A

PROJECT REPORT ON

"Isolation and Characterisation of Pathogen causing Black Sigatoka Disease in *Musa spp*"

SUBMITTED TO

VIVEKANAND COLLEGE, KOLHAPUR

(EMPOWERED AUTONOMOUS)

DEPARTMENT OF BIOTECHNOLOGY

SUBMITTED BY

Miss. Sakshi Anil Kathare

FOR THE PARTIAL FULFILMENT OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

For the year

2023-2024

UNDER THE GUIDANCE OF,

Mr. A.L.Upadhye & Ms. R.M.Shetty

Asst. Professors,

Department Of Biotechnolog

"Education for Knowledge, Science and Culture"

~Shikshan Maharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

Vivekanand College,Kolhapur

(Empowered Autonomous)

Certificate

This is to certify that, **Miss Sakshi Anil Kathare**, Exam Number_____ has satisfactorily completed a Project report on **"Isolation and Characterisation of Pathogen causing Black Sigatoka Disease in *Musa spp*"** as a part of syllabus prescribed by BoS Department of Biotechnology, Vivekanand College, Kolhapur for B.Sc III course in Biotechnology(Entire) and this project represents her bonafide work of the year 2023-24.

Place: Kolhapur

Date :

Teacher In Charge

17/03/24

Examiner

16/3/24

HEAD
DEPARTMENT OF BIOTECHNOLOGY (ENTIRE)
VIVEKANAND COLLEGE, KOLHAPUR
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Head Of Department

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Record my since you thanks to Vivekanand College, Kolhapur for allowing me to carry my project works successfully in the college labs.

Kathare

Miss. Sakshi Anil Kathare

DECLARATION

I hereby declare that the project work entitled ""Isolation, Identification and Characterisation of Pathogen causing Black Sigatoka Disease in *Musa spp.*" is submitted to Vivekanand College, Kolhapur for the award of degree of 'Bachelor of Science,Biotechnology'. This is the result of bonafide work carried out by me under the guidance of assistant professor A.I. Upadhye and Ms. R.M Shetty

I further declare that the results presented here are not the basis for reward of any other degree.

Place: Kolhapur

Date:

Miss. Sakshi Anil Kathare

CONTENT

INTRODUCTION

Fruits are an excellent source of essential vitamins and minerals as well as the important and beneficial part of our day to day life. There are around 2000 fruits all over the world. India is known for its fruit production rate of 11% amongst all nations and hence is called as the 'Fruit Basket of The World'. *Mangifera indica* (Mango is the most cultivated crop of India followed by the second most *Musa spp*. (Banana). Globally banana crops rank fourth after rice, wheat and maize.

Musa spp. belongs to the monocotyledons class, *Zingiberales* order and to the *Musaceae* family. The genus *Musa* has more than 50 species which are further classified into numerous sub species. The genus includes 83 species of flowering plants including edible fruits that is banana and plantains. Banana is grown in tropical and subtropical areas and is a staple food crop in most of the countries like Africa and Asia. Biotic and abiotic factors do affect in the cultivation of banana. The environmental factors like drought salinity and heat stress as well as cold temperature is also a major a biotic factor which has severe affects on the banana production .31 to 32°c temperature is favourable for the growth of the plant as well as its development with the essential availability of the factors nutrients and water. This leads to the higher yield of the crop. Due to this there are many cultivars of banana are present. Parents of the cultivars *Musa acuminata* and *Musa Balbisiana*, two wild species which are usually seedy. Cause of the binomial Latin nomenclature for edible varieties of the cultivars they are referred to as Musa spp(AAA Group, Cavendish Group).*Musa acuminata*(genomeA) is known for its fruit quality characters whereas *Musa Balbisiana (*Genome B) is known to be more tolerant to biotic and abiotic stress. The nature of the fruit does depend upon the species it is from. M. acuminata gives sweet fruit while *M. Balbisiana* gives starchy fruit. There are approximately 500 varieties of bananas and plantains, about 150 of these are primary clones and rest of them other somatic mutants. Banana cultivars are complex, diploid, triploid and tetraploid hybrids. Fo example AB is diploid,an AAB is a triploid and AABB, a tetraploid. Most familiar seedless, cultivated varieties of banana are triploid hybrids (AAA, AAB, ABB). Diploid (AA, AB, BB) and tetraploids (AAAA, AAAB, AABB, ABBB) are much rare. In India Bananas based on the cultivars are divided into following groups:

- **Dwarf Cavendish / G9 variety (AAA)**

The AAA genome variety also known as Dwarf Cavendish or G9 variety is a popular commercially grown banana in many states of India. This type of Banana is grown extensively for table and processing purposes. Is mostly found cultivated in the states like Maharashtra, Gujarat, Bihar and West Bengal. Also it is popular in Tamilnadu, Karnataka and Andhra Pradesh. 'Basrai' is the leading commercial variety of this group found in Maharashtra. This plant is usually dwarf which makes it less susceptible to the wind damage. The size as well as the length of the fruit is quite good. The average bunch weight is about 15 to 25 kgs. Dwarf Cavendish is becoming an highly successful as it gives high yield. But this kind of variety is highly susceptible to various diseases mainly the black sigatoka, a leaf spot disease in humid regions.

- **Robusta(AAA)**

The Robusta is is mostly grown in the Tamilnadu and Karnataka region for table purposes it is high yielding and produces bunch of large size with well fruits. This type of variety of banana does produce semi tall variety of Banana plants. The bunch weighs about 25 to 30 kg. These are initially dark green in colour and turn up to bright yellow upon right name depending up on the writing. Robusta variety is very sweet with a good Aroma. Thekeeping qualities quite poor which leads to the breakdown of pulp after ripening hence it is not good for transportation. It is another variety of plants susceptable to sigatoka disease.

- **Nendran(AAB)**

It is a popular Kerala which is used for processing as well as is the consumption as a fruit. The cultivation is quite increased in the Tamil Nadu region since a few years. It has considerable diversity in plant stature stem colour presence and absence of male access bunch size etc. the fruits of this variety have distinct neck with thin green skin turning yellow on ripening.It weighs about 12-15 kgs per bunch.The fruit is starch during ripening and is highly susceptible to Banana Bract mosaic Virus.

➢ Red Banana(AAA)

The Red Banana is the most relist and high cost variety of Kerala and Tamilnadu. It is mainly cultivated in Kanyakumari and Tirunelveli districts of Tamil Nadu. It's also popular in some Southern States as well as Western and Central India. It is also popular by the name Lal velchi in Bihar while, Chandrabale in Karnataka. The colour of this Banana is reddish;fruit is sweet in taste;orange yellow coloured with a pleasant aroma. Bunch of these fruits do weigh 20- 30 kg. This variety is highly susceptible to *fusarium wilt* and nematodes.

➢ Rasthali (Silk AAB)

It is a medium tall variety of plant grown in the southern regions as well as in Bihar. It is a unique fruit quality and highly priced cultivar for table purpose. The colour is yellowish green which is further turned into pale yellow to golden yellow after ripening. It has a very good Aroma long crop duration etc. It has susceptibility to *fusarium wilt* requirement of bunch to covered to protect fruits from sun tracking and formation of hard lumps in fruits makes the crop production expensive.

➢ Poovan (Mysore AAB)

The poovan it is commercially cultivated with location specific ecotypes like Palayankodan in Kerala. It is also found in several states of South India. It is a perennial crop. Tamilnadu is the leading producer of poovan cultivar owing its climatic and marginal soil condition. Fruits are slightly acidic form and has a typical sweet and sour aroma. Fruits turn attractive golden yellow on ripening medium size bunch has closed packed fruits. It is highly susceptible to Banana bract mosaic virus and banana streak virus which reduces the yield.

➢ Ney Poovan (AB)

Ney Poovan is the deployed cultivar which is under the commercial mono cultivation and large scale production.In few regions of South India it is grown in the backyards too. It is a slender, having bunch of 15 to 30 kgs after almost 12 to 14 months. The colour of the fruit is initially dark green which intern turns into golden yellow and has a good

- **Safed Velchi Musa(A B Group)**

This Safed Velchi Musa is a good quality fruit cultivated in the Thane, Nashik districts of Maharashtra for the table purpose.It is an grown under the shade of arecanut gardens in the South Kanara districts of Karnataka. Variety is medium sized with slender yellowish green pseudostem and can be recognised by the reddish petiole margin, large fruits, very thin and papery rind and white firm flesh that is very sweet. The average bunch about 12 KG with 150 fruits per bunch in duration of 13 months.

- **Exotic varieties:**

1) USA-Dwarf Cavendish ,giant Cavendish, Pisang masak hijau, Ice cream, 'Enano Gigante, Macho, Orinoco
2) Brazil- Robusta, Santa catarina silver, Brazilian,
3) China- Dwarf Cavendish
4) South Africa- Dwarf Cavendish,golden beauty
5) Australia- Robusta,Williams,cocos
6) East Africa, Thailand- Bluggoe, Maricongo, Common Dwarf
7) Phillipines- Common dwarf, Phillipine Lakatan
8) 8)Taiwan - Giant Cavendish

FUNGAL DISEASES IN BANANA:

- **Leaf Spot, Leaf Streak or Sigatoka Disease (black leaf streak-*Mycosphaerella fijiensis* or yellow sigatoka-*Mycosphaerella musicola*; *black sigatoka*)**

Black and yellow sigatoka is one of the serious diseases affecting the banana crop. Initial symptoms appear in the form of light yellowish spots on the leaves. A small number of these enlarge, become oval; the colour also changes to dark brown. Still later, the centre of the spot dies, turning light grey surrounded by a brown ring. In severe cases, numerous spots coalesce, killing large parts of the leaf. Rainfall, dew and temperature determine the spread of the disease. Conditions favouring mass infection are most common during the rainy season with temperature above 21°C.

- **Panama Wilt (*Fusarium oxysporum f. sp. cubense*):**

This is a soil-borne fungal disease and gets entry in the plant body through roots. It is most serious in poorly drained soil. Initial symptoms are yellowing of lower leaves, including leaf blades and petioles. The leaves hang around the pseudostem and wither.

In the pseudostem of the diseased plant, yellowish to reddish streaks are noted with intensification of colour towards the rhizome. Wilt is severe in poor soil with continuous cropping of banana. Warm soil temperature, poor drainage, light soils and high soil moisture are congenial for the spread of the disease.

- **Anthracnose (*Gloeosporium musae*) :**

The disease attacks banana plants at all stages of growth. Disease attacks the flowers, skin and distal ends of banana heads. The symptoms appear as large brown patches covered with a crimson growth of the fungus. The disease fruit turns black and the fruit is shrivelled.

- **Cigar End Tip Rot (*Verticillium theobromae, Trachsphaera fructigena and Gloeosporium musarum):***

A black necrosis spread from the perianth into the tip of immature fingers. The rotted portion of the banana finger is dry and tends to adhere to fruits (appears similar to the ash of a cigar).

- **Crown Rot (*Colletotrichum musae, Fusarium sp., Verticillium theobromae, Botryodiploidia theobromae and Nigrospora sphaericu) :***

The characteristic symptoms are blackening of the crown tissues, which spreads to the pulp through the pedicel resulting rotting of the infected portion and separation of fingers from the hand.

- **Stem-end Rot (*Thielaviopsis paradoxa*) :**

The fungus enters through the cut stem or hand. The invaded flesh becomes soft and water-soaked.

Plant disease being an impairment of the normal state of the plant that is susceptible to each and every species. These diseases interrupt and modify in the integral functions of the plants. The food crops form an essential part of the diet of humans. During the production, harvesting and post harvesting processes these crops are highly susceptible to the contamination by pathogens with the potential to cause diseases and severe impacts in the environment. The awareness is spreaded globally as it is an exigent topic and hence biological controls are of great importance. The present attempt is to isolate and characterise pathogen causing Black Sigatoka Disease in Musa spp and to find a biocontrol for such a disease causing pathogen.

AIM AND OBJECTIVES

2.1.AIM:

To isolate, identify and characterise the pathogen causing Black Sigatoka disease in *Musa spp*.

2.2.OBJECTIVES:

1. Collection of plant sample from Kolhapur.
2. Isolation of fungus *Mycosphaerella fijiensis*.
3. Study of morphological characters.
4. Formulation of Biocontrol.
5. Induction of disease in plants and its study.

REVIEW OF LITERATURE

Black Sigatoka is considered the most damaging and costly disease of banana] because its control accounts for 27% of total production costs [1]. It has been estimated that the disease causes greater than 38% yield loss on plantain [2,3], and even greater losses may occur on export bananas when control measures fail. The disease affects the leaves of banana plants, destroying the foliage very rapidly if control measures are not applied. Both growth and yield are affected because of the reduction in the photosynthetic area Although the disease significantly reduces yield, the greatest loss probably occurs as a result of the premature ripening of fruit, which can occur in the field and during transport and storage. Hence it is a destructive foliar disease that affects the plants of genus musa and plantain. Caused by the estimate of the fungus of the genus *Mycosphaerella figinesis* Morlet (anamorph *Pseudcercospora fijiensis*) presents the principle phytopathological problem for the crops.It is identified as a major constraint to global production of banana and plantain.[4]. The *Mycosphaerella fijiensis* M.Morlet is a sexual heterothalic fungus having Pseudcercospora *fijiensis* (M.Morlet) Deighton stage. It is haploid hemibiotropic ascomycete within the class of dothideomycetes, order capnodiales and family *Mycosphaerellaceae*. Taxonomic placement is based on DNA phylogeny morphological analyses and cultural characteristics. Sigatoka name comes from sigatoka valley in Fiji where it was first identified in 1912. In next 40 years the disease was spreaded to all banana producing countries. [5]The banana is grown throughout the tropical and subtropical regions of the world. Because of the bulk of world banana production which is approximately 85% comes from relatively small plots and kitchen or back years in which bananas are grown for local consumption the production consumption(Arias et al., 2003) and export by developed countries are dangerously impacted by Black leaf Sigatoka ka disease. The 15% of bananas are grown for export developed and developing countries estimated at 14.6 million tons in 2008 (Anonymous,2009b). Findings indicate that under the right climate conditions the impact of Black Sigatoka Leaf Disease can be substantial, currently resulting in an average 3% reduction in global annual production, i.e., a loss of yearly revenue of about USD 1.6 billion. [3] The disease is widespread in South-East Asia, India, China, the southern Pacific islands, East and West Africa, USA (Hawaii), Grenada (Caribbean), Trinidad, and Central and South America. It also occurs in Papua New Guinea and on several islands in the Torres Strait.[6,7]

herein were focused on the activity of *Trichoderma* isolates under field conditions without determining the mechanism of action at this moment. Further studies should be directed to reveal the mechanisms by which isolates such as 2.047 utilize to suppress M. *fijiensis*. Nevertheless, recent studies show that *Trichoderma* is essentially mycotrophic (ATANASOVA et al., 2013) and thus, mycoparasitism is expected to be involved in the activity of this antagonist against *M. fijiensis*. Coincidentally, among the three species profiled in their mycoparasitic activity, *T. atroviride* was shown to be the most aggressive (ATANASOVA et al., 2013).

5.2.METHODS:

➢ ISOLATION OF THE FUNGUS

The black sigatoka diseased leaves where washed mildly and crushed sterile mortar and pestle with the time to time addition of sterile suspension. The crushing was carried out between two bunsen burners for creating a sterile environment so that there could be no contamination. The extraction of liquid through crushing included the disease causing components. Sterile pipetes were used to take a drop on the Potato Dextrose Agar Plates. Drop was further spreaded on the petriplate with the help of a sterile spreader. The plate was further subjected to incubation at 37 degree Celsius for 24 hours.

Fig.1 Crushing Of the Diseased sample

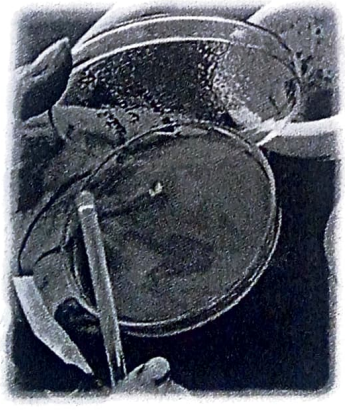

Fig.2 Spreading of the extract for fungus isolation

➢ TEST FOR THE PHENOLIC COMPOUNDS

The diseased leaf and the healthy plant's leaf were crushed separately in the mortar and pestle.The addition of distilled water was done as required inorser to get a liquid solution at the end. 2.5 ml of these liquids were added separately into two test tubes. 2% dilute ferric chloride solution of 1ml were added into the same test tubes. Results were observed.

Fig.5 Extract of the diseased leaf

➢ THE CFU TEST

The test for colony forming units was performed by serially diluting fungal spores in the test tubes.1ml Suspension was spreaded on to the PDA medium and the markings were done accordingly to identify the petridish belongs to which diluted spore suspension. The Petri dishes containing the spreads were subjected for incubation at 37°c. The results were observed after 24 hours.

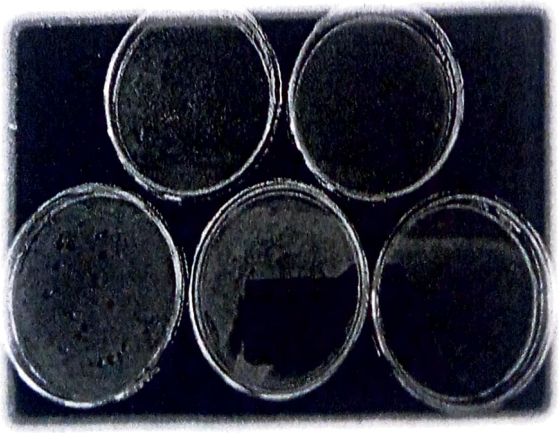

Fig.6 Suspension Spreaded petriplates

RESULT AND CONCLUSION

6.1.RESULT:

➢ ISOLATION OF THE FUNGUS

After 24 hours incubation at 37 degree celcius, a group of fungi species were observered on the fungal growth medium PDA. Accordingly one of the isolated fungal spore was selected depending upon its morphological and biochemical tests *Mycosphaerella* was isolated and partially characterised. This spore was subcultured into another petridish which gave result to the pure culture of fungus *Mycosphaerella*.

Fig.10 Isolation of colonies of different Fungus

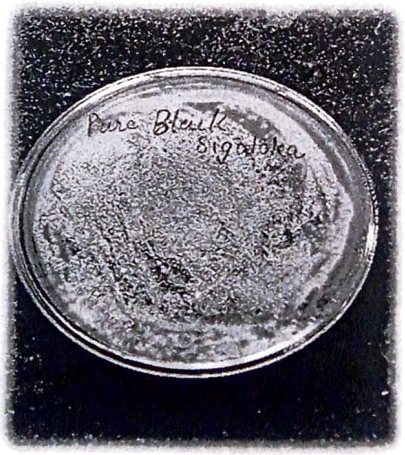

Fig.11 Isolation of Mycosphaerella

➢ TEST FOR PHENOLIC COMPOUNS

The reddish brown colour was produced on the addition of Ferric chloride into the solution. Thus the presence of phenolic compounds was determined.

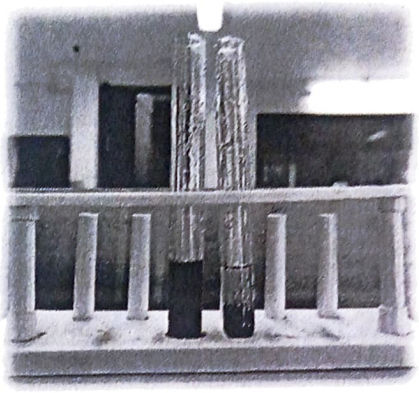

Fig.14 Determination of Phenolic Compounds by the colour change.

➢ THE CFU TEST

The serially diluted solutions showed the variations among the spreaded PDA petridishes. The -1 petridish showed dense growth, gradual decrease in -2 and noticeable huge decrease in the next 3 petridishes.The results are as follows:

	No. Of Colonies	Colony Forming Units
10^1	493	4.93 x 10^3
10^2	391	39.1 x 10^3
10^3	265	265 x 10^3
10^4	89	890 x 10^3
10^5	42	4300 x 10^3

Calculations:

CFU=4.93+39.1+265+890+4300 x 10^3 / 5

= 1099.8 per ml

Therefore, Colony Forming Units per ml are 1099.8

6.2 CONCLUSION

The fungus was isolated from the diseased plant and grown on PDA and was partially characterised and mineral oil was found to be potential candidate for the biocontrol against Black Sigatoka