Province.



January-February

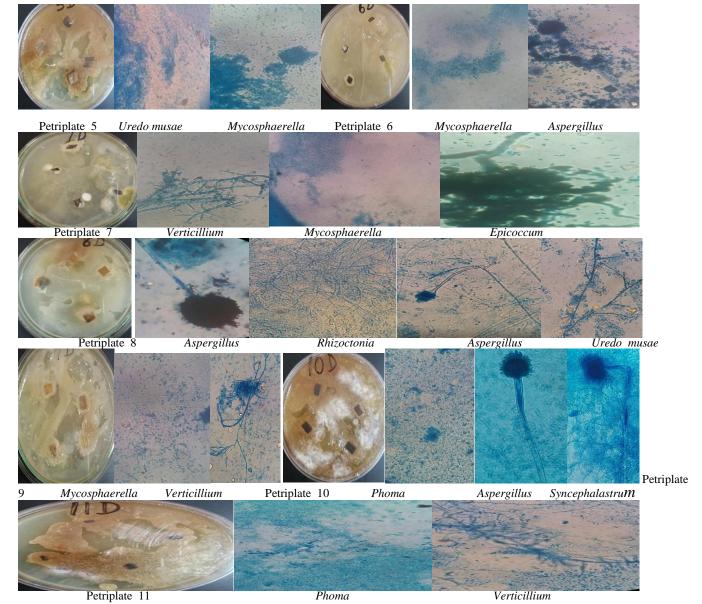
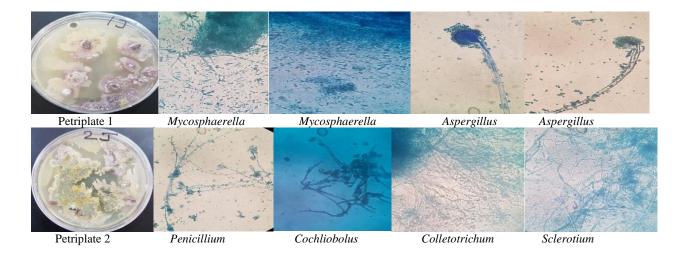
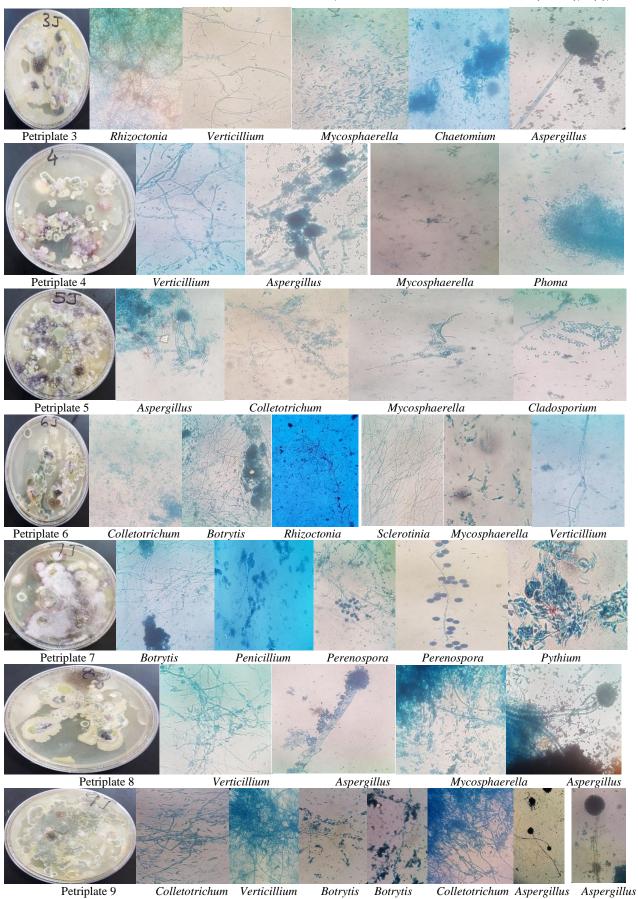


Figure 10. Photomicrographs of the Mycoflora from the different parts of the Banana plant from Ad Darb region of Jizan Province.





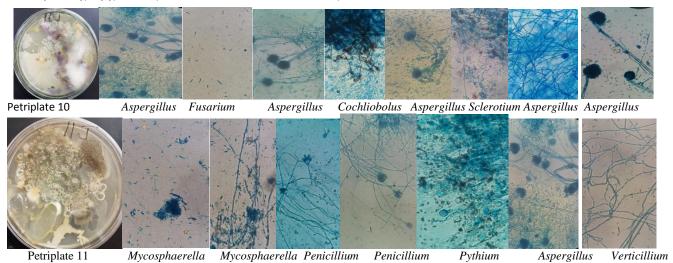


Figure 11. Photomicrographs of the Mycoflora isolated from the infected parts of the Banana plant from Jizan region of the province

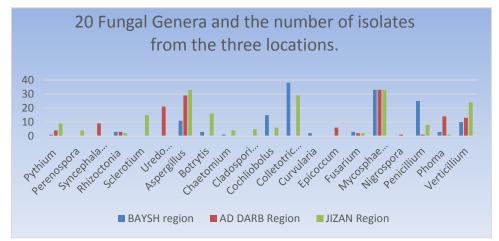


Figure 12. Bar Diagram to represent the different fungal genera and the number of isolates from Baysh , Ad Darb and Jizan regions of the Province

Among diseases caused by fungi, the leaf spot (Sigatoka) caused by Mycosphaerella fijiensis (Black Sigatoka), and M.musicola (Yellow Sigatoka), (Septoria leaf spot) are considered to be the most serious. Recently Septoria leaf spot has also been recorded to cause significant loss. Panama wilt (Fusarium oxysporum f.sp.cubense) Symptom: Fusarium wilt is lethal disease. Leaf Spot /Anthracnose (Colletotrichum musae (Berk. & Curt.) v. Arx) .Cigarend rot (Verticillium theobromae(Turc.) Masonet Hughes) .Finger tip or black tip rots (Botryodiplodia theobromae Pat.) [12,13].

Sigatoka or Leaf spot or Leaf Streak Disease: It is one of the major diseases of Banana in the region. It is caused by *Mycosphaerella musicola*. The leaves are severely affected by this diseases .The young leaves show small pale spots which coalesce and enlarge into spindle shaped intense streaks .The spots are black or yellow in color with grey center which are surrounded by brown ring. The whole plant is affected and gives a burnt appearance. It leads to death of leaves and fruit.

The fruits are undersized and poor in quality due to this diseases Figure 13-i,ii,iii, x,xi,xii,xiii,xiv,xv .

Panama disease or Banana Wilt (Fusarium wilt): It is caused by Fusarium. This disease affects the leaves, leaf sheaths, pseudostem and other plant parts. The leaves turn pale and yellow beginning with the oldest leaves and moves towards the center. The yellowing starts from the leaf margin and extends towards the midrib region. The leaves droop, wilt, hang down and break leading to the death of the entire canopy Figure 13-.xvi, xvii, xviii, xix.

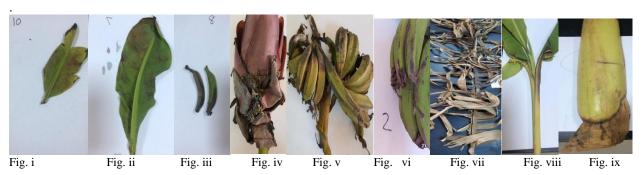
Cigar End Rot: It is caused by *Verticillium*. It is a dry mummified necrotic rot spreading from the perianth towards the tips of the fingers on the inflorescence and fruit bunch. The rotten finger ends adhere to the fruit which gives the characteristic appearance of cigar end Figure 13- iv, v.

Anthracnose of Banana: It it is one of the severe diseases affecting Banana crop in the region. It is caused by *Colletotrichum musae*. The leaves, pseudostem, fruit, pedicel and flowers are infected by this fungus at all stages of growth. Initially small black or brown lesions appear which enlarge and lead to shrivelling, withering, dropping off and finally death of the plant parts Figure 13 vi, vii, viii, ix.

Banana Rust: It is caused by *Uredo musae* and was isolated only from Ad Darb region during this study. Dark brown to black lesions which are surrounded by yellow margin appear on the phylloplane and other

infected parts. The spore masses are raised on the surface and impart a rough texture to the infected leaf Figure 13- xx .

Hence this study reports Black Sigatoka disease caused by *Mycosphaerella* from all the three locations of study whereas Anthracnose caused by *Colletotrichum* which is a major disease of Banana is absent in Baysh and Jizan regions. *Uredo musae* the causal phytopathogen is restricted only to Ad Darb region. Panama disease or Banana Wilt caused by *Fusarium* and Cigar end rot caused by *Verticillium* are the other diseases reported from the three regions of the province during this study.



Figs. i ii,iii Anthracnose and Sigatoka on phylloplane and fruit . Figs. iv and Fig. v Cigar End Rot on Inflorescene and Fruit bunch Figs. vi,vii,viii,ix Anthracnose on phylloplane , pseudostem and fruit bunch



Fig. x Fig. xii Fig. xiii Fig. xiii Fig. xiii Figs. x,xi,xii,xiii,xii,xiv,xv Sigatoka or Leaf Streak on Phylloplane and Fruit



 $Figs.xvi\ , xvii, xviii, xix\ \ Panama\ disease \quad or \quad \textit{Fusarium}\ \ Wilt\ Fig.xx\ \ Banana\ Rust\ by\ \textit{Uredo musae}$

Fig. 13. Important Diseases of Banana (Musa paradisiaca) from the Sampling Sites at Jizan region of the Province).

Table 1. Fungal Genera Isolated from the different parts of the Banana tree from Baysh region of Jizan Province.

Sr.N	Sample	Oomycotina	Basidiomycotina	Ascomycotina												
0		Pythium	Rhizoctonia	Aspergillus	Botrytis	Chaetomium	Cochliobolus	Colletotrichum	Curvularia	Fusarium	Mycosphaerella	Penicillium	Phoma	Verticillium		
1	Inflorescence	+(1)	-	-	-	-	-	-	-	-	-	-	-	-		
2	Phylloplane	-	+(1)	-	-	-	-	+(1)	-	-	+(3)	+(2)	-	-		
3	Pseudostem	-	-	-	-	-	+(4)	+ (6)	-	-	-	+ (4)	-	+(3)		
4	Flower	-	-	-	-	-	-	+(3)	-	-	+(7)	-	+(3)	-		
5	Leaf Sheath	-	-	-	-	-	+(8)	+(6)	-	-	+(8)	+(4)	-	-		
6	Pseudostem	-	+(1)	-	-	-	-	+(3)	-	-	-	+(3)	-	+(7)		
7	Phylloplane	-	-	+(1)	-	-	-	+(3)	-	-	+(3)	-	-	-		
8	Fruit	-	+(1)	-	-	+(1)	-	+(4)	-	-	+(5)	-	-	-		
9	Fruit Bunch	-	-	+(5)	-	-	-	+(3)	-	-	+(1)	-	-	-		
10	Phylloplane	-	-	-	-	-	+(3)	+(4)	-	+(3)	+(6)	+(6)	-	-		
11	Phylloplane	-	-	+(5)	+(3)	-	-	+(5)	+(2)	-	-	+(3)	-	-		
Total r	number of isolates	1	3	11	3	1	15	38	2	3	33	25	3	10		
Total fungal genera = 13								(+) F	resent	(-) Absent					

Table 2. Fungal Genera Isolated from the different parts of the Banana tree from Ad Darb region of Jizan Province.

Sr.N	Sample	Oomycoti	Zygomycotinamyco	Basidiomycotina		Ascomycotina									
o		na	tina												
		Pythium	Syncephalastrum	Rhizoctonia	Uredo musae	Aspergillus	Epicoccum	Fusarium	Mycosphaerella	Nigrospora	Penicillium	Phoma	Verticillium		
1	Phylloplane	-	-	+(2)	-	-	+(2)	-	+(6)	-	=	-	-		
2	Leaf Sheath	-	-	-	+(6)	-	-	-	-	-	+(1)	-	-		
3	Pseudostem	-	-	-	+(8)	+(1)	-	-	+(2)	-	-	-	-		

			IS	ISSN 1013-5316;CODEN: SINTE 8			Sci.I	nt.(Lahore),3	31(1),123-136,2				
4	Pseudostem	+(4)	-	-	-	-	-	+(2)	+(4)	+(1)	-	-	-
5	Pseudostem				+(6)	-	-	-	+(14)	-	-	-	-
6	Phylloplane	-	=	-	=	+(1)	-	-	+(1)	=	-	-	=
7	Phylloplane	-		-	-	-	+(4)	-	+(1)	-	-	-	+(4)
8	Phylloplane	=	-	+(1)	+(1)	+(4)	-	-	-	-	-	-	-
9	Phylloplane	-	-	-	-	-	-	-	+(5)	-	-	-	+(7)
10	Inflorescence	-	+(9)	-	=	+(22)	-	-	-	=	-	+(6)	=
11	Inflorescence	-	-	-	-	-	-	-	-	-	-	+(8)	+(2)
Total	number of isolates	4	9	3	21	29	6	2	33	1	1	14	13
			Total fungal genera = 12						(-) <i>A</i>	Absent			

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Table 3. Fungal Genera Isolated from the different parts of the Banana tree from Jizan region of the Province.

Sl.N	Sample	Oomycotina		Basidiomycotina		Ascomycotina										
o		Pythium	Perenospora	Rhizoctonia	Sclerotium	Aspergillus	Botrytis	Chaetomium	Cladosporium	Cochliobolus	Colletotrichum	Fusarium	Mycosphaerella	Penicillium	Phoma	Verticillium
1	Fruit Bunch	-	-	-	-	+(3)	-	-	-	-	-	-	+(12)	-	-	-
2	Fruit Bunch	-	-	-	+(4)	-	-	-	-	+(3)	+(3)	-	-	+(4)	-	-
3	Phylloplane	-	-	+(1)	-	+(2)	-	+(4)	-	-	-	-	+(5)	-	-	+(4)
4	Inflorescence	-	-	-	-	+(2)	-	-	-	-	+(2)	-	+(5)	-	+(1)	+(10)
5	Inflorescence		-	-	-	+(5)	-	-	+(5)	-	+(2)	-	+(5)	-	-	-
6	Leaf Sheath	-	-	+(1)	+(1)	-	+(8)	-	-	-	+(1)	-	+(6)	-	-	+(6)
7	Fruit Bunch	+(7)	+(4)	-	-	-	+(3)	-	-	-	+(3)	-	-	+(1)	-	-
8	Fruit Bunch	-	-	-	-	+(5)	-	-	-	-	+(6)	-	-	-	-	+(3)
9	Phylloplane	-	-	-	-	+(5)	+(5)	-	-	-	+(5)	-	-	-	-	+(1)
10	Phylloplane	-	-	-	+(3)	+(6)	-	-	-	+(3)	-	+(2)	-	-	-	-
11	Phylloplane	+(2)	-	-	+(7)	+(5)	-	-	-	-	+(7)	-	-	+(3)		-
Total n	umber of isolates	9	4	2	15	33	16	4	5	6	29	2	33	8	1	24
Total fungal genera = 15								(+) Present	(-)	Absent						

CONCLUSION: Banana fruits are grown commercially in Jizan province. Twenty one fungal genera are isolated from the three locations of the province . Sigatoka , Anthracnose , Cigar end rot , Fusarium wilt and Banana rust are the diseases reported from this nutritionally important crop in the region. The plant is highly susceptible to many diseases which lead to fruit deterioration leading to economic losses which ultimately lead to high market prices. Therefore it is necessary to study the diseases associated with this plant and take the necessary precautionary and control measures . The diseases must be checked and controlled in order to produce high yielding , disease resistant and improved quality of the fruit.

Conflict of Interest: Authors declare that we have no conflict of interest

ACKNOWLEDGEMENTS: The authors from the College of Science and Arts at Ad Darb , Jizan University , Jizan , Saudi Arabia take this opportunity to thank the Dean Dr. Ayesha Khasim Ali Al Shamakhi, the Vice Dean Dr. Wafa'a Asiri and the Head of the Research Committee Dr. Fatimah Qassadi for their immense support . The authors also thank (Dr.) Isra Syed Siraj for her help in the preparation of this manuscript.

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