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THE EFFECT OF DIFFERENT PHYSICAL FACTORS ON GROWTH AND MORPHOLOGY OF SIX PATHOGENIC FUNGAL SPECIES

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ABSTRACT

The project study was to examine, how some physical factors such as different pH, incubation period, media, light source, sugars on growth and developments of six pathogenic fungi, *calonectria, clonostachy, diplodia, fusarium, paramyrothecium, pestalopsis* in vitro condition. The growth of fungi was obtained by the daily measurement. The uniform pH ranging from pH3 to pH14, incubation period ranging from 2 to 14 days. The media such as potato dextrose agar, malt extract agar, corn meal agar, oat meal agar, sabraoud dextrose agar. The light sources are white light, green light, blue light and yellow light. The report has shown that the growth of six fungal isolates affected by pH. The selected six fungus grows at the pH ranging from pH 3 to pH 12, but at pH 14 no growth was observed and the pH ranging from pH 5 to pH 10 exhibit good growth except the fusarium sps which also shows well growth in pH 12. The influence of incubation period on the fungal isolates growing on potato dextrose agar media, as a result all six fungal isolates showed initial growth after 2nd day and between 4 to 8th days all fungal isolates were showed approached growth compared to control. After 12th and 14th day the growth of the fungal isolates reach maximum and come to an end. The effect of different media on the growth of fungal isolates giving the report reveals those considerable changes. All media are good for the fungus but the diplodia shows highest growth in malt extract agar and cornmeal agar, *calonectria* has highest growth in corn meal agar and oat meal agar, potato dextrose agar, cornmeal agar and oat meal agar is suitable for pestalopsis. Fusarium shows highest growth in all media. Sugars are the important physical factor that effects the growth of the fungi. *Diplodia fusarium and pestalopsis* well grown on glucose and sucrose containing media, dextrose containing media is suitable for *calonectria and clonostachy*. The *paramyrothecium* shows good growth in presence of glucose.

1. INTRODUCTION

Fungi form a large and heterogeneous eukaryotic group of living organisms characterized by their lack of photosynthetic pigment and their chitinous cell wall. Fungal kingdom contains more than 1.5 million species, but only around 100,000 have so far been described, with yeast, mould, and mushroom being the most familiar. Although the majority of fungal species are saprophytes, a number of them are parasitic, in order to complete their biological cycle, animals or plants, with around 15,000 of them causing disease in plants, the majority belonging

Fungi are among the dominant causal agents of plant diseases. To colonize plants and cause disease, pathogenic fungi use diverse strategies. Some fungi kill their hosts and feed on dead material which are called necrotrophs, while others colonize the living tissue called biotrophs. For successful invasion of plant organs, pathogenic development is tightly regulated and specialized infection structures are formed. To further colonize hosts and establish disease, fungal pathogens deploy a plethora of virulence factors. Depending on the infection

strategy, virulence factors perform different functions. While basically all pathogens interfere with primary plant defense, necrotrophy secrete toxins to kill plant tissue. In contrast, biotrophs utilize effector molecules to suppress plant cell death and manipulate plant metabolism in favour of the pathogen.

After sexual and asexual reproduction, fungi, like all other living things, require energy and food sources to continue their growth and life cycles. These nutritional components include minerals, vitamins, nitrogen, and carbon. Likewise, for them to develop and flourish, the environment must have the appropriate pH, temperature, humidity, and oxygen levels. Because of their hydrolytic enzymes, fungi can devour both vegetable and animal carbon sources. Monosaccharides and polysaccharides such as glucose, fructose, chitin, cellulose, hemicellulose, and lignin can be utilized.

Fungi can endure a wide range of environmental conditions and stresses. They are capable of surviving and procreating in adverse circumstances, including the poles, regions that are extremely cold, and areas that are excessively hot, such deserts. Despite their ability to live in cold, alkaline, and anaerobic environments, fungi typically thrive best in warm, acidic, and aerobic habitats. The greatest growth was recorded at 25°C, irrespective of the fact that the fungi's growth temperatures range extremely wide Fungus that can survive below the temperature where they grow greatest are referred to as psychrotolerant, while those that can survive at temperatures of 40°C or above are considered to as thermotolerant fungi. Fungi that reside in or are exposed to temperatures above 40 °C can survive by generating heat shock proteins to defend themselves from heat stress. According on the temperature of the environment they are in, fungus can be found in yeast or mould forms; these fungi are known as dimorphic fungi.

At pH levels between 1 and 13, which are harsh for many creatures, certain fungus may persist and even procreate. In the pH range of 3 to 10, the majority of fungus thrive and grow. The optimum pH range is between 5 and 7. Fungi may develop to their best ability in an alkaline environment by using the organic acids they release to change the pH of the surrounding area to the ideal growth PH.

The growth and development of fungi are greatly influenced by a number of external elements, but medium is the most important one. The quality of the media in which a fungus develops has a big impact on how well it grows. The morphological and physiological characteristics of fungus depends heavily on the growing medium. Fungi normally grow on many types of substrate and have distinct characteristics, but in the lab, they were isolated and multiplied on growth media for diverse investigations. Therefore, fungi exhibit a variety of morphological and physiological traits that are essential for their characterization in various growing mediums. The necessary elements and components present in the medium which are required for the growth and development of fungi.

The majority of living things, including fungus, depend on light as a signal in several metabolic

processes, making it an essential environmental component. All phyla of fungi, including more than 100, have been discovered to respond to light. Numerous metabolic processes, including as circadian rhythms, asexual conidiation, pigmentation, secondary metabolism, and sexual development are all regulated by it. According to recent studies, light regulates a number of pathogenic fungi's metabolic processes and plays a significant role in interactions between plants and pathogens.

Fungi are important environmental microbes, particularly in the natural ecosystem where they cause spoilage, produce mycotoxins, and occasionally engage in advantageous bioconversions. The main kind of carbohydrates found in fungus is saccharide. The following categories apply to knowledge of sugar's structures and characteristics: In contrast to disaccharide, which is digested into two molecules of monosaccharaides, monosaccharide is a type of absorption that is accessible. (iii)Oligosaccharide. In nature, filamentous fungi are able to utilize a great variety of carbon sources of secreting a wide range of different enzymes in large amounts of their environment.

In the present study aims to investigate the influence of various physical factors such as different PH, media, light sources, incubation period, sugars on growth, morphological characters and activity of selected six plant pathogenic fungus such as *Diplodia sps, calonectria sps, clonostachy bysicola, fusarium sps, pestialotiopsis sps, paramyrothecium sps.*

2. AIM & OBJECTIVE

The study aims to investigate the effect of different physical factors on growth and morphology of six different fungal species with following considerations

1. EFFECT OF DIFFERENT PH RANGES FROM ACID TO BASE ON FUNGAL GROWTH WITH CONTROL PH 7
2. EFFECT OF INCUBATION PERIOD ON FUNGAL GROWTH
3. EFFECT OF DIFFERENT LIGHT SOURCES SUCH AS GREEN, WHITE, YELLOW AND BLUE ON THE FUNGAL GROWTH
4. EFFECT OF DIFFERENT MEDIA ON FUNGAL GROWTH
5. EFFECT OF DIFFERENT SUGARS ON FUNGAL GROWTH

3. REVIEW OF LITERATURE

Under in vitro circumstances, the development of *Alternaria solani* was evaluated for their effects on temperature, pH levels, and light intensity**.** Temperature has a substantial impact on the *Alternaria solani* mycelium development and sporulation. At 25°C, colony growth was at its highest, followed by 30°C, and at 40°C, it was at its lowest**.**

Excellent sporulation was seen at temperatures of 20°C and 25°C. At 40oC, however, no sporulation was seen. *Alternaria solani* colony diameter, sporulation, and mycelium dry weight were all affected by pH levels when grown on PDA medium. The results show that the maximum colony diameter and dry weight were seen at a pH level of 6.5, followed by a pH level of 6.0, and the minimum colony diameter and dry weight were seen at a pH level of

8.5. Poor sporulation was reported at pH levels of 4.0, 8.0, and 8.5 whereas excellent sporulation was seen at pH levels of 6.0 and 7.0, Poor sporulation was seen at pH levels of 4.0, 8.0, and 8.5. On PDA medium, light had an impact on the colony development and sporulation of *Alternaria solani*. Maximum colony growth and excellent sporulation were observed at 12 hours of darkness and 12 hours of light, while minimum colony growth was noted at 8 hours of darkness and 16 hours of light. Good sporulation was noted at 24 hours of light and 24 hours of darkness, while poor sporulation was noted at 8 hours of darkness and 16 hours of light and 8 hours of darkness and 16 hours of light. (Rajendra Kumar Bais, Ved Ratan1, Sumit Kumar and Somesh ISSN: 2319-7706 Volume 8 Number 12 (2019).

The goal of the current study was to determine the impact of various culture medium, temperature, and pH on *Fusarium oxysporum f. sp. udum* mycelia development, sporulation, fresh weight, and dried mycelial weight. PDA and Czapek's Dox agar medium outperformed the other seven culture media in terms of fungal growth. The pH levels between 6.0 and 7.0 were ideal for fungal development and great for sporulation. After seven days of inoculation, *Fusarium oxysporum f. sp. udum's* growth peaked at 30 °C but rapidly decreased below 20 °C and above 30 °C. (R. Poorvasandhya, Bireswar Sinha, Ph. Sobita Devi ISSN: 2319-7706 Volume 9 Number 4 (2020**)**

Fusarium oxysporum f.sp. lini (Bolley) wilt, one of the linseed's fungal diseases, is a significant barrier to high output and productivity. To determine the ideal temperature and pH for Fusarium oxysporum f.sp. lini growth and sporulation, an in vitro experiment was carried out. The maximum growth of the fungus was found to be 88.33 mm at 24° C after 9 days of incubation, with the greatest growth rate of 9.81 mm per day and the highest sporulation of

7.9 × 106 per ml. After 9 days of incubation at 25±2°C, the maximum growth of the fungus was 86.33 mm at pH 5.5, with a greatest growth rate of 9.59 mm per day and a peak sporulation of 8.2 × 106 per ml**. (N Pal, A Kumar, AB Malannavar, IJCS: 21-04-2019).**

The impact of various culture, medium, pH, and temperature settings on the mycelia development of Fusarium oxysporum f. sp. zingiberi was investigated in laboratory investigations. Ten different culture medium were investigated, and Potato dextrose agar and Richards's agar produced the greatest results for the fungal growth. After seven days of inoculation, F. oxysporum's growth peaked at 25 °C (90.00 mm), and it significantly decreased below 15 °C and above 40 °C. The pH level with the best conditions for fungal development was 7, followed by 6.5, where mycelia grew to lengths of 86.17 mm and 84.83 mm, respectively. In contrast to other alternative light and dark conditions, continuous light promotes the fastest development of *F. oxysporum f. sp. zingiberi*. (Chaithra J, Sunil Kulkarni, Gururaj Sunkad SB, Amresh YS and Shekhar Patil (Journal of Pharmacognosy and Phytochemistry 2020; 9(1): 786-790)

A large percentage of *aspergilli*, particularly *Aspergillus niger*. Here, we offer proof that the filamentous fungus *A. niger* was used as the only carbon source in the investigation of sugar absorption. This study line aims to determine how quickly fungi develop by measuring the colony diameter every 24 hours (cm). Two types of culture medium were injected with A. niger: While Czapeck Dox Agar was utilised to investigate their carbon needs, employing five different carbon sources, PDA: for maintaining a strain as pure (vizs. glucose, fructose, sucrose, maltose, and starch). The test fungus grew sporadically on the basal media devoid of carbon, which served as the control. However, it was discovered that this fungus differed in its capacity to utilise the provided carbon sources. While glucose and maltose showed to be good carbon sources with a greater affinity, fructose and sucrose were discovered to be appropriate sources of carbon for a fungal isolate. Starch, a polysaccharide, provided this isolate with inadequate carbon for development. Contrary to prior assertions, a wide variety of extracellular enzyme activities from Aspergillus niger were used to break

down saccharides rather than monosaccharides extracellularly. (Hewa O. HAMAD, M. Hakki ALMA², Hero M. ISMAEL, Ali GOCERI, KSÜ Doğa Bil. Derg., 17(4), 2014).

In Southeast Asia and China, Penicillium marneffei is a significant thermal dimorphic fungus that produces penicilliosis, a condition that is uniquely associated with AIDS. Dimorphic switching is regarded as a crucial growth trait linked to its pathogenicity. In recent years, the molecular processes enabling both dimorphic switching and monomorphic growth have been investigated. However, little is understood about the physical and chemical elements that affect this organism's monomorphic development or dimorphic flipping. The disease's natural history is also unknown. Our research focuses on how *P. marneffei's* two development stages respond to temperature, pH, and salinity. We investigated 11 *P. marneffei* isolates and discovered that while all could grow at a wide range of temperatures (8.03-39.8 C), growth was severely reduced at 40 C. At 32C, the morphological transition from hyphae to yeast growth began. The susceptibility to high temperatures during this transition, however, differed amongst isolates. In comparison to alkaline settings, both hyphae and yeast growth forms thrived substantially more in an acidic (pH 5, 6) and neutral pH environment. Although equivalent sensitivities to both NaCl and CaCl2 were seen at high concentrations, yeast cells were generally more sensitive to both chemicals. Our findings show that discrete variations in development patterns exist. The growth needs outlined in our work are significant because they may give insight on the environmental factors that support its survival, a topic that has not yet been fully addressed in the literature. **(**CUNWEI CAO, RUOYU LI, ZHE WAN, WEI LIU, XIAOHONG WANG, JIANJUN QIAO, DUANLI WANG, GLENN BULMER & RICHARD CALDERONE
Medical Mycology August 2007, 45, 40140715 **)**

Trichoderma is a genus of fungus that is used to manage plant diseases biologically, and a variety of its bio-formulates are sold on the market. However, its effectiveness in real-world settings is yet unknown, particularly in terms of safeguarding grapevine plants from Grapevine Trunk Diseases (GTDs). These illnesses are brought on by a group of fungi known as fungal pathogens, and their primary entry point into the afflicted plants is through pruning wounds. The ability of several Trichoderma native strains to proliferate at various temperatures and their potential to colonize pruning wounds under challenging climatic circumstances have been assessed in this study. Trichoderma section strains have evolved to colder environments. Conversely, strains from the group Harzianum/Virens thrive in warmer environments. Differences across strains within the same

clade or section do exist, though. In wintertime, more than 70% of vine pruning wounds might be colonized by native strains. The Trichoderma strain T154 demonstrated a noticeably greater degree of re-isolation from vine plants, and its concentration was ideal for spraying onto vine plants. In conclusion, Trichoderma local strains may provide superior protection to grapevine plants in co-evolution with each unique vineyard since they are more equipped to live in a changing environment. (Guzmán Carro-Huerga, Sara Mayo-Prieto, Álvaro Rodríguez-Gonzále, Samuel Álvarez-García , Santiago Gutiérrez and Pedro A. Casquero 3 September 2021)

Trichoderma harzianum is a biocontrol agent that has a negligible impact on other beneficial soil organisms and a moderate impact on soil balance. In this work, we looked at how pH and temperature affected the development of T. harzianum's mycelia and spore output in batch and fed-batch cultures. Depending on the culture method, the pH and temperature had a big impact on T. harzianum's growth and sporulation. The maximum mycelial growth and spore yield produced by T. harzianum in batch and fed-batch cultures were observed to be produced at pH 4 at the optimum temperature of 25 oC and 45 oC, respectively. The optimum pH for mycelial growth in batch and fed-batch cultures was observed to be pH 4, and the optimum temperature for mycelial yield was produced at pH 4 at the optimum temperature of 30 oC. The findings demonstrated that fed-culture outperformed batch culture in terms of spore output, whereas batch culture achieved high mycelial growth. This research has shown the critical impact that environmental factors play in the mycelia development and spore output of the biocontrol agent T. harzianum. (Abiodun
A. Onilude and Damilola O. Seyi-Amole, ISSN: 2319-7706 Volume 7 Number 04 (2018)

In this study, three species of Pestalotiopsis (P. fici, P. guepinii, and P. palmarum) had their mycelial development affected by factors such as nutritional medium, temperature, relative humidity, light, and pH. These factors were examined for improved growth. The PLA seems to be the optimum medium for the production of conidiomata, whereas PSA was optimal for mycelial growth. The fungus expanded from 10 to 40 °C, with the best growth occurring between 20 and 30 °C and stopping at 5 °C. When the relative humidity was increased from 35% to 100%, it was found that the fungus' development accelerated. The development of mycelium was impacted by various light regimes. Darkness was better for development, but artificial light in cultures caused the establishment of growth rings. At pH 4, no growth is apparent. At pH 4.5 to 7, the fungus flourished. After 5 days of

incubation at 25oC, pH 7 resulted in 94% for P. palmarum and 74% for P. guepinii growth at its optimal level. When the pH went from 7.5 to 9, there was a noticeable drop in growth. (Zahra Ibrahim El-Gali, September 2, 2017)

In controlled climatic settings, the impact of inoculum concentrations, temperature, relative humidity (RH), incubation duration, and leaf age on Pestalotiopsis disseminata sporulation and the development of grey blight disease on som (Persea bombycina Kost. The spore germination and germ tube growth of P. disseminata, the disease's causative organism, were significantly influenced by these variables. The pathogen's ideal inoculum concentration for achieving the highest infection percentage was determined to be 1 107 spores ml1. Temperature and the proportion of spores that germinate at a particular relative humidity were shown to have a nonlinear connection (RH). However, the pathogen's spores germinated best at a temperature of 25 °C (2) and a relative humidity of 70%. In a controlled setting, young leaves (numbered 1-4 from the top) were more vulnerable to the development of the illnesses than older leaves. After 8 hours of incubation, the spores began to germinate; this process continued until 20 hours had passed (maximum). The ideal temperature ranges of 25 2 °C and RH (70%) had a combined impact to favour the severity of the condition. These epidemiological variables could aid in illness management strategies. (RanjanaDas[a]M.Chutia[a]K.Das[a]D.K.Jha[b] ,_Volume 29, Issue 9, September 2010)

Investigated were the effects of temperature and mycological medium on the mycelial development and spore generation of three fungal infections that have recently been described on Ricinodendron heudelotii. The pathogens were identified as Pestalotiopsis microspora (isolate PMHP 109L), Lasiodiplodia theobromae (isolate LTHP 110L), and Fusarium oxysporum (isolates FOBR 164S, FOBR 049L, and FOHP 121L) based on the ITS sequences of their ribosomal DNA. On three culture mediums, including potato dextrose agar (PDA), malt extract agar (MEA), and V8 juice agar, conidia concentrations (number of conidia/ml of solution) and radial growth of the fungus (mm/day) were evaluated (V8). All of the medium were appropriate for L. theobromae growth, although P. microspora grew mycelially at the highest rate (13.4 mm/day) on V8 juice agar. The media PDA and MEA were suitable for F. oxysporum. Temperature affected the growth rate and conidia concentration, which peaked at 23°C for P. microspora and L. theobromae strains and 28°C for Fusarium oxysporum strains. P. microspora and L. theobromae did not grow at 33oC, although Fusarium oxysporum strains did continue to develop and generate spores at this

temperature. P. microspora, F. oxyporum, and L. theobromae all produced spores best at 23oC, 28oC, and 21, 23, and 28oC, respectively. The most conidia were generated by F. oxysporum isolates across all culture medium. These findings advance our understanding of the biology of these recently identified parasitic fungus on R. heudelotii and demonstrate that species like F. oxysporum have a high degree of phenotypic flexibility that enables them to persist and spread under a variety of environmental circumstances.(Joseph Djeugap Fovo, Daniel Dostaler and Louis Bernier ISSN: 2319-7706 Volume 6 Number 6 (2017) pp. 3098-3112)

Fusarium head blight (FHB), often known as head scab, is a destructive disease that is affecting the quantity and quality of wheat throughout the world. Numerous Fusarium species have been linked to the illness, although their composition changes throughout seasons and geographical areas. Fusarium species are affected by climatic conditions such as temperature, pH, and humidity for their development, survival, and infestation. By analysing the in vitro growth rate (mm/day) on potato dextrose agar (PDA) medium, 36 isolates of the Fusarium spp. F. graminearum, F. oxysporum, and F. pallidoroseum (F. semitectum) were subjected to different temperature and pH conditions in the current investigation. All of the isolates behaved differently, but intriguingly, F. graminearum isolates demonstrated greater tolerance to a wider range of temperature and PH. Due to these factors, F. graminearum is a common and dangerous pathogen of wheat.

On Czapek-Dox peptone-supplemented medium, Fusarium sambucinum Fuckel 8099-1 was cultivated for 14 days at 15 degrees C. The cultures were then examined for the synthesis of diacetoxyscirpenol (DAS) using liquid-liquid extraction and gas chromatography. A tricarboxylic acid cycle inhibitor called sorbic acid, 150 mg/liter, was added to encourage the development of fungi and the generation of DAS. Among the coenzymes that include beta-hydroxy-beta-methylglutaryl The results of the precursor tests revealed that neither L-leucine nor isovaleric acid catabolisms induce trichothecene biosynthesis, with isovaleric acid completely inhibiting fungal growth and DAS production, ethyl isovalerate not supporting a significant increase in DAS production, and L-leucine partially inhibiting DAS production. Although shaken cultures did not affect DAS output, stationary cultures required solid particles (cork powder). Shaking significantly increased DAS synthesis and fungi growth. (D. MONNET, D. VIDAL, AND0.CREACH 22February1988/Accepted3June1988)

The pathogen Fusarium oxysporum (Fo) is significant because it causes root rot and seedling disease, both of which lower soybean production. This study examined how pH and temperature affect seedling disease and fungus development. In an in vitro experiment, 14 Fo isolates from symptomatic soybean roots across Iowa in 2007 were cultured on artificial culture medium at four different temperatures (15, 20, 25, or 30°C) and five different pH values (4, 5, 6, 7, and 8). In a rolled-towel experiment, soybean seeds from the Fo-susceptible cultivar Jack were infected with a suspension of either a pathogenic or a nonpathogenic Fo isolate; both isolates had previously been categorised according to how aggressively they caused root rot at 25°C. The seeds were put in rolls of germination paper, which were then incubated in various combinations of buffer solutions at four different temperatures and four different pH values (4, 5, 6, and 7). For in vitro radial development and the degree of root rot, temperature and pH had a significant interaction (P 0.05). The isolates that underwent incubation at pH 6 and 25°C exhibited the greatest in vitro radial growth. At pH 6 and 30°C for the rolled-towel experiment, the pathogenic isolate produced the worst root rot. According to estimations from Gaussian regression analysis, the ideal pH for maximum fungal development was estimated to be 6.3 at 27.1°C, and the pH for the most severe root rot to be 5.9 at 30°C. These findings show that the ideal pH and temperature ranges for Fo development and illness in soybean seedlings are comparable, and they also imply that Fo may be a more significant seedling pathogen when soybeans are planted in warm, moderately acidic soils. (David R. Cruz, Leonor F. S. Leandro, and Gary P. Munkvold, 2019).

From tomato plants exhibiting symptoms of wilting and root rot at various locations in the Dakahlia governorate of Egypt, 23 isolates of Fusarium oxysporum, 8 isolates of Fusarium solani, 2 isolates of Verticillium dahliae, and 4 isolates of Rhizoctonia solani were found. The aggressiveness of these isolates toward tomato plants varied. In a lab setting, either in Petri dishes or in liquid culture, the effects of temperature, pH, light regime, sealing culture plates with Parafilm (1–10 layers), and media type on the growth of two isolates of F. oxysporum f. sp. lycopersici (isolates 14 and 19), F. solani, V. dahliae, and R. solani were assessed. All of the investigated fungus grew best when incubated at 25°C and with better aeration (achieved by not wrapping the culture plates). All of the fungi studied, with the exception of F. oxysporum f. sp. lycopersici (isolate 14), grew best on lima bean agar, however potato dextrose agar (PDA) was the best culture medium. For F. oxysporum f. sp. lycopersici (isolate 19), F. solani, and R. solani, constant light produced the greatest

development. However, whilst diurnal light was optimum for V. dahliae development, continuous darkness was better for F. oxysporum f. sp. lycopersici (isolate 14). F. oxysporum
f. sp. lycopersici (isolate 19), F. solani, V. dahliae, and R. solani all grew best at pH 8 (initial level), with the exception of isolate 14 of F. oxysporum f. sp. lycopersici, which grew best at pH 9 (initial level).(El-Sayed A. Fayzalla, Yasser M. Shabana and Nasser S. Mahmoud, 2008

This study's main goal was to ascertain how the pathogenicity of the hemibiotrophic fungus Fusarium oxysporum f. sp. Schlecht lupini related to the quantities of soluble sugars (sucrose, glucose, or fructose) in yellow lupine embryo axes. Finding out how exogenous saccharides affected F. oxysporum's development and sporulation was the initial stage in this work. The second experiment measured the amounts of ergosterol, a fungal growth indicator, in infected embryo axes that were cultivated in vitro on or without a medium that included sugar. The final goal of this research was to measure the quantities of moniliformin, a mycotoxin that is the most distinctive secondary metabolite of F. oxysporum, in both the infected embryo axis and the high sugar medium. The final goal of this research was to measure the quantities of moniliformin, a mycotoxin that is the most distinctive secondary metabolite of F. oxysporum, in both the infected embryo axis and the high sugar medium. Additionally, measurements of the length and fresh weight of the embryonic axis, or morphometric measures, were made. The infected embryo axis with a sugar deficiency had the greatest ergosterol levels. The mycotoxin moniliformin also accumulated significantly in those tissues at the same period. Additionally, it was shown that the inclusion of sugars in water agar medium decreased the sporulation of the pathogenic fungus F. oxysporum in comparison to the control (sporulation of the pathogen on medium without sugar), with glucose having the largest inhibitory impact. The development of embryo axes was greatly slowed down by F. oxysporum infection, however this impact was more pronounced in infected axes that were grown in sugar shortage as opposed to soluble sugar cultures. The acquired results therefore demonstrated that high sugar levels may result in decreased mycotoxin formation by F. oxysporum, hence preventing the development of infection and fusariosis.(Magda Formela-Luboińska, Dorota Remlein-Starosta Agnieszka Waśkiewicz, Zbigniew Karolewski,Jan Bocianowski, Łukasz Stępień, Mateusz Labudda, Philippe Jeandet, and Iwona Morkunas Int J Mol Sci. 2020 Oct; 21(19): 7258)

In nature, light is a significant information carrier. Virtually all living things can adapt to their environment by adjusting their electromagnetic energy (photons) into the chemical language of their cells through the use of molecular machinery. Fungi respond to

illumination in a variety of ways, and we discovered that upon growing in light or after perceiving a light pulse, they begin significant modifications in their metabolic pathways. Most often, carotenoid metabolism, polysaccharide and carbohydrate metabolism, fatty acid metabolism, nucleotide and nucleoside metabolism, and control of the generation of secondary metabolites have been found to change in response to light. Within minutes, transcription of genes begins, abundance and activity of metabolic enzymes are modified, and ultimately, levels of metabolites are changed to deal with the negative effects of light or to get ready for reproduction, which is frequently dependent on light. This article seeks to provide an overview of how light affects metabolic pathways and to show the physiological importance of light for fungus. offer a foundation for determining if a certain metabolic pathway may be vulnerable to light control and how these characteristics might be used to improve biotechnological operations.(Doris Tisch and Monika Schmoll 2009 Nov 14).

When cultured on diverse nutritional conditions, the fungi shown variance in their growth and development. This study looks at seven distinct fungi's growth and development in defined media. By tracking daily variations in pH and fresh and dry weights, the fungal growth was determined. Two fungi Fusarium sp. and Antrodia sitchensis having slow growth rate in compare to rest of the five and preferred neutral to slightly alkaline condition for their growth. The remaining five were discovered to thrive better in a somewhat acidic environment, including Aspergillus niger, Curvularia intermedia, and three Macrophomina phaseolina isolates (a, b, and c). The initial pH of the media significantly changed as the fungi expanded. It is described how pH changes affect the growth and development of fungi. (Anjisha R. Maharshi and Vrinda S. Thaker European Journal of Experimental Biology, 2012, 2)

4. MATERIALS AND METHODS

1. FUNGAL CULTURE

The fungal culture of ***calonectria sps, clonostachys bysicola, lasido diplodia, fusarium sps, pestalopsis sps, and paramyrothecium*** was obtained from the pathology department of Kerala Forest research institute peechi. These strains were maintained on potato dextrose agar medium

2. FUNGAL INOCULATION

Six fungi were studied for their ability to develop linearly under various environmental conditions including different media, light source, sugars and ph. A sterile cork borer was used to extract 5mm diameter agar plugs for the border of pure colonies and one of these plugs was then inserted in the middle of 90mm Petri plate containing medium. After that clim film was used to cover the plates for incubation.

❖ EFFECT OF DIFFERENT PH

Potato dextrose agar medium was used to study the impact of ph on the pathogen growth. The media's ph was adjusted to from **acidic (3, 5, 6) to basic (8, 9.10, 12, 14) condition** by using 0.1N hydrochloric acid and 0.1N sodium hydroxide and PH paper. The PH 7as the control plate.10 ml of agar is placed into the sterilized Petri plates then they are let to set up. 5mm disc from each six fungal culture that had been developing for 14 days was placed on solidified medium. The inoculated Petri plate was incubated at room temperature for 24 hours and observes the growth of colonies was taken at one-day interval up to 14 days.

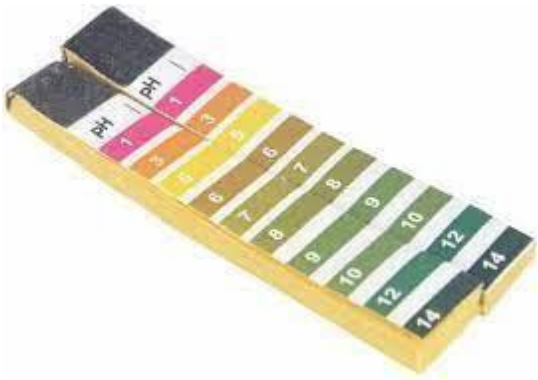


Figure :1 pH paper

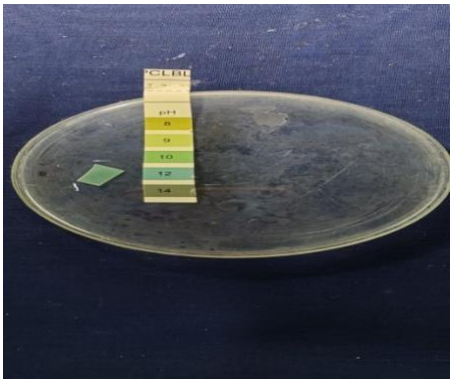


Figure: 2 pH adjustment with pH paper

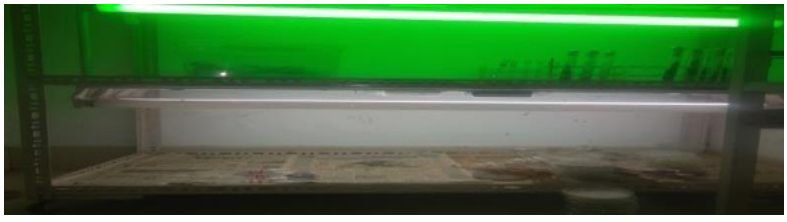
❖ EFFECT OF DIFFERENT MEDIA

The morphological traits of six fungi were assed on **potato dextrose agar, malt extract agar, oat meal agar, corn meal agar, sabaurod dextrose agar medium.** In each Petri plates 10 ml of each sterile medium were added and allowed to solidify. Then 5mm diameter each plug was taken with the help of cork borer from the margin of 14 days old isolates grown on potato dextrose agar and placed at the centre of each set of Petri plates containing different media. Petri plates were incubated at room temperature. The diameter of each isolate was recorded at one-day interval up to 14 days.

❖ EFFECT OF DIFFERENT LIGHT SOURCE

The growth was tested on potato dextrose agar with selected light sources having different intensities such as **blue light, white light, green light and yellow light**. These light conditions were adjusted artificially. From 14-day old culture, 5mm mycelia disc of selected six fungi was inoculated into Petri plates containing 10 ml of potato dextrose agar. These inoculated Petri plates were placed under the selected light source. After incubation at room temperature for 24 hours and observe the growth were taken at one-day interval up to 14 days.

Figure:3 cultural plates of six fungus under different light source



❖ **EFFECT OF DIFFERENT SUGARS**

The growth and morphological characters of selected six fungi were assed on potato dextrose agar plates with selected sugars including **glucose, sucrose, and dextrose**. 5mm mycelia disc of the six fungi were inoculated in to the Petri plates containing 10 ml of potato dextrose agar with different sugars. These plates after incubation at room temperature for 24 hours and record the diameter and morphology were taken at one interval up to 14 days.

5. RESULT AND DISCUSSION

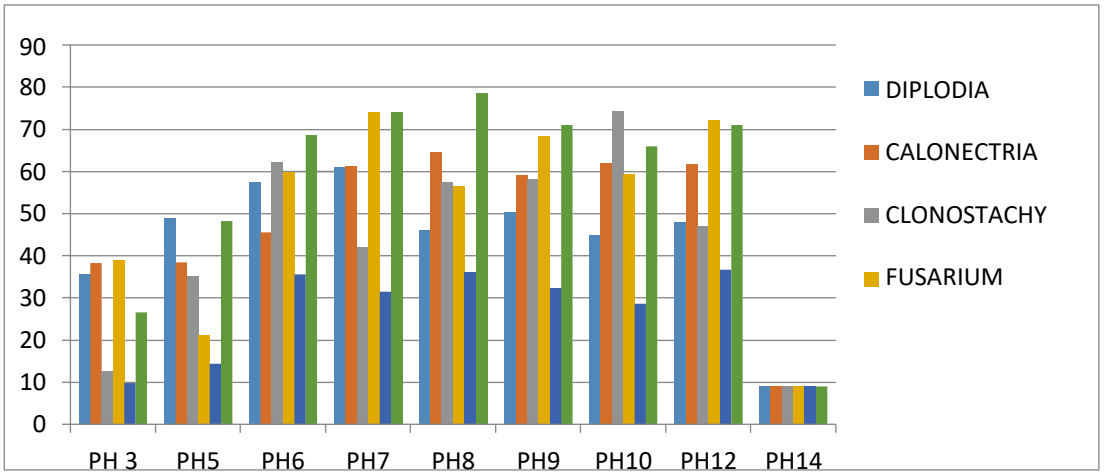
➢ **EFFECT OF PH ON FUNGAL GROWTH**

Fungi generally grow well in acidic conditions, but some species favour neutral to slightly alkaline condition. The PH of the medium has a profound effect upon the rate and extent of growth and many other lives process of fungi. The fungi generally utilize substrate in the form of solution only if the reaction of solution is conducive to the fungal growth and metabolism. This shows the importance of hydrogen ion concentration for better growth of fungi. The result of the present study of effect of different PH Values on the growth of selected six fungi are shown *in Table;1 and figure;1*

TABLE;1 SHOWING THE EFFECT OF PH ON FUNGAL GROWTH

NAME OF FUNGI	COLONY CONTROL DIAMETER (mm) PH 7	PH3	PH5	PH6	PH8	PH9	PH10	PH12	PH14
		AVG GROWTH DIAMETER (mm)							
DIPLODIA	61	35.6	49	57.5	46	50.5	45	48	NG
CALONECTRIA	61.3	38.3	38.4	45.5	64.6	59.2	62	61.9	NG
CLONOSTACHY	42.1	12.7	35.1	62.1	57.4	58.3	74.4	47.2	NG
FUSARIUM	74	39	21.2	59.9	56.6	68.3	59.3	72.2	NG
PARAMYROTHECIUM	31.5	9.8	14.4	35.6	36.3	32.4	28.6	36.7	NG
PESTALOPSIS	74	26.6	48.3	68.7	78.6	71	6.6	71	NG

FIGURE;4 EFFECT PH ON FUNGAL GROWTH



The **TABLE;1** and **FIGURE;1** indicate the effect of PH on fungal isolates. The maximum growth of *Diplodia (*57mm) occurred at PH 6, whereas the lowest (35.6mm) was observed at PH 3. However, the PH increased towards the control PH 7 and then it declines to high basic condition. Highest growth of (64.6) was observed at PH 8 followed by PH 10 and PH12 where growth of 62mm & 61.9mm was recorded. Lowest growth (38.3mm &38.4mm) of fungi was observed at a PH 3 & PH 5. Clonostachy shows maximum growth at PH 10 (74.4mm) followed by PH 6 which recorded 62.1mm growth. The lowest growth of fungi was observed at a PH 3. (12.7mm). Maximum growth (74mm) of fusarium sps was recorded at PH 7 followed by PH 12(72mm) and the lowest growth (21.2mm). The paramyrothecium shows highest growth (36.7mm) at PH 12. Lowest growth (9.8mm) at PH 3. The highest growth of pestalopsis at PH 8 (78.6mm) followed by PH 7 (74) & PH 6 (68.7). Lowest growth at (26.6).

This study interpreted that the selected six fungi are doesn't grows in high basic condition and minimum growth shows in high acid condition. The growth is well near to control PH7.

➢ EFFECT OF INCUBATION PERIOD ON FUBGAL GROWTH

Fungi develop best under certain circumstances, and they get all of their energy and nutrients from the growing media through biochemical reactions that break it down. However, the growing medium must already contain all the components necessary for growth. Depending on the species and variety, different time periods between three and seven days are needed**.** Figure;2 illustrates the results of testing the duration of six fungi at 2, 4, 6, 8 10 ,12,14 days.

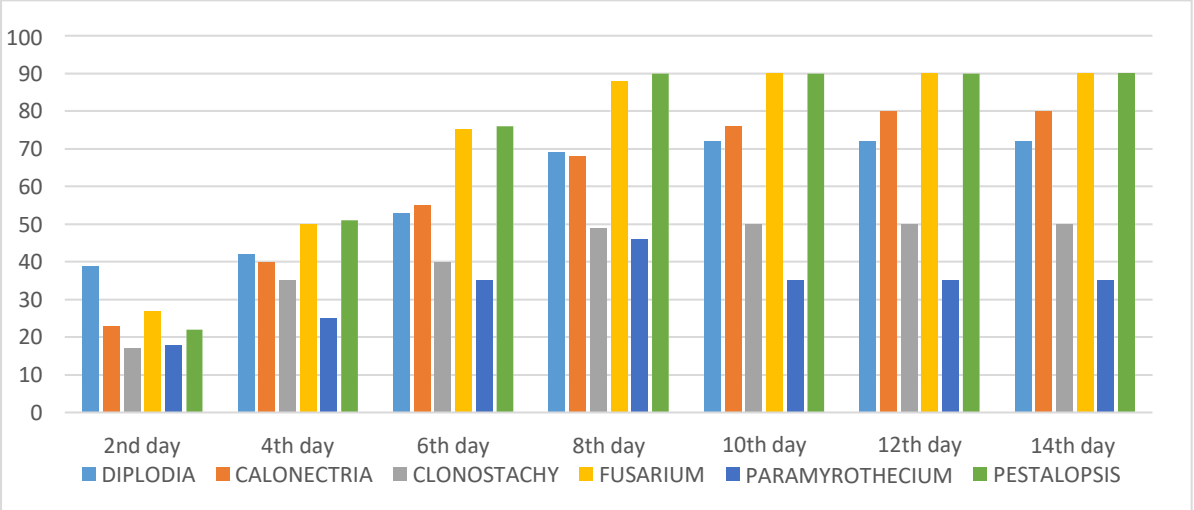
The findings indicated that 6 to 10 days was the ideal study time, with development occurring progressively each day. As shown in table 2 the statistical analysis reveals that the fungal growth curves at various incubation temperatures differ significantly from one another.

TABLE ;2 SHOWING EFFECT OF INCUBATION PERIOD ON FUNGAL GROWTH

NAME OF FUNGI	AVG COLONY CONTROL DIAMETER AT14 DAYS	2ND DAY (mm)	4TH DAY (mm)	6TH DAY (mm)	8TH DAY (mm)	10TH DAY (mm)	12th day	14TH DAY (mm)
DIPLODIA	61	39	42	53	69	72	72	72
CALONECTRIA	61.3	23	40	55	68	76	80	80
CLONOSTACHY	42.1	17	35	40	49	50	50	50
FUSARIUM	74	27	50	75	88	90	90	90
PARAMYROTHECIUM	31.5	18	25	35	46	35	35	35
PESTALOPSIS	73.9	22	51	76	90	90	90	90

FIGUR;2 EFFECT OF FUNGAL GROWTH

Fungi require different conditions for optimal growth, fungi derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes. However, all the materials for growth must already be present in the growth



medium. Specific time spans required during 3-7 days vary respective to species and variety Fungi require different conditions for optimal growth, fungi derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes. However, all the materials for growth must already be present in the growth medium. Specific time spans required during 3-7 days vary respective to species and variety

EFFECT OF DIFFERENT MEDIA ON FUNGAL GROWTH

usually, the fungus is well grown in potato dextrose agar media but in some are also grown on various other media such malt extract agar media, corn meal agar media, oat meal agar media and saboraud. In present study indicate the, five cultural media used to test the growth and morphology of selected six fungi to various degrees shown in *Table;3 and Figure; 3*

TABLE;3 SHOWING THE EFFECT OF DIFFERENT MEDIA ON THE FUNGAL GROWTH COMPARED WITH CONTROL PDA PLATE

NAME OF FUNGI	COLONY CONTROL DIAMETER (mm) POTATO DEXTROSE AGAR	MALT EXTRACT AGAR	CORN MEAL AGAR	OAT MEAL AGAR	SABOURAUD DEXTROSE AGAR
	AVG DIAMETER OF THE COLONY (mm)				
DIPLODIA	61	75.4	76	53	68.6
CALONECTRIA	61.3	48.6	73	70.9	34.6
CLONOSTACHY	42.1	47	54.5	46.5	35.3
FUSARIUM	74	74.7	72.6	71.5	71.3
PARAMYROTHECIUM	31.5	45.4	42.1	36.4	37.6
PESTALOPSIS	73.9	48.6	73	70.9	34.6

FIGURE 3 SHOWING THE EFFECT OF DIFFERENT MEDIA

The graph and the table reveals, how the medias influence the growth of selected fungus. Almost all fungus exhibit growth on adopted five media. The diplodia grows well in cornmeal agar and malt extract agar which compared to the growth in control potato dextrose agar media plate. The highest growth of calonectria on corn meal agar and oat meal agar. To compare with control potato dextrose agar plate, the clonostachy indicate maximum growth in corn meal agar followed by malt extract agar and oat meal agar. The fusarium exhibit well growth in each media. The malt extract media is best for the growth of paramyrothecium.

Pestalopsis is significantly well grows on corn meal agar media and oat meal agar media

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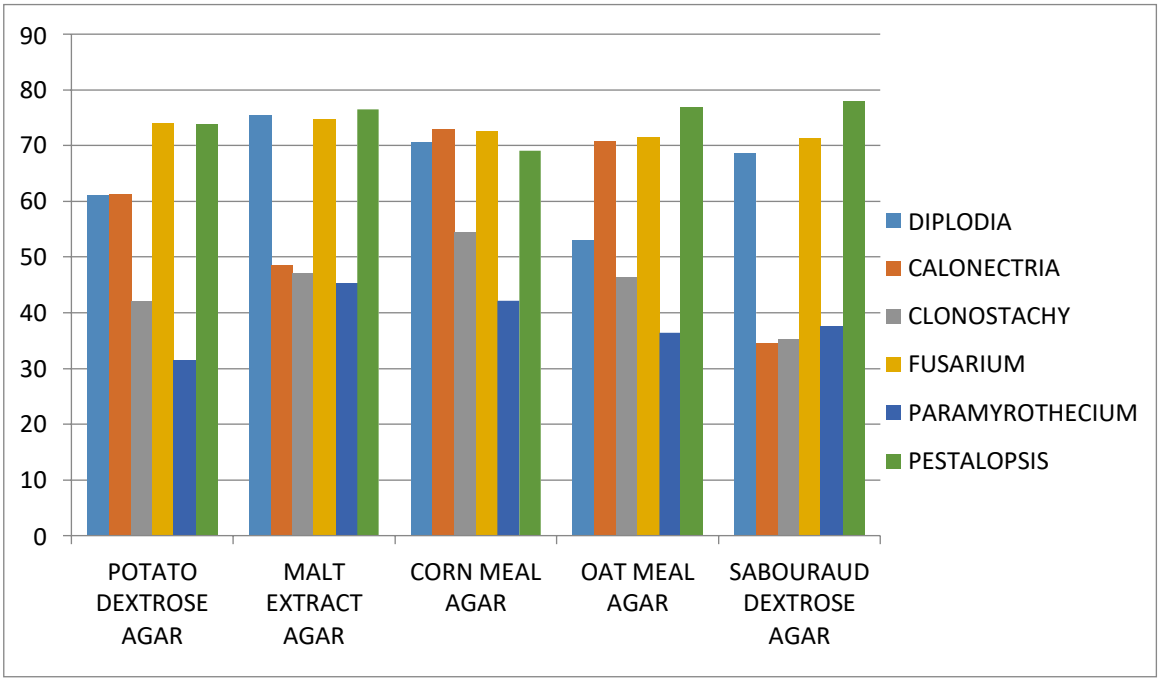


EFFECT OF DIFFERENT LIGHT SOURCES ON FUNGAL GROWTH

The fungi use light as a source of information but not as a source of energy. During decades of studies on fungi, at least 100 fungal species, representing all phyla, have been found to react to light. They have perception mechanisms for blue, near UV, green, and red light. This experiment study carried out the effect of white, blue, green, yellow light on the growth of fungi. The result of the experiment shows in the *Table;4 AND Figure*

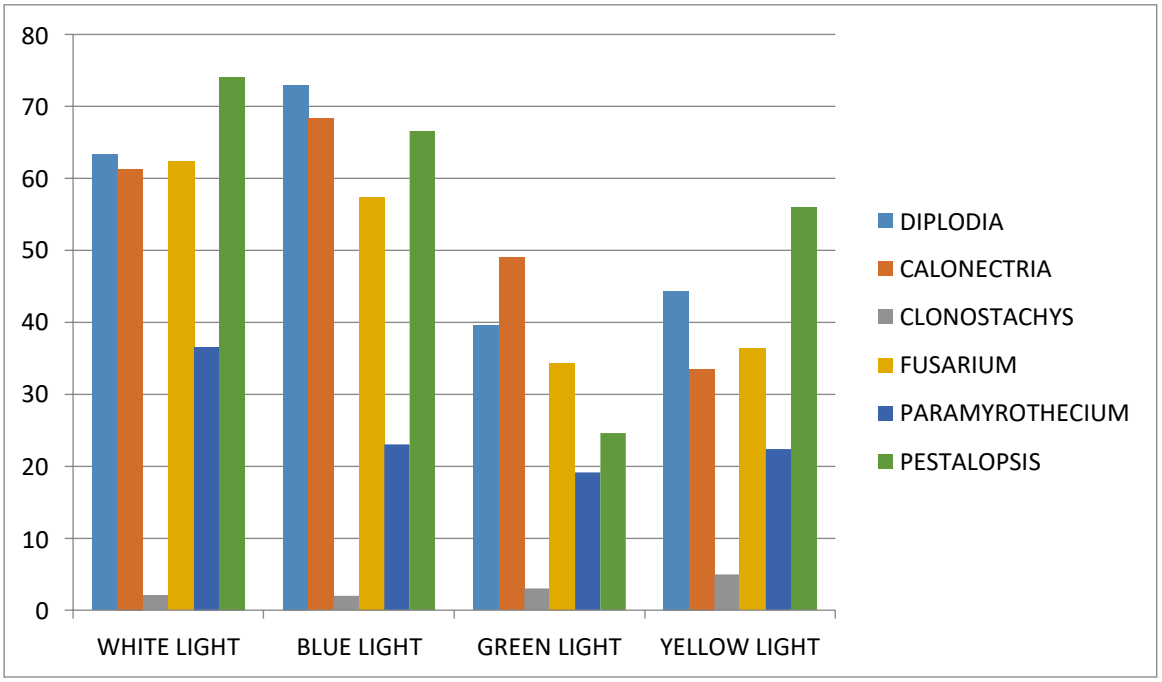
TABLE;4 SHOWING THE EFFECT OF LIGHT SOUCRCE ON THE FUBGAL GROWTH

NAME OF FUNGI	WHITE LIGHT (mm)	BLUE LIGHT	GREEN LIGHT	YELLOW LIGHT
	AVG DIAMETER OF THE COLONY (mm)			
DIPLODIA	63.3	73	39.5	44.3
CALONECTRIA	61.3	68.3	49	33.5
CLONOSTACHY	77.9	52.1	39.9	30.4
FUASARIUM	6.23	57.4	34.3	36.4
PARAMYROTHECIUM	36.6	23	19.1	22.4
PESTALOPSIS	74.1	66.6	24.6	55.9

FIGURE;4 SHOWING THE EFFECT OF LIGHT SOUCRCE

EFFECT SUGARS ON THE GROWTH OF FUNGI

Sugars are essential nutrients for the development of fungal pathogens because these metabolites are used as carbon skeletons for the biosynthesis of other compounds as well as serving of substrates for energy production and being involved in cellular signalling pathways in fungi. The key target of fungal infections during their life cycle is to colonise, grow, and reproduce in host plant cells by using their resources. The main elements for carbon partitioning at the level of the entire plant, at the commencement of sugar flow towards the attacked tissues, and during interactions with fungus are sucrose and monosaccharide transporters in host cells. Additionally, cell-wall invertases are important enzyme regulators of carbon partitioning during fungal infection, cleaving sucrose into glucose and fructose in the apoplast of plant cells. This study aims to the impact of the different sugars such as



glucose sucrose and dextrose on fungal growth. Outcome of this experiment mention in the *Table;5 and figure;5.*

TABLE;5 SHOWING THE EFFECT OF SUGARS ON THE GROWTH OF FUNGI

NAME OF FUNGI	COLONY CONTROL DIAMETER (mm) PDA	GLUCOSE	DEXTROSE	SUCROSE
	AVG DIAMETER OF COLONY (mm)			
DIPLODIA	61	59.6	34.6	59.9
CALONECTRIA	61.3	33.3	61.3	44.1
CLONOSTACHY	42.1	54.9	59.3	51.3
FUSARIUM	74	56.6	23.3	57.3
PARAMYROTHECIUM	31.5	36.3	35.2	30.3
PESTALOPSIS	73.9	73.6	73.5	69.9

FIGURE;5 SHOWING THE EFFECT OF SUGARS

To analysing the table5 and figure 5, giving the report contain the growth of fungi has influenced by the sugars, sucrose glucose and dextrose.

Figure6; showing growth of calonectria

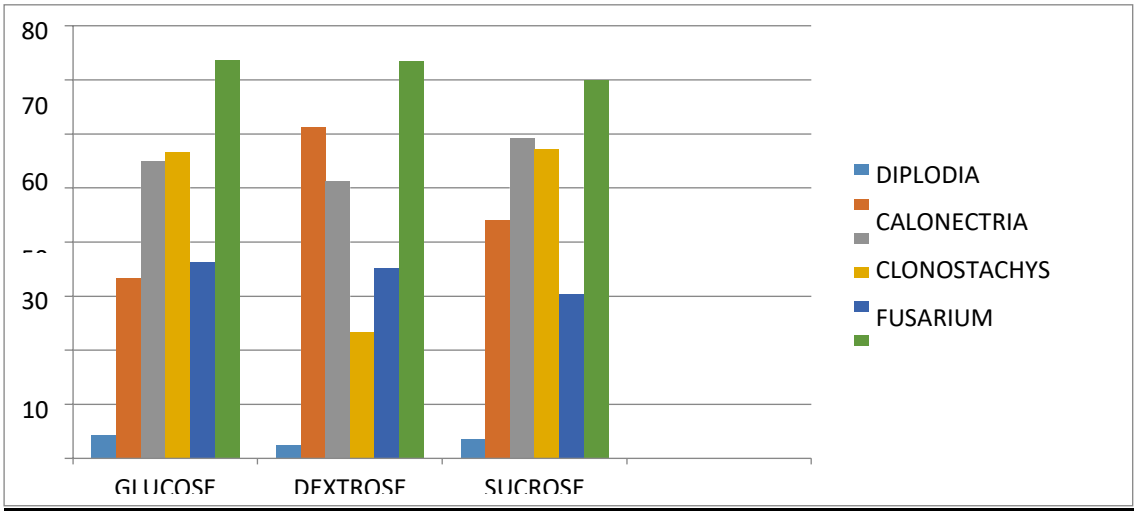
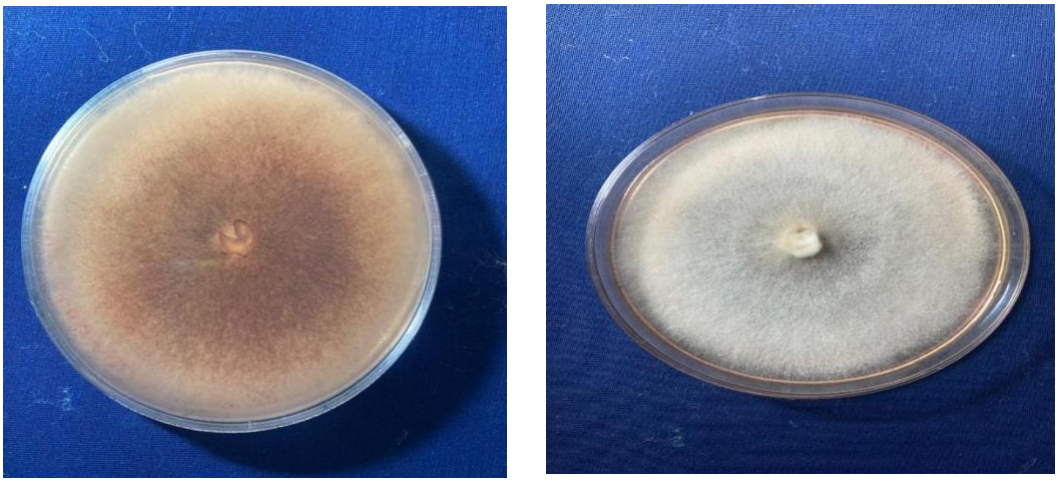


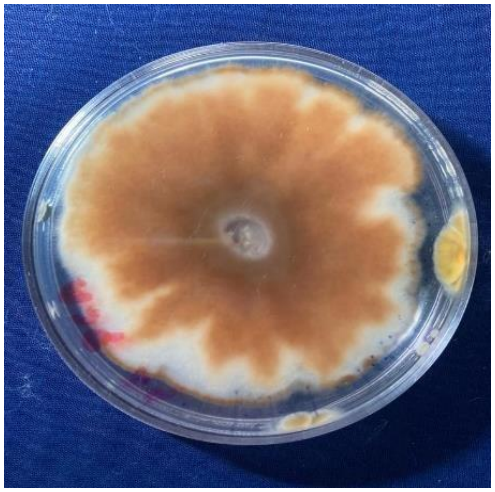
Figure ;7



The morphology of calonectria in cornmeal agar is entirely different compared with control pda plate and other media. colony is changed into the circular form having flat elevation with filiform margin and white hairy appearance on upper and brown pigmentation in lower side.

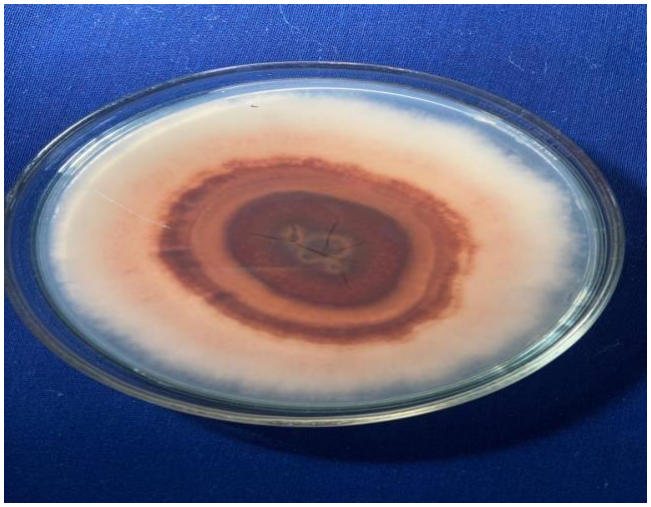
Figure ;8

Compared to other sugars containing media , the growth in dextrose containg media has irregular form with flat



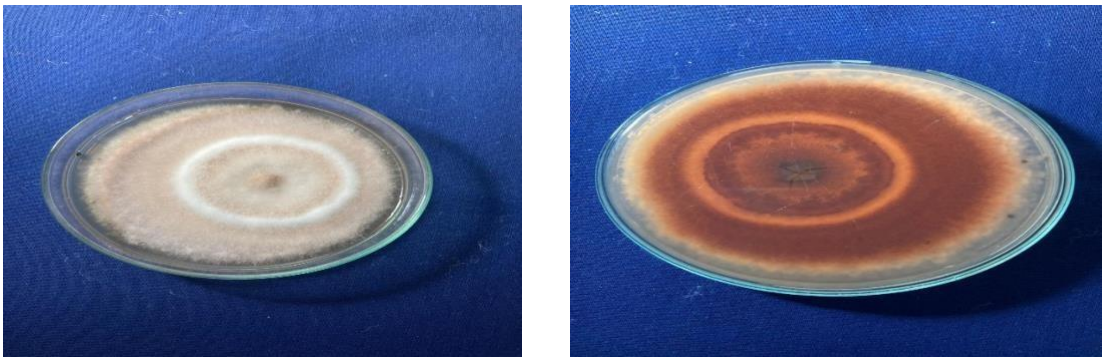
elevation having undulate margin and the colony apperaed as white colour with light brown shade on upper side and brown coloured sulcatus appearance in lower side .

Figure ; 9



In ph 12(basic condition), completely interchange their morphology. It is changed into circular form with convex elevation having filliform margin and brown coloured ring are formed on the center along with white puffy apperance on upper side and the lower side containing dark brown coloured rings on center

Figure 10 ph 8



The ph 8 showing circular form with flat elevation with filliform margin and white coloured ring appearance on upper and brown colour ring on lower side

figure11 showing pestalopsis

Figure 12 control plate of pestalopsis

Circular for flat elevation with entire margin and white coloured powdery apperance on upper and green coloured rosette apperence on lower side.

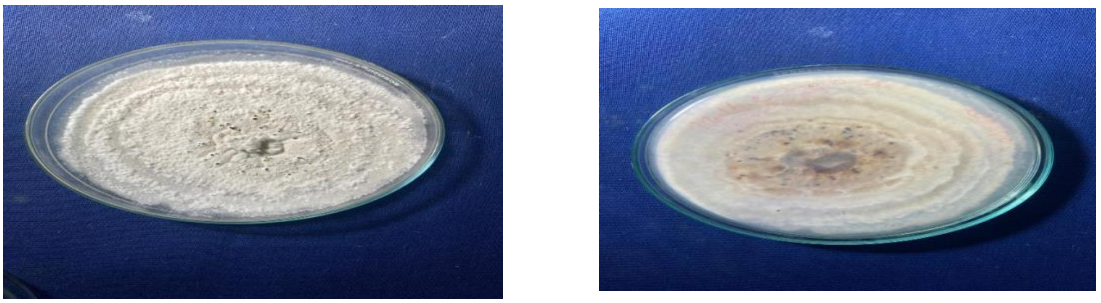


Figure 13 dextrose media

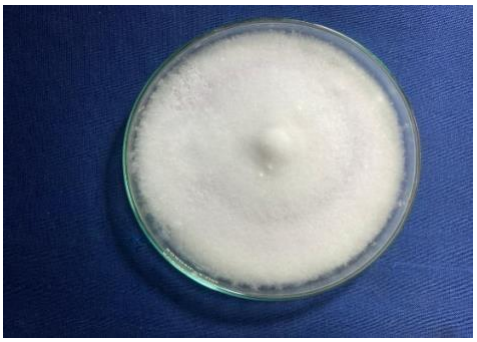
White Rosett appearence with green colour on center and green rosett appearance on lower side Circular form,flat elevation umbonate margin .

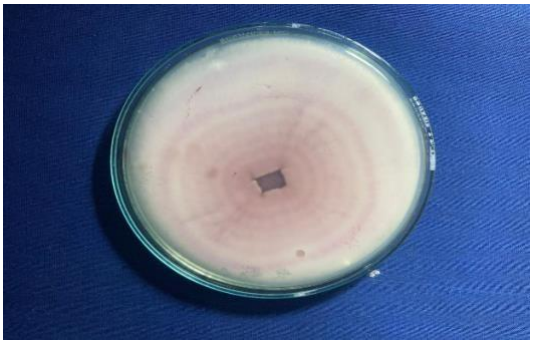
Figure 14 ph 12

Irreugular form, flat elevation , filliform margin,

Figure 15 *fusarium*

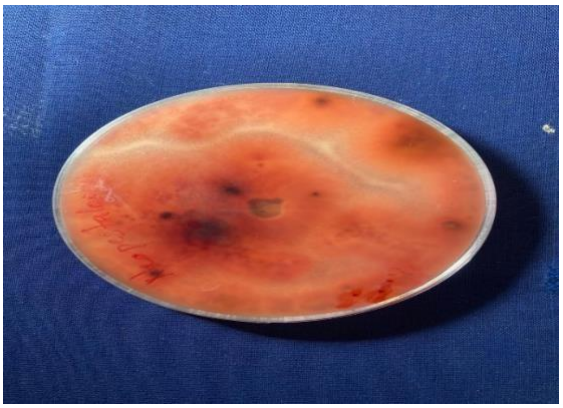
Figure 16 control plate





Puff like appearance,white coloured colony with purple shade on upper side ,purple coloured ring are appeared on lower side ,circular form ,umbonate elevation,entite margin

Figure 17 MEA MEDIA



white pinkish colony ,puff like appearance,circular form,dark pinkish colour on lower side,entire margin,umbonate elevation.

figure 18 *paramyrothecium*

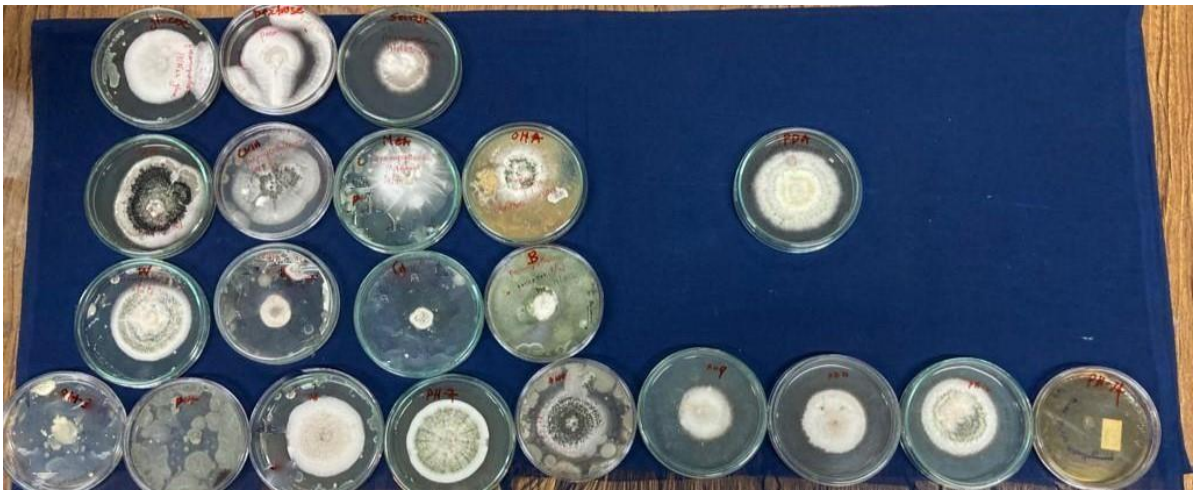
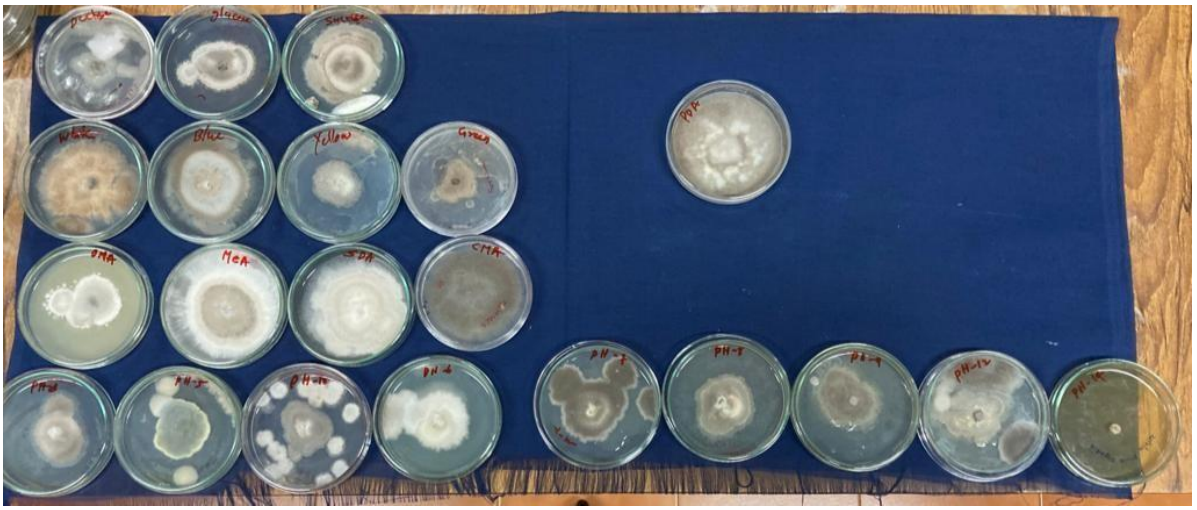


Figure 19 *clonostachy bysicola*

Figure 20 *diplodia*



6. CONCLUSION

The plant pathogenic fungi are ability to produce mycotoxins, secondary metabolites, volatiles and other compounds. The Production of the mycotoxins are causing several diseases in plants. Some physical factors are affecting the production of these mycotoxins. This study is the start-up work related to mycotoxins. The influence of physical factors and different media on the growth of selected six pathogenic fungi is only studied in this work. The result of this study shows the selected six pathogens are grows at the pH ranging from ph5 to pH 12. No growth is observed at ph 14 and less growth is identified in ph3 and the glucose, dextrose sucrose containing Medias and other media such as PDA, MEA, OMA, CMA, and SDA are suitable for the growth of these fungi. Under the white light and blue light these fungi are shows maximum growth and morphological changes but under the yellow and green light, less growth is observed.

Further this study aims to select an organism from the selected six pathogenic fungi which shows wide difference in their growth and morphology and identify the mycotoxins and their amount using GCMS (Gas chromatography – mass chromatography.

This study gives an idea about which condition is more suitable for the growth of this fungus. Thereafter, using some antagonistic organisms isolated from the soil/compost/other environment to check their ability to inhibit the action of the selected plant pathogenic fungi.

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