



## Impact of Plant Pathogen *Fusarium oxysporum* on Wetland Plant *Acanthus ilicifolius*

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### Abstract

Wetlands are considered as kidneys of the landscape. It include various vegetation like Acanthaceae, Aviceniaceae, Verbenaceae etc. This paper deals with the effect of plant pathogen like *Fusarium oxysporum* on wetland plants like *Acanthus ilicifolius* and results in the exhibition of various antifungal activity under stress condition.

**KEYWORDS:** *Wetlands, Acanthus ilicifolius, Fusarium oxysporum*

### Introduction

Wetlands are considered the most biologically diverse of all ecosystems. They are the most important ecosystems on the earth. They are sometimes described as the 'kidneys of the landscape' for the functions they perform in hydrologic and chemical cycles.

Wetland vegetation includes mangrove, water lilies, cattails, sedges, tamarack, black spruce, cypress, gum etc. They play a major role in erosion control, which in turn contributes to shorelines stabilization and storm protection, by helping dissipate the force and protect the coast by reducing the damage of wind and wave action.

A mangrove are trees and shrubs that grow in saline (brackish) and mangal is a plant community and habitat where mangroves thrive.

## Types of Mangroves

Slnno	Family	Genus	Common name
1	Acanthaceae Aviceniaceae Verbenaceae	<i>Avicenia</i>	Black mangrove
2	Combretaceae	<i>Conocaepus</i> <i>Lagun cularia</i> <i>Lumnitzera</i>	Button wood White mangrove
3	Arecaceae	<i>Nypa</i>	Mangrove palm
4	Rhizophoraceae	<i>Burguiera</i> <i>Ceriops</i> <i>Kandelia</i> <i>Rhizophora</i>	Red mangrove

There are three key elements which identify wetlands. They are,

- Hydrophytes: Aquatic plants which grow in water or in soil too water logged for most plants to survive.
- Hydric soil: Soil with an abundance of moisture.
- Hydrology: Water distribution and circulation on and below the earth's surface and in the atmosphere.

There are five major wetland classifications,

- Marine: Associated with the open ocean and high energy coastline waters(include coastal wetlands, such as tidal marshes)
- Estuarine: Consisting of deep water tidal habitats and adjacent tidal wetlands
- Riverine: Found along rivers and streams
- Lacustrine: Associated with lakes.
- Palustrine: May be isolated or connected wet areas including marshes, swamps and bogs.

Functions of wetland includes,

1. It provides food sources for thousands of species including humans.
2. They are the principle habitats for migrating water fowl.
3. They hold water like sponges and prevent soil erosion.
4. They provide crucial habitats for a multitude of plant and animal species.
5. They replenish ground water supplies with cleaner water after filtering it.
6. They help control flooding and flood damage.
7. They provide direct spawning and rearing habitats for fisheries.
8. They absorb and filter pollutants that would contaminate ground water, rivers, lakes and estuaries.

Wetland plant species exhibit higher antifungal activity when collected late in the growing season from wetland soil areas of high altitude meadows. Also, large number of saprobic fungi was isolated from sediments of the marine and estuarine waters. However, these saprobes also occur on plant fragments in the soil under the vascular halophytes.

A fungus is a eukaryotic organism that is a member of the kingdom fungi. The fungi are heterotrophic organisms possessing a chitinous cell wall, with majority of fungal species growing as multicellular filaments called hyphae forming a mycelium. Sexual and asexual reproduction of fungi normally through spores. Most of the fungi are invisible to the naked eye, living for the most part in the soil, dead matter, and as symbionts of plants, animals or other fungi. Several species of fungi are significant pathogens of plants, animals, and humans' etc.: *Fusarium* sp. Important *Fusarium* sp include *Fusarium oxysporum*, *Fusarium solani*, *Fusarium chlamydosporum* etc.

*Fusarium oxysporum* appears to be the second most common species recorded. They are also referred to as Panama disease or Agent Green is a fungus that causes Fusarium wilt disease in more than a hundred species of plants. It does so by colonizing the water conducting vessel (xylem) of the plants .As a result of this blockage and breakdown of xylem, symptoms appear in plants such as leaf wilting, yellowing and eventually plant death.

*Fusarium oxysporum* exhibit rapid growth on potato flakes agar at 25°C. Colonies initially white, becoming tinged with salmon and lavender at maturity. Hyphae are septate and hyaline. Conidiophores are short and simple. Macro conidia usually produced abundantly having slightly rickle shaped, thin walled, with an attenuated apical cells and a foot shaped basal cells. Micro conidia are abundant mostly non-septate, ellipsoidal to cylindrical, chlamido conidia are present and often abundant occurring ringly and in pairs.

## *Acanthus ilicifolius*

*Acanthus* is a genus of about 30 species of flowering plants in the family Acanthaceae, native to tropical and warm temperate region, with the highest species diversity in the Mediterranean region and Asia.

Kingdom : Plantae

Order : Lamiales

Family : Acanthaceae

Genus : *Acanthus*

Species : *ilicifolius*

Common name : Sea Holly, Holly mangrove

### **Morphology**

Habitat : Shrub, 1-5m tall often inhabits the soft muddy soil of river banks.

Root : Usually it as no ground roots but may have small prop roots.

Leaves : Glossy green, prickly leaves (hence the name Holly mangrove. When the plant is growing deep shade the leaves tend to develop fewer prickles.

Flowers and Fruits :

Purple or white flowers that are pollinated by bees or small birds. The seed pods is a shiny green oval shaped capsule that propels the seeds away from the plant using a spring loading mechanism.

### Ecology

Sea Holly grows on mud near the high tide mark, often on mud lobster mounds. It can grow equally well under trees and in open areas. But it grows especially well in areas with more fresh water input. The plant can sometimes cover large areas and form thickets, particularly in distributed mangrove. They also grow along river banks.

### **Medicinal importance**

- The leaves of *Acanthus ilicifolius* are used to treat Rheumatism, neuralgia and poison arrow wounds.
- Chewing the leaves will protect against snake bite.
- To treat kidney stones.
- Water extracted from the bark is used to treat cold and skin allergies.

- Tea brewed from the leaves relieves pain and purifies the blood.
- Ground fresh bark is used as an antiseptic.

Kingdom : Fungi

Division : Ascomycota

Class : Sordariomycetes

Order : Hypocreales

Genus : *Fusarium*

Species : *F.oxysporum*

*Fusarium oxysporum* can survive either as mycelium or as any of its three different spore types. Healthy plants can become infected by *Fusarium oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with its sporangial germ tube or mycelium by invading the plant's root. Once inside the plant, the mycelium grows through the root cortex intercellularly. As it grows, the mycelium branches and produces micro conidia.

Due to the growth of the fungus within plants vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaf's stomata to close, the leaves wilt and the plant eventually dies.

Panama disease of banana caused by *Fusarium oxysporum* is a serious constraint both to the commercial production of banana and cultivation for subsistence agriculture (J.N.Thakker et al,2007). Infected plants are usually stunted their leaves turn pale green to golden yellow and later wilt, die and drop progressively.

Wilt of chick pea caused by *Fusarium oxysporum* is one of the most important diseases causing moderate to extensive damage to the crop. (Tejal.N.Sheth and A.G.Desai, 2007)

## Review of Literature

Wetlands are areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary with water that is static or flowing, fresh, brackish or salt. (C. Rajasekara Murthy and N. Ahalya, 2000)

Wetlands play a very important role in stabilizing the coastal areas. They have respiratory roots coming out of the trees and utilize the atmospheric oxygen during low tides. They create unique ecosystems and provide habitat to a variety of reptiles and insects. (Hosetti B.B, 2002)

Wetland plant species exhibited higher anti-fungal activity when collected late in the growing season from wetland soil areas of high altitude meadows (Kuhajek J.M et al, 2003).

*Fusarium* is a filamentous fungus widely distributed on plants and in soil. It is commonly seen in tropical and sub-tropical areas. They produce both macro conidia and micro conidia. Micro conidia are mostly 3 septate but some have 4-5 septa. It grows rapidly on potato Dextrose Agar (PDA) at 25°C. It produces woolly,

cottony, flat, spreading colonies colony colour many be white, cream yellow, red pink or purple. It has septate hyphae. It is one of the most drug resistant fungi.

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Efficacy of different microbes for their antagonistic ability was determined invitro against *F.oxysporum* by dual cultural method (Verma and Dohroo N. P, 2007). Seeds treated with Trichoderma showed reduced pre emergence and Fusarium wilt under green house and field conditions. Trichoderma species showed the maximum antagonistic activity invitro.

*Fusarium graminearum* can cause Fusarium head blight (FHB) in wheat. These was evaluated in seven wheat cultivars (Bekele G.T *et al*, 1996).FHB was most sever in cultivar wheat on least severe in cultivar pioneer 2375 and of intermediate in the remaining cultivars..

Physiological studies on four species of *Fusarium* pathogen to soybean was studied by Agarwal D .K and Sarbhoy A.K in 1979. *Fusarium oxysporum*, *Fusarium solani*, *Fusarium graminearum* and *Fusarium equiseti* did not grow when the incubation temperature was below 15°C. But growth and sporulation was recorded at 28°C. *Fusarium oxysporum* and *F.solani* grew best at pH 4.5 whereas *F.graminearum* and *F.equiseti* at pH 3.5

Physiological requirements of *F.oxysporum* causing wilt of potato was studied by Ganacharya, 1979. *Fusarium oxysporum* responded well to wide range of pH varying from 2.5 to 7.2 with an optimum range of 4.6 to 5.1. Minimum and maximum temperature at which growth occurred 14°C and 32°C respectively. Optimum temperature was 29°C for growth as well as sporulation.

Zucchini plant collapse has been associated with *Fusarium solani* f.sp *cucurbitae* Race 1, which is the causal agent of Fusarium crown and foot rot disease of cucurbits. Isolation from necrotic vessels gave more than 20 single spore cultures. On the basis of morphological characteristics they were identified as *Fusarium solani* and *Plectosporium tabacinum*. (S.Vitale *et al*, 2007).

Wetland plant species exhibited higher anti-fungal activity when collected late in the growing season from wetland soil areas of high altitude meadows (Kuhajek J.M *et al*, 2003).

Polyamine accumulating transgenic plants exhibited an increased tolerance levels to multiple abiotic stresses such as salinity, drought, low and high temperature and heavy metal resistance against fungal wilt disease caused by *Fusarium oxysporum*. (Venkat Raman Prabhavathi, Malchikatta Vekat Rajam, 2007).

## Objective

To study the impact of the plant pathogen *Fusarium oxysporum* on the wetland plant *Acanthus ilicifolius*

## Materials Required

- Culture of *Fusarium oxysporum* grown in Potato Dextrose Agar
- Leaves of *Acanthus ilicifolius*
- Isopropyl alcohol
- Chloroform
- Methanol
- Potassium tri iodide
- 1,2-dichloro ethane
- Distilled water, cold distilled water
- Spectrophotometer, Cold centrifuge, Rota vapour, Water bath
- Pestle and mortar, conical flasks, micro pipettes, test tubes, beakers and other
- Routine microbiological facilities

Chemicals	Concentration/litre
Sodium Nitrate( $\text{NaNO}_3$ )	0.34g
Calcium Chloride( $\text{CaCl}_2$ )	0.1665g
Potassium dihydrogen Phosphate( $\text{KH}_2\text{PO}_4$ )	0.214g
Magnesium Sulphate( $\text{MgSO}_4$ )	0.514g
Ferrous Sulphate( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	5.5mg
$\text{H}_3\text{BO}_3$	0.57mg
Manganese Sulphate( $\text{MnSO}_4$ )	0.57mg
Zinc Sulphate( $\text{ZnSO}_4$ )	0.57mg

## Methods

### Collection

Five or six *Acanthus ilicifolius* of same age were collected from Kadalundi Wetlands, Calicut district, Kerala and were allowed to grow in both pot and in nutrient solution (hydroponics solution).

### a) Pot culture

The pot is filled with mixture of soil, sand and manure in 3:2:1 ratio. *Acanthus ilicifolius* were planted in two pots.

### b) Hydroponics solution

The composition of nutrient solution was prepared according to the Shive and Robbin (1942). *Acanthus ilicifolius* were allowed to grow in the two set of nutrient solution. The composition of nutrient solution is as follows:

#### Isolation of *Fusarium oxysporum*

Infected ginger was obtained from IISR (Indian Institute of Spices Research Centre) in Calicut. It was surface sterilized using 1% HgCl<sub>2</sub> after thoroughly washing with tap water. Then it was taken out and washed with distilled water. A small portion of it was cut with sterilized knife. Using a sterile forceps it was directly placed over Potato Dextrose Agar (PDA) and incubated at 28°C for 2-3 days and result were observed.

#### Infection transferring to the host plant

The mycelium of *Fusarium oxysporum* were separated from the Potato Dextrose Agar plate and were mixed in the beaker containing distilled water. It was then poured into one of the pot planted with *Acanthus ilicifolius*. While the other were kept as control.

Other method of transferring infect is by Disc cutting method. Discs from the Potato Dextrose Agar plate containing *Fusarium oxysporum* were cut and placed on the surface of the leaves. Other plants in the hydroponic solution were kept as control.

The above all experiment setup were placed under laboratory condition for seven days.

#### Result

After incubation, growth on Potato Dextrose Agar plate were observed

#### Morphology

Woolly, cottony, flat, spreading colonies. Colony colour is white, septate hyphae, filamentous.

From the above morphological characteristics, fungal growths on Potato Dextrose Agar plate were confirmed as *Fusarium oxysporum*.

#### Discussion

Wetland plants can grow with normal metabolic activity under normal condition. Alteration to these activities occurs when any stress conditions like salinity, drought, pathogenic stress, osmotic stress etc. exist. As a result of change in metabolic path way, plants exhibit various defense mechanism to resist stress condition

From the study conducted, the stress was given by infecting *Fusarium oxysporum* into plant by disc cutting method and pouring mycelium dissolved distilled water directly into the soil. After infection, plants



can be observed. No morphological changes can be observed in the test plants. This indicates that resistance mechanism have been taken place.

A high level of fungal resistance was usually obtained by combining the expression of different enzymes or other anti-microbial proteins. The partial elucidations of some biochemical pathways are important for stress resistance. (M.Lorito *et al*, 2003).

## Summary

The mangrove *Acanthus ilicifolius* were collected from the Kadalundi wetlands of Calicut district and was grown in pot and hydroponics condition. It was then subjected to experimental study with reference to the plant pathogen *Fusarium oxysporum*.

The test plant was infected with *Fusarium oxysporum* by disc cutting method and by direct pouring of mycelial solution of the plant pathogen to the soil and was kept at proper laboratory conditions for seven days. Uninfected plants were kept as standard. No morphological changes were occurring on plants.

Wetland plant *Acanthus ilicifolius* has peculiar biochemical characteristics and is found to be tolerant to common fungal pathogens like *Fusarium oxysporum*.

The results of the study can be applied in the agricultural field and drug development. Further research to be carried to transfer the tolerant genetic characteristics to sensitive crop plants to control diseases.

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