STUDIES ON MYCOTOXINS PRODUCING MICROORGANISM AND MUCORMYCOSIS DURING COVID-19

Project report submitted in fulfilment of the requirement for the degree of

Bachelor of Technology

in

Biotechnology

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CERTIFICATE

This is to certify that the work titled **"Studies on mycotoxins producing microorganism and Mucormycosis during COVID-19"**, submitted by **"Ritika Shridhar(181827) & Mahima Singh Thakur(181828)"** in fulfilment for the award of the degree of B. Tech in Biotechnology of Jaypee University of Information Technology, Solan has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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CANDIDATE'S DECLARATION

We hereby declare that the work presented in this report entitled **"Studies on mycotoxins producing microorganisms and Mucormycosis during COVID-19"** in fulfilment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Department of Biotechnology and Bioinformatics**,** Jaypee University of Information Technology Waknaghat is an authentic record of our work carried out over a period from August 2021 to May 2022 under the supervision of **Dr. Jata Shankar** (Associate Professor) Department of Biotechnology and Bioinformatics.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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This is to certify that the above statement made by the candidates is true to the best of my knowledge.

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ABSTRACT

Mycotoxins are secondary metabolites produced by various fungi that may prove to be fatal to humans and other animals. Toxic fungi and mycotoxins originally appeared in the human food supply around the time when humans began to cultivate and store crops. It also created a wide new ecological niche for fungi that were harmful to grain crops or saprophytic on harvested grain, many of which produced mycotoxins. Coronavirus has spread rapidly to the world and became a pandemic within no time it affected countries economically and its public health affecting daily life significantly. Protein interactions of the coronavirus play a very important role in the studies and in drug therapy. Protein interactions play a very important role in examining deep protein functions and the pathways that are involved in the biological processes in order to know how they cause the progression of diseases. Fungal toxin producing microorganisms are screened using the soil sample collected from upper Shimla (HP) region. Further, these fungal species will subjected for molecular identification and their role in pathogenesis in the crops of upper Shimla region.

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A. Introduction

1. Mycotoxins

The term 'mycotoxin' refers to the harmful chemical compounds generated by fungi that invade crops easily (1). These biological chemicals are frequently necessary for fungi to survive and, as a result, are harmful to their environment (2). Various types of environmental stress may cause the synthesis of these harmful chemicals, implying that they play a protective role, for example, in oxidative stress (3). Moulds can be found on a wide range of crops and foods, including cereals, nuts, spices, dried fruits, apples, and coffee beans, and they grow in warm, humid environments. Fungal toxins (mycotoxins) are not proteins, unlike bacterial toxins. As a result, they are hardly ever detected by human and animal immune systems. The outbreak in the 1960s killed poultry and was therefore named Turkey X disease. After looking for a cause mycotoxins were found in the feed that came from brazil. Aflatoxin- class 1 carcinogen; was present in it. Scientists have only been able to isolate these toxins for the last 30-40 years from their fungal sources. The research ideas and methodologies are still changing frequently.

2. Mycotoxins Entering Food Chain & Prevention

The continued and inevitable development of mycotoxigenic Aspergillus species as a result of Earth's changing climate has increased the risk of mycotoxin contamination in the feed and food production chain. Since their discovery, these dangerous mycotoxins have caused major health and economic consequences(4). They may enter pre- or post-harvest(5). Many biological and environmental factors affect the production of mycotoxins but temperature and humidity play a major role in it. Post-harvest contamination can occur if crop drying is delayed, as well as during crop storage if water levels are allowed to rise over threshold levels for mould growth. Mould growth is aided by insect or rodent infestations in some stored goods. Actions that begin with planting and end with feed and food processing can help to reduce mycotoxin contamination in the food chain. Agronomic approaches such as crop variety, fertiliser application, and pesticide application can alter a crop's vulnerability to fungal assault and, as a result, the development of mycotoxins in the field. Fortunately, the transformation and detoxification pathways existing at the moment are capable enough to prevent these toxins from entering the food chain via mainstream livestock like poultry, pigs, etc. For minimising mycotoxin contamination in crops, genetic modifications and biotechnology are helpful strategies. The fight against fungal toxins can also be sustained during storage and food processing, when innovative technology can significantly lower mycotoxins levels. In the field of
Animal nutrition, the use of isolating compounds, as well as vaccination against mycotoxins, have revealed new options to limit the carry-over of these toxins.

3. Different Types of Mycotoxins

3.1. Aflatoxins

Aflatoxin is the name of a group of toxins that are produced by *Aspergillus flavus and Aspergillus parasiticus.* Two major aflatoxins are- B1 and G1. When these toxins are exposed to U.V.Light, they show Blue and green fluorescence respectively. The dihydro derivatives of B1 and G1 are- B2 and G2. The hydroxyl derivatives of B1 and B2 are- M1 and M2. Aflatoxins are furocoumarin lactones chemically (furanocoumarin derivatives). In B and M aflatoxins, a bifuran ring is fused to a coumarin nucleus with a pentane ring, while G aflatoxins have a six-membered lactone ring. Aflatoxin B1 is the most toxic than other forms of aflatoxin. European Commission set strict max level for these toxins- 2 µg/kg for AFB1; 4 µg/kg for total aflatoxins [sum of AFB1, AFB2, AFG1, and AFG2] for cereals and nuts for direct human consumption. M1 and M2 are less toxic than parent compounds. They are all highly oxygenated heterocyclic compounds. The primary target organ for aflatoxin is the liver, so it causes liver cancer(6). By far the two agricultural commodities with the highest risk of aflatoxin contamination are groundnuts and groundnut meal.

3.2. Ochratoxin A

These toxins are now gaining importance through coffee. It can be isolated from food like corn, wheat, white beans, and peanut. Scott D.B(7) in 1965 isolated 22 fungi which are toxic to ducklings. This toxin is specially produced by *Aspergillus ochraceus*. It is most commonly produced by *A. ochraceus*. Some of the other producing species are- *A.ostianus, A .petrakii, A. alliceus, P.cyclopium, P. commune.* There are 2 forms of ochratoxin- Ochratoxin A(green) and Ochratoxin B(blue). Ochratoxin A is structurally achlorinated isocoumarin derivative with an amide bond to phenylalanine. Ochratoxin B on the other hand is a dichloro derivative of ochratoxin A. It is toxic for ducklings, rats, chicks, and other animals. The toxicity is 1/3rd of that of aflatoxin(8).

3.3. Patulin

It was first isolated and described as an antibiotic on the account of being the structural synonyms of clavantin, mycoin C, penicillin. In its pure form, it is a white crystal with a melting point of 110.5°C and a molecular weight of 154. They are sensitive to Sulphur dioxide, unstable in alkali, and stable in acid. Some animals are sensitive to patulin. It is considered a potential carcinogen for humans and mice. Patulin-producing strains can be isolated from food and animal feed like- apple sap, apple cider, apple juice. It is used as an antibiotic, effective against many microorganisms. An example of it can be seen when a 0.1% concentration of patulin inhibits the E.coli and S.aureus.Produced by the following species- *Penicillium expansum, Penicillium claviforme, Penicillium patulum, Penicillium urticae, Aspergillus giganteus, Aspergillus clavatus.*

3.4. Fumonisins

Fumonisin is a mycotoxin produced by the fungus *Fusarium verticillioides*, a common contaminant of corn and corn products. The different forms are- A1, A2, B1, B2, B3, and B4. The fumonisins are structurally similar to sphingosine, the major long-chain base backbone of cellular sphingolipids(9). Therefore act as a competitive inhibitor for sphingolipid biosynthesis. The clinical signs associated with fumonisin toxicity will vary significantly between species depending on the primary target organ, and safe levels of fumonisin in the feed are quite variable between species. For humans, the limit is set at 2 µg/kg bw/day of fumonisin. No specific treatment for fumonisin toxicity in animals has been described apart from removing the contaminated grain source.

4. Aflatoxins in Food and Feed

Temperature, moisture, soil type, and storage conditions all influence the degree of fungal development and AFs formation in cereals. Rice and corn are the cereals most commonly infected by AFs under natural environments as a result of changes in agricultural practices(10). The fungal growth is caused by inappropriate rice grain drying, which results in a greater moisture content (>14%). As a result, these fungi produce grain discoloration and/or husk, as well as a reduction in grain quality(11). A report found AFs contamination in black pepper, cardamom, cinnamon, clove, cumin, coriander, and ginger, among other spices. In Cameroon, AFs were found in eggs gathered from a chicken farm as well as raw cow milk(12). As a result, the crops that are impacted allow AFs to enter the food chain, which is heavily influenced by climatic circumstances.

The table below shows the Occurrence of Aflatoxins in food and feed around the world(13):

Country	Food matrix	Aflatoxin	Range (µg/kg)
Turkey	Almond	AFB1	1–13
Turkey	Butter	AFM1	<0.001–0.100
Brazil	Cashew nuts	Total AFs	0.60–31.50
United States	Chilies	AFB1	<2
Costa Rica	Corn	Total AFs	24
Zimbabwe	Corn	AFB1	0.75–26.6
India	Corn	AFB1	48–383
Serbia	Corn	Total AFs	1.01–86.10
Vietnam	Corn	AFB1	1.0–34.80
Turkey	Cream cheese	AFM1	0.1–0.70
Pakistan	Dried Fruits	AFB1	0.04–9.80
Turkey	Feed	AFB1	0–5
Turkey	Figs	Total AFs	0.1–28.20
Nigeria	Ginger	Total AFs	0.11–9.52
Ethiopia	Groundnuts	Total AFs	15–11,900
Turkey	Hazelnut	AFB1	0.07–43.60

Table 1-aflatoxin found in differnt food items

5. Occurrence of AF in the human body

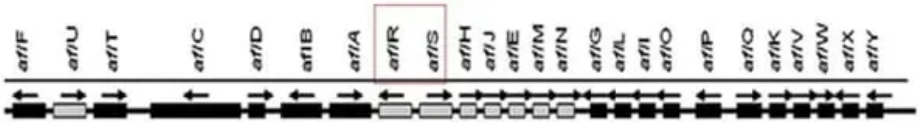
AFB1 is a potent human carcinogen that has been associated with major health problems(14). It has been linked to liver cancer, severe hepatitis, and frequent outbreaks of acute aflatoxicosis that result in death. Aflatoxin M1 is formed when

aflatoxins enter the body and are metabolized by the liver into a reactive epoxide intermediate or hydroxylated. After ingesting AFB1, cytochrome P450 enzymes convert it to an active intermediate, AFB1-exo-8,9-epoxide, through a series of metabolic steps(15). The detoxification process is catalyzed by glutathione transferases (GSTs). AFB1-exo-8,9-epoxide's detoxifying process might be connected to the mechanism that inhibits liver cancer.

6. Climate Change's Impact on Aflatoxin Production

Climate change has an influence on the complex communities of AF-producing fungi by altering the amount of AF-producers and hence reshaping the fungal community. Warm, humid, and even deserts and drought conditions increase contamination. *A. flavus* surpasses other fungal species due to its highly evolved physiological systems for adapting to unfavorable environmental conditions(16).When opposed to an alkaline environment, acidic environments promote a greater drop in AFB1 production.In all of the toxigenic strains studied, AFB1 production was dramatically reduced at pH 5.5 (acidic) and pH 8.0 (alkaline).With raising pH from acidic (pH 4.0) to alkaline pH, A. paraciticus and A. nidulans produced 5 times less mycotoxin (pH 8.0).(17) Climate change affects the environment's temperature and water activity (aw), which regulates gene expression to create AFs. The AF-producing genes are clustered on the chromosome and express both primary regulatory genes (aflR; aflS) and structural genes (aflD) that are regulated by temperature and raw circumstances(18).

The figure given below helps us to understand the effect of temperature on the expression of aflatoxin regulatory genes(19).



Ratio of *aflS/aflR* in relation to aflatoxin biosynthesis and different parameter combinations

Parameter combination	Ratio *aflS/aflR*	*Aflatoxin [ng/g]*
25°C/0.90	0.5	3.7
35°C/0.90	0.3	4.7
25°C/0.95	7.4	830.2
30°C/0.95	7.1	3016.9
25°C/0.99	1.5	1957.3
30°C/0.99	2.7	2758.7

Table 2- Ratio of aflS/aflR in relation to aflatoxin biosynthesis

Bernáldez et al. (2017) investigated the influence of temperature and raw interactions on the expression of the biosynthetic regulatory gene (aflR) and the synthesis of AFB1 by A. flavus in maize(20). They discovered that A. flavus grows best at 30°C/0.99 aw, with no growth at 20°C/0.90 aw. According to a study, the likely appearance of AFB1 in cereals in the European Union as a result of climate change is increasing the danger of AFs in various regions of Spain, Italy, Greece, Portugal, Bulgaria, Albania, Cyprus, and Turkey with every 2°C increase in temperature. (in the next 30 years)(21).

B. PROTEIN-PROTEIN INTERACTION OF HOST PROTEIN AND COVID-19 VIRAL PROTEIN

INTRODUCTION

A deadly outbreak of severe acute respiratory syndrome that is known as SARS coronavirus 2 became a global threat and caused millions of deaths worldwide. This repeated global red shows that the invincible nature of the virus has highly evolving characteristics. The efficiency of this virus means unrecognised in the host cell and it hijacks the cellular mechanism which is very crucial for the violins this has increased the demand for the pre-empty prediction that is predicting the pathway and the mechanism of the virus to predict such future pandemics. The treatment and diagnosis of this viral disease are very challenging because of the complex biology

of the breeze viral particles and their effect inside the host machinery to decode this cellular machinery. We need to know and examine the protein- protein interactions involved and the targeting mechanism that is involved in the pathogenesis so that drug designing can be effective, efficient and long-lasting.

Coronavirus symptoms can range from mild to viral pneumonia which has the power to affect the lungs, as well as the acute respiratory distress syndrome to sepsis and septic shocks in further addition to this activity and multi-organ involvement. Previous coronavirus outbreaks did create havoc in the world but the current version of Covid has affected the world in the worst way. Study is going on worldwide but unfortunately, the fatalities were extreme and it was out of control because of lack of proper treatment and diagnosis. Why this is a positive sense and single-stranded which are the envelope and they belong to family Coronaviridae. Coronavirus emerges from its zoonotic infections in which the animal virus gains the ability to affect humans we can say that except animals to humans all the three lost viruses which created havoc are supposed to be originated from the animals themselves even this current coronavirus is considered to spread from bad and religion Bengal and coronavirus.

73 different factors are considered important and can spread the coronavirus more vigorously and are considered as immune response subversion by a virus or some external factors which are associated with the viral severity because of involvement of common versions of beta coronavirus in their recent outbreak the study shows that it has the similarity between MERS, SARS, and SARS2 coronavirus. The virus shows that it has a remarkable capacity to spread vigorously and create problems with the development of anti-violence is a strategy that is involved in targeting the host and that is less susceptible to variation.

The NSP1 to NSP16 forms the replication transcriptase complex. The majority of violence factors are considered to be associated with the host membrane interactions. This makes it more viable for the lipid interacting interface of the proteins of the virus, which can provide useful information into the knowledge about the host-pathogen interaction. Knowledge of this functional interface can give

us a view of the therapeutic targets. In the M proteins, the protein is particularly the protein of interest because of its role in hampering the integrity of host greens and it manipulates it into favorable membrane parts to particles of virus generating factories. This viral structural protein gives us essential knowledge for future strategies where the protein is considered a less recognized component and now it is significantly involved in the valence factor in the host.

The protein is being considered an important part in determining the Covid viability and it has the power to target the effective therapeutic agents in the recent outbreak as it is considered it is the smallest trance membrane protein of size 8.4 to 12kDa. And it has a variable structure propensity if the protein can alter the host cell membrane permeability by forming oligomeric cation-selective ion channels. The amino acid sequence tells us about the potential purposes that are yet to be considered and can help develop the moon's efficient and effective therapeutic strategies. The characterization of these transient horse membrane conformers of protein E at different levels of protein synthesis like folding final packaging can help gain a clear knowledge of how the viral system works. The protein can acquire multiple topologies while the infection is in its peak stage and is highly conserved at the C terminal region of the protein at amyloidogenic propensity.(22-24)

1. SARS-CoV-2 Infection Leads to MHC-I Down-Regulation by ORF8

T lymphocytes have a very significant role in controlling the infections spread by the virus as they directly eliminate the cells that are infected by the virus. If a virus is infected by the cell the MHC class one molecule gets present in a peptide derived from the different weight of virus proteins. If the cell receptors once recognize the CD8 positive produces the special signal is generated by the MHC1 peptide complex and the cytotoxic T lymphocytes then release various toxic substances for example perforin and FasL etc which directly induces the death of the cell that is infected by the virus and the cytokines like interferon Gama and IL2. So the sales that support the replication of the virus get eliminated and in this way, the spreading

of the viruses is prevented. Some of the viruses which cause very chronic infections like HIV type one and KSHV Will disrupt the presentation of the antigen which is required for the activation of immune response by downregulating MHC1 expression on the cell surface by evading the surveillance of the immune system.

Covid 19 leads to the downregulation of MHC1 in both the infected human as well as in the infected lung epithelial cells of the mice. The structural proteins and the identified ORF in which the ORF8 shares the least homology with the virus can directly interact with the MHC1 molecule and can initiate the down-regulation by the pathway known as autophagy. Sales expect Singh over if it ends SARS Covid two infected cells were discovered that they were more resistant to words the CTL Lysis when what if it protein was knocked down it was found that its expression in the coronavirus infected cells would be restored in MHC1 expressions and consequently cell sensitivity to the lysis of CTL.

What are eight expressions that were knocked down in COVID-19 that affect infected cells by specific interfering RNA which is used to decrease the subgenomic RNA? When the studies were conducted the results were not satisfactory therefore the knockdown system at the protein level was developed and others incorporated the specific dilatation of this protein by using the engineered E3 ubiquitin-protein ligase. For this ORF8 is enriched by using 4 rounds of page display planning against this protein it was then fused with the C terminal of HIV1 F, which interacted with Cul5, Elongin C, and Elongin B, leading to ubiquitin-proteasome system-mediated degradation of the natural target and its artificial target protein Kras.

For confirming this process, ORS8 mediated MHC one down regulations the cells were treated with different kinds of inhibitors which are used to block the membrane protein

degradation by different pathways such as N2, etc that blocks the associated proteins and buffalo Myson even that is used to block the lysosomal deglutition. In all the inhibitor is the most important contraction of this MHC1 protein expression by what if it is mediated by BAF-A1, which clears that the addition of lysosome is the major process or the pathway for the downregulation of MHC1 by ORF8 protein. The top enrichment of Covid 19 what if it routines interacting with each other was observed

in the endoplasmic reticulum indicates that this host interaction with the overall if it does facilitate the significant reconfiguration of endoplasmic reticulum trafficking while the infection is in place. Also, this all of it showed very strong colocalization with the endoplasmic reticulum rather than the Golgi and into zooms which suggests that this ORF8 protein is responsible for down-regulate the MHC1 expression in the lysosome not in the goal G or the plasma membrane and also this knocked down of vesicle trafficking which is related to proteins like AP 1-3 fails to counteract this down regulation mediated by or of eating protein, not including the possible involvement of this vesicle in transport from the Golgi body plasma membrane or endosomal network.MHC- I ABOUT ABOUT ABOUT ubiquitination didn't change this what if its overexpression in a significant way and excluded the involvement of the ERED pathway.

Thus it was concluded that if it can mediate this MHC1 trafficking from the endoplasmic reticulum to the lysosome for the breakdown. And this trafficking is mediated by the Endoplasmic Reticulum page which is a kind of selective autophagy. 6 types of autophagy receptor proteins are useful in minding with the substrates to proceed with autophagosomal membranes.

After deeper examinations, a substantial fraction of this protein was colocalized with LC3B labelled autophagosome in the cell expressing what if eight. Then a substantial fraction of MHC1 was also localised with this LC3B labelled autophagosome. In addition to these specific autophagy inhibitors, chloroquine and E64 or PEP restored MHC1 impression on the surface of the cell and was also restored at the protein level. When we talk about LC3B it is highly rich in the lysosomes in the cells that express ORF8. After some research, it was observed that the knockdown of ATG 5 and 7, and this autophagy c in the Golgi like RB1CC1 were restored in the MHC1 expression both in the case of cell surface and in the protein level as well.

It was also concluded that this fraction of ORF8 colocalized the Beclin 1 protein, which is very essential for the initiation of autophagy in the formation of the autophagosome. CT else I directly involved in the immune-mediated protection cargo protein against the COVID-19

virus hence the downregulation of MHC1 by what if it leads to the hampering of CTL mediated killing of these infected cells. The epitope of SARS Covid 19 is SSP1 and can be characterised by the immune response.

Viral infection is effective in it and adaptive immune responses towards the replication of the virus. SARS Covid 19 can lead to the adaptive moon in this order while it's maintaining its active viral replication. Thus, we can conclude that ORF8 of Covid 19 mediates the downregulation of MHC1, which is not observed in other strains of devices. And what if it is the only protein that has about 20% homology with the covid that's why what if it is a new protein. And can be used to predict the spectrum of the disease. After studying closely it was observed that why is it of that Covid19 can mediate down-regulation upon the viral infection and plasmid infection, this infection can induce the downregulation by a single dose, and its time-independent so its effect is not consistent and can be different in different environmental settings.

After using HIV1 Nef as the control it was well observed that the molecules to mediate immune invasion through downregulation of MHC1 are similar to that of HIV1. It was also observed that all the killing essays showed the target cells can evade the recognition by the CTL in the presence of this ORF even at a very low concentration, together with what if it is one of the reasons which explains the immune evasion caused by the virus. The anti-viral T cells which are infected by the host cells that are infected are very important for viral clearance. These virus reactive sales include the ones that are responsive and what was detected in the patients that were recovered from the COVID-19. All other different viruses have developed this ability to evade immune surveillance by hampering their antigen presentation and their underlying mechanisms are very different from each other. The HIV1 Nef mechanism mainly inculcates in the direction between AP1 and MHC which prevents the movement of these MHC1 molecules in the plasma membrane and it reroutes the MHC1 from the trans-Golgi network to the lysosomes for degradation. Proteins such as K3 and K5 of KSHV proteins ubiquitinated MHC1 in the plasma membrane to facilitate endocytosis. It was also observed that MHC1 pulled down the covid 19 as bait, along with the other proteins in the endoplasmic reticulum. And the ER-resident proteins want to eat and use the De De addition of

MHC1. It is also examined that the ORF8 and MHC1 are colocalized with the LC3 labelled autophagosome.

Beclin 1 knocks out the MHC1 that was restored on the cell surface and it interacts with different regulatory proteins and acts as a scaffold to form the multiprotein complexes. Also,

This is very important during autophagy for initiating and its nucleation. The utilisation of these SARS Covid antigen-specific CTL was observed that CTL failed to identify the ORF8 expressing target cells. SARS-CoV2 uses the ORF8 protein as a unique process for altering the expression but it's not limited to the MHC1 surface only to evade the immune surveillance. It also enhances the autophagy pathway which functions as the anti-viral strategy and reaches its purpose. (22)

2. NSP1 and NSP13 (ORF1ab) block the interferon activation

The antiviral system gives the first barrier of protection against all the viruses in which the RNA virus infection, the final pathogen which is associated with the molecular patterns like while or any can we sent through the cytosolic pattern recognition receptors, that is RIG1, TLR, MDA5 in which this RIG1 I are in a binding initiates the downstream signaling by MAVS which is an adapter protein. This adaptive protein gets activated, which leads to the nuclear translocation and transcriptional induction of interferon-beta. Now, this binds it into Beta and gets attached to IFN-α/β receptors on the surrounding cells which elevate and initiate the response of the interferons by STAT receptor kinase which produces interferon-stimulated genes. In the RIG1 signaling pathway the TBK1 acts like it act in other pathways like in autophagy this TBK one helps to fulfill NF-κBsignaling by forming a complex which is called NEMO. Where is complexity lead to the initiation of downstream phosphorylation and ubiquitination which results in degradation of κB and translocation of NF-κB? This process produces ISGs that function as an inhibitor for viral spread and viral replication. No, I studied further we have seen that the coronavirus has also avoided pathways to bypass the signaling programs. For example, MERS Coronavirus can inhibit this RIG1 activation with the help of accessory proteins that is protein for a. It also can block IFN signaling by

preventing the IRF3 dimerization and phosphorylation. what is viruses do is that they use TB K-1 signaling to elope the immune responses by the host. Now MERS Coronavirus protein that is ORF4b prevents the TBK1 interactions by binding with TBK1.

After the RNA virus infection, the signaling from the immune system is initiated by a multi-step procedure where this recognization receptor identifies the internal cellular infection which results in the nuclear translocation of the anti-viral transcription factors like IRF3 and NF-κB. Which shows that immune activation has taken place. SARS Coronavirus can inhibit the interferon activation, after screening all the 10 to 16 nonstructural proteins, and it was observed that NSP1 and NSP 13 inhibited SUV-mediated NF-κB TVT by 2.5 fold and two-fold respectively. NSB 13 inhibited both NF-κB promoter activity as well as nuclear translocation whereas it was observed that NSP1 enhanced this whole thing. This TBK1 only infected cells but this got reduced after the addition of NSP 13 which shows that NSP13 limits this activation of NF-κB it was also observed that the NSP1 antagonizes as a step in the activation pathway which proceeds to nuclear translocation. (22)

2.1. NSP1 hinders the nascent protein synthesis.

Now as there are important amino acids, two sets of NSP1 protein sequences and three different related Coronaviruses were used, for example, K164/H1650 is very important for the binding of the ribosomes whereas if you talk about R124/K125D are important for the cleavage of the RNA. It was also observed that the new protein synthesis was what is removed in the cells which express the wild type NSP1 and it was the opposite in the case of NSP1 that was mutant which suggests that the nascent protein production is by the SARS Coronavirus2 is purely independent of the cleavage activity of RNA. (23)

2.2. NSP 13 decreases the TBK1 and IRF3 phosphorylation

TBK1 is a regulatory kinase that acts in the many pathways and signals IRF3 and NFκB. Is this take a note that NSP 13 organizes both the TVK1 induced IFN beta and ISRE promoter activity in basically a dose-dependent fashion which suggests that NSP 13 can stop the downstream IFN response by inhibiting dephosphorylation by TBK1 it was also seen that the pTBK1 and what is four IRF three protein levels were significantly reduced when the dose was of NSP 13 in a dependent manner which suggests that the NSP 13 can limit the TBK1 and IRF3 activation. (23)

3. Spike protein expression induces epithelial cell senescence

SARS coronavirus can cause very severe diseases and can induce a substantial cytopathic effect and even did this regulation in the immune system of the host. The mechanism that is involved in this preparation is also responsible for the enhanced cytolysis in the host and even in the strong production of the inflammatory cytokines by the infected epithelial cells. The major role of SARS CoV-2 is in the inducing of excessive inflammatory responses which act as a factor that contributes to the disease severity. Deciding you just send a sense is initiated by the stressful insults and different physiological processes which is because of the prolonged and irreversible cell cycle with the secretory features. SARS CoV-2 spike protein and the pancreas associated response that is generated in the endothelial cell leads to the initiation of the senescence.

This CoV2 spike protein expressed in the A549 cells and induces the markers that lead to senescence. Spike protein shows the enhanced expression of this marker which reduces in senescence that is senescence-associated beta-galactosidase. Andon similar grounds, it reduced the expression of senescence-associated with galactosidase in the absence of spike protein expression in the cells which is because of the paracrine effect. This increased expression was prominently seen in the single cells that had less efflorescence as compared to those of the surrounding cells also

the lies from the cells that are infected with the virus showed enhanced P 21 and P 61 expression. All the cells that were A59 like that are A59 cells that are expressing spike protein also showed p 21 and p 16 markers. We can identify the sense and sells by the increased level of ROS this generation of ROS is linked with the inducing of the senescence and maintaining the whole senescent state in those A549 cells. (24)

4. Binding of the N viral protein to 14-3-3γ host protein

SARS CoV2 N protein basically binds with different types of cell whose proteins include this 14-3-3 gamma which is a regulatory protein. Mutations in these binding regions of 14-3-3 proteins affect the binding energy and it changes the affecting capacity and the interaction.
Defining the end to 14–3–3 Gamma is very important for the virus and can be used as a targeting strategy to produce the antiviral drugs.

This 14 –3–3 is of the family of highly conserved equally or take proteins which has more than 300 binding partners. In its binding to specific phosphorylated motives changes the activities stability of the target protein. It also contributes to the pathogenesis and progression of the viral infections, these infections with 14 –3–3 proteins basically changes the original distribution and the function of the host family. N protein of this virus is basically phosphorylated on different Ser/Thr residues of the host cells and here they are basically cytoplasmic, it eventually binds with the nucleic acids where it controls the replication of the virus and its assembly. These phosphorylated proteins bind to the 14–3–3 and regulate the subcellular localization. If we decrease the level of 14– 3–3 by siRNA it causes N protein nuclear concentration.

Isoforms of 14–3–13 interact with different affinities like with 14 –3–3 Gamma is the strongest amongst them all and is enriched in RNA processing protein. And this is a form that even has P bodies which are especially specific for the translational repression, surveillance of mRNA, and even the degradation. SARS CoV2 basically uses this 14–3–3 in its life cycle for replication and even for the assembly by interacting with the N with 14-3-3 Gamma proteins. 14–3–3s is basically involved in autophagy and also in the other host defense mechanisms. (25)

5. E protein in viroporin activity of the virus

The coronavirus Genome basically has 6 to 10 open reading frames in which there are 16 nonstructural proteins and 4 structural proteins; these four structural proteins are Spike, envelope, membrane, and nucleocapsid protein and also have nine accessory factors. In which the NSP1 to 16 forms a replication transcriptase which promotes the multiplication of the virus in the host cells and all the structural proteins are involved in host-pathogen interaction like packaging and propagation in the host cell. Where its protein is involved in the integrity of the host membrane where it manipulates it into favorable membrane compartments to virus particle generating factories. It significantly contributes to the balance factor, it displays lower titer and is immature and is very important for the viability and can be used as a target for the preparation of therapeutics

E protein also maintains the integrity of the host membrane; it also has the ability to alter the host cell permeability of the cell membrane. The protein of the coronavirus is the most important membrane protein and has about 76 to 109 long residues and is known as the most dynamic add the membrane functional interface. This structure makes it important for the studies as it is very important for viral propagation. The SARS coronavirus 2, E protein has about 97% similarity in the sequence with the SARS coronavirus. E protein is found in the perinuclear region of the cells, E protein is a viroporin and is present in low copy numbers in a virus but still has a dominant role as a mediator for the virus assembly and for the release at its location and if there is increase lipid content in these locations will be higher virus budding. This protein is self-sufficient to produce the packaging and the assembly of the coronavirus particles and can effectively manipulate the host cellular machinery. It is involved in the application of the virus cycle like in host cell response that is apoptosis in inflammation and also in the autophagy with the other nonstructural proteins.(26)

6. ORF7a

The post translational modification that forms many aspects of being biology, alongside small organism infection is understood as macromolecule ubiquitination. In tempering the immune reaction, and viruses to escape immune reactions, protein ubiquitination is extremely vital.

The IFN-I signal gets smothered by the distressful cellular processes by SARS-CoV-2 ORF7a. it's assumed that SARS-CoV-2 commandeers the host ubiquitin structure to spice up ORF7a's capacity to alienate IFN-I reactions.

To develop effective vaccines and medicine it is important to grasp relations between the host and SARS-CoV-2. The SARS-CoV-2 seizes the host ubiquitin arrangement at Lys119 to polyubiquitinate through macromolecule ORF7a. The ORF7a polyubiquitination in the main contains K 63 allied ubiquitin chains. Such ubiquitin via STAT2 phosphorylation enriches ORF7a to obstruct type-I antiviral drug communication. the 2 plasmids articulating FlagORF7a and angular distance-tagged ubiquitin infected HEK293T cells, and therefore, ORF7a macromolecule was prodded for ubiquitin by HA antibodies and immunoprecipitated victimization anti-Flag.
ORF7a can also be ubiquitin by endogenous ubiquitination and for that alone immunoprecipitate Flag-ORF7a with an anti-Flag antibody and prodded for ubiquitination conjugated ORF7a. TheORF7a was smartly ubiquitin by endogenous cellular ubiquitination and it's shown by the Western Blot technique. ORF7a consists of 7 Lys remains and they perform as ubiquitinsites. The individual Lys residues were mutated to identify that Lys has ubiquitinated Ala and evaluated the mutant ORF7a for ubiquitination. The malformed K119A ORF7a showed reduced ubiquitin and this proves that ubiquitin happens at excess K119.

The 7 Lys excesses of Ubiquitin function as polyubiquitination points. The foremost conjoint excesses for ubiquitination chain development are K48 and K63. ORF7a was ectopically expressedto point out linkage-type, with varied ubiquitin K-to-R mutants together with co-immuno-precipitation analysis. Ubiquitin of ORF7a wasn't reduce within the existence of Ub-K48R however reduced for Ub-K63R. The K63-linked polyubiquitin chains ubiquitinated ORF7a. The different polyubiquitin kinds could boot be a gift as a result of Ub-K63-only slightly reduced ORF7a ubiquitination. The IFN-I communication gets smothered by ORF7a and therefore the important question here is whether ubiquitination is important or not.

The repressing actions amongst WILD TYPE and ubiquitin-defective K119A ORF7a are compared by ISRE promoter-driven luciferase assay. every macromolecule restrained stimulation of the ISRE promoter in a {very} very dose depend manner by IFN-α treatment. Throughout the informational RNA levels of WILD TYPE, ORF7a were relatively in a lesser range than IFN stirred up genes (ISGs), alongside ISG56, IFITM1, and OAS1, expressing mutant ORF7a. These effects counsel that ubiquitin at K119 is extremely vital and aimed at

optimum antagonism of IFN-I communication by ORF7a. The IFN-I signal pathway we have a tendency tore then checked if ORF7a restrains them or not. During a very dose-dependent manner upon IFN-α stimulus, WILD TYPEORF7a repressed STAT2, not STAT1 phosphorylation.

In contrast, mutant K119A ORF7a did not significantly have a bearing on phosphorylation of STAT2; it suggests that the ubiquitination for optimum STAT2 phosphorylation suppression is important. as a result of phosphorylating STAT1 or STAT2 inside the ISGF 3 sophisticated translocates to the nucleus to activate ISG transcription, we examine the impact of WILD TYPE/mutant ORF7a on STAT1 nuclear translocation.

The nuclear translocation of STAT1is restrained by WILD TYPEORF7a and it's not suffering from mutant K119A. ORF7a needs ubiquitination to prevent IFN-α communication by obstructing phosphorylation of STAT2. it's clear from the higher than an experiment that to push antagonism of IFNI responses, SARS-CoV-2 ORF7a commandeers the host ubiquitination system. Among totally different SARS-CoV-2 proteins with IFN-I antagonism, nsp13, ORF7a, and ORF3a to boot appear to be ubiquitinated. (27)

7. ORF6

SARS-CoV is an RNA virus, it contains many accessory proteins, and one of those accessory proteins is ORF6. It is shown that ORf6 disrupts bidirectional Nucleocytoplasmic Transport. ORF6 too exhibits similar relations with Nup98 and Rae1. In comparison to ORF6, ORF6 suppresses reporter expression more strongly and co purifies with Nup98 and Rae1. ORF6 interacts with Rae1 and Nup98 to prevent the nuclear import of a wide range of host factors.

Recently, it was discovered that SARS-CoV-2 ORF6 co-purifies with Nup98 and Rae1, suggesting a plausible tool for officious with nuclear transportation. Nup98 works together with the ribonucleic acid distribute issue Rae1 to bind single-stranded Ribonucleic acid and enhance the translocation of messenger RNA through the nuclear pore complex, suggesting that it is an element of the nuclear pore complex. The Membrane protein of VSV and ORF10 of KSHV virus both target Nup98 related Rae1 to lure messenger RNA transcripts into the nucleus.

The barrier to STAT1 nuclear import, these interactions are likely to impair nuclear messenger RNA export. ORF6 downregulated the manifestation of co-infected concepts, resulting in the possibility of a bi-directional barrier of nucleocytoplasmic transport, according to previous research.(28)

8. ORF10

A thorough investigation of ORF10 protein was conducted to assess its impending for yielding variations that could modify viral pathogenicity. Each ORF10 sequence has 1 different metamorphosis, according to findings. Each of the 22 ORF10 variations distinct point in the genome. However, none of these changes in the SARS-CoV-2 ORF10 contribute to the classification of SARS-CoV-2 clades. In the MoRF area of ORF10, two additional mutations, I4L and V6I, were discovered, both of which may contribute to the IPD. Because of their sensitivity to trypsin action, the alterations at positions 20 and 24 were also noteworthy. At these two sites, four ORF10 variants (QNC04532.1, QMT54534.1, QMU93213.1, and QLA48060.1) have 4 mutations. Three variations with the R20I, R24L, and R24C alterations are resistant to trypsin, while the 4th(QLA48060.1) with the R20K mutation is sensitive to protease destruction.

The amino acid homology amongst Pangolin-CoV ORF10 and SARS-CoV-2 ORF10 was found to be 97.37 percent. Even though most physicochemical and peptide parameters are identical, SARS-CoV-2 ORF10 has a higher probability of antigenicity than Pangolin-CoV ORF10, and hence a sturdier immune response is expected. At position 26 (tyrosine (Y)), a transition from strand to the coil is anticipated, reflecting the protein's higher disordered state. In SARS-CoV-2 ORF10, a sequence with the Y26H mutation was found, indicating that hydrophobic amino acid was substituted by a hydrophilic amino acid, accumulating the likelihood of additional ionic interactions.

ORF10 mutations were discovered that were projected to influence the binding affinity of SARS-

CoV-2 ORF10 to respective HLA alleles and, as a result, the immunogenicity of SARS-CoV- 2 ORF10. This could indicate that ORF10 mutations primarily decrease epitope affinity to evade the host-immune host immunereas in mixed si, situations, the effect of mutations increasing attraction is offset by the presence of mutations decreasing affinity. It is expected that obtaining more than one mutation in a single

sequence in the forthcoming will neutralize mutations that merely improve binding affinity epitopes. Furthermore, the immunogenicity mark calculation revealed that a large number of mutations had a lowered or no effect, while just a handful had an enhanced immunogenicity score, suggesting that SARS-CoV-2 may have used this method to elude the host immune response. SARS-CoV-2 and Pangolin-CoV ORF10 proteins are identical. Though, there are expected significant changes in loop/coil

shape, solubility, mutational diversity, and antigenicity of SARS-CoV-2 between these two ORF10 proteins. In spite of an amino acid homology of 97.37 percent amongst the Pangolin- CoV and ORF10 proteins of SARS-CoV-2, there are considerable differences in several physicochemical, structural, and immunological aspects, which merit further investigation.

The manifestation of ORF10 in vivo and virally infected cell lines is a subject of debate. It's worth noting that both people infected with this strain exhibited mild symptoms, and the VeroE6 cells employed for viral culture don't produce native interferon (IFN).

In this vein, ORF10 expression has been discovered in immune cells of SARS-CoV-2-infected individuals, and ORF 10 expression levels are linked to illness severity. Finally, T cells from SARS-CoV-2 infected acute and convalescent patients react to ORF10 in vitro. These findings indicate that ORF10 articulated during infection and may have a role in illness severity. The investigation of the mutations revealed in our study using various in vivo and in vitro models of COVID-19 infection and disease severity should be pursued, as they appear to be critical in our understanding of disease pathophysiology.(29)

9. Observation

S.no	PMCID	Host protein	Viral protein
1	PMCID: PMC8201919	Down-regulating MHC-1	ORF8
2	PMCID: PMC8224853	RF3 and NF-κB downregulates IFIT1 protein expression	ORF1ab (NSP1&NSP13)

7	PMCID: PMC7836370	involved in virus replication and release	ORF3a
8	PMCID: PMC7815971	inhibit type-I interferon (IFN-I) signaling via STAT2 phosphorylation.	ORF7a
9	PMCID: PMC8092196	translate their own transcripts and prevent host antiviral responses Nuclear entrapment by co purifying Rae1 and Nup98	ORF6
10	PMCID: PMC7373048	CARD11, CAMK4, and BTN3A1	ORF10
11	PMCID: PMC7373048	BCL11B	ORF7b

Table 3 Immunological profile during Covid 19

The complete knowledge of all the coronavirus factory proteins and all the mechanisms and how the viral infection really occurs is very important to determine the therapeutics and the target approach that one should have. This

strategy can only be effective if there is complete knowledge about wholesale integrity and the viral propagation mechanism.
Having complete knowledge and understanding of the functional interface and the host membrane will help get better possibilities and the attributes of the required functions in order to get the crucial protein domains that are linked with the host membranes in a different way for the therapeutic design. Because proteins plays important role in the wild packaging in propagation, the concert sequences in the terminal C region can help get the evolutionary and its important role of this compartment for the studies. The total knowledge about the protein's functional interface will provide better opportunities to understand the structure and the function correlation which can be used as an effective design against the virus. Characterization of the transient host membrane which is associated with all these proteins and different stages of the protein synthesis even in the folding and the final packaging helps to know about the viral system.

10. Observations

1. ***SARS-CoV-2-infected cells gather messenger RNA in the nucleus:***

Infected cells are blocked from exporting host messenger RNA by a variety of RNA viruses, together with Zika virus and VSV. We looked at the messenger RNA distribution in COVID-infested and mock-infested cells 24 hours after infection. messenger RNA was largely localized to nuclei in SARS-CoV-2-infested cells, but messenger RNA was more equally dispersed in the cytoplasm in mock-infected cells and nuclei. Both COVID infested HBEC3- ACE2 and Calu3 showed this nuclear messenger RNAaccumulation pattern.

2. ***SARS-CoV-2 ORF6 lumps nuclear export of host messenger RNA:***

ORF6 interrelates with nuclear pore complex component Nup98 and messenger RNA export element Rae1. Both KSHV ORF10 and VSV M interact with Nup98 and Rae1, resulting in the increase of messenger RNA in infected cells nucleus. By quickly infecting human embryonic kidney cells with a GFP, GFP-tagged VSV M, or GFP-tagged ORF6, we explored whether ORF6 was accountable for the nucleus localizing of messenger RNA detected after COVID infection. The distribution of messenger RNA in GFP-infected cells was vague from the

messenger RNA localization arrangement in not infected cells. messenger RNAwas found in several foci within the nucleus in cells articulating wild-type VSV M and ORF6, indicating that the messenger RNAin these cells was gathering in the nucleus. The lung epithelial cancer cell line and Calu3 cells rapidly infected with ORF6 showed identical messenger RNA nuclear accumulation characteristics.

3. ***SARS-CoV-2 ORF6 downregulates protein manifestation of freshly transcribed genes:***

It was looked at in what way host messenger RNA gathering in the nucleus affects host protein expression in ORF6-expressing cells. It was rapidly infected 293T cells with m Cherry, m Cherry-tagged VSV M, or m Cherry-tagged ORF6, then evaluated emerging protein manifestation in these cells 24 hours later using the Click-iT labeling assay, which uses L-azidohomoalanine instead of methionine to label freshly produced proteins. By labeling the L-azido homoalanine remains with a fluorescent marker and comparing circumstances by normalizing to the complete number of cells labeled, nascent protein synthesis can be detected. In cells expressing m Cherry, ORF6, and VSV M, analogous levels of emerging protein manifestation were detected, implying that ORF6 doesn't affect the translation of current cytoplasmic messenger RNA transcripts and only prevents the manifestation of freshly generated messenger RNA transcripts.

Cells articulating the GFP-ORF6 concept exhibited a substantial drop in m Cherry manifestation when matched to cells infected with GFP, similar to VSV M. Following that, Western blotting demonstrated that m Cherry manifestation was reduced in cells articulating Wild type ORF6. A549 cells rapidly infected with Wild Type ORF6 showed a similar drop in m Cherry expression.

ORF6 D1-16 and the clinical segregate variant ORF6 D22-30, which contain omissions in the protein N terminus, showed a three- to a fourfold lessening in m Cherry manifestation, analogous to Wild Type ORF6, demonstrating that the N terminus of ORF6 is not convoluted in downregulating protein production. In the existence of ORF6 hypotheses with deletions in the C terminus, ORF6 D38-61, and D50-61, m Cherry manifestation was reduced 1.4-1.5- fold.

ORF6 Met58Ala was created by changing the methionine remainder in ORF6 to alanine. ORF6

Met58Ala infection didn't reduce m Cherry manifestation, implying that Met58 is required for the ORF6 function. We subsequently confirmed that variations in messenger

RNA localization were to blame for the detected increase in m Cherry manifestation in cells infected with Met58Ala matched to those infected with Wild Type ORF6. In momentarily infected Calu3 cells, 293T, and A549, messenger RNA localizing configurations in Wild Type ORF6- and Met58Ala-infected cells revealed different messenger RNA localization patterns. Nothing like Wild Type ORF6-expressing cells, Met58Ala-expressing cells did not accumulate messenger RNA in the nucleus, demonstrating that Met58 is critical for ORF6 function.

4. ***The C terminus of SARS-CoV-2 ORF6 interacts with Rae1 and Nup98:***

Collaborations with the nuclear messenger RNA export factor nuclear pore complex component Nup98 and Rae1 cause downregulation of co-infected luminescent and fluorescent reporters and inhibition of messenger RNA nuclear export in KSHV ORF10and VSV M. We theorized that the ORF6 C-terminal deletion inability to downregulate m Cherry manifestation in the same way as WILD TYPE ORF6 was due to the forfeiture of dealings amongst Nup98 and Rae1 and ORF6 constructs. We infectedGFP-tagged ORF6 constructs into 293T cells and affinity-purifythe GFP-tagged proteins quickly. WILD TYPE ORF6, as well as ORF6 paradigms with N-terminal deletions, interacts with Nup98and Rae1, as verified by Western blotting on the eluates. Rae1 and Nup98 were not pulled down by the C-terminal deletion constructORF6 D50-61 and ORF6 D38-61. These findings imply that ORF6 interacts with Rae1 and Nup98 at its C terminus, but that the N terminus isn't required for the communications to occur. This is in line per the finding that ORF6 C-terminal deletion mutants had little effect on m Cherry reporter expression.

5. ***Overexpression of Rae1 reestablishes m Cherry reporter manifestation in cells infected with ORF6:***

It was then looked at if overexpressing Rae1 in 293T cells infected with ORF6 could restore m Cherry manifestation. In a dose-dependent fashion, Rae1 overexpression

restored m Cherrymanifestation. Rae1 dose-dependent rescue of m Cherryexpression was confirmed by Western blotting.

6. *SARS-CoV and SARS-CoV-2 ORF6 block the nuclear importation of a wide- ranging of host factors:*

During infection in cell culture, both SARS-CoV-2 and SARS-CoV have been shown to impede nuclear importation of the transcription factor STAT1. In cells articulating either SARS-CoV-2 ORF6 or SARS-CoV ORF6, the nuclear import of STAT1 was inhibited, which is consistent with prior results. STAT1 accumulated in the nucleus in cells articulating SARS-CoV-2 ORF6 Met58Ala or GFP after interferon-beta (IFN-b) stimulation but remained in the cytoplasm in cells articulatingSARS-CoV-2 ORF6 or SARS-CoV ORF6.

It was consistent that the nuclear import barrier was not likely to be limited to STAT1. Following steroid activation, the transcription factor glucocorticoid receptor (GR) is transported into the nucleus via contacts with nucleoporin Nup62 and importin b. Following dexamethasone stimulation, GR was trans located into the nuclei. In the cells, articulating SARS-CoV-2 ORF6 Met58Ala or GFP.

However, after dexamethasone stimulation, GR continued in the cytoplasm in cell articulating SARS-CoV ORF6 or SARS-CoV-2 ORF6, indicating that ORF6 blocks nuclear import broadly. These importins bind cargo proteins and aid in their translocation into nuclei. In cell articulating SARS-CoV-2 ORF6 Met58Ala or GFP, KPNA2 and KPNA3 were nuclear-localized (29).

C. EXPERIMENTAL WORK

Collection of soil samples to identify pathogenic fungi

Introduction

Apples are one of the most important fruits grown in the state of Himachal Pradesh. Its production almost takes first place in the state amongst all the other fruits that are grown in Himachal, having a total production of almost 4,92,100 metric tons (anonymous 2017). The main reason for low productivity of Apple production is different kinds of fungal, bacterial, and viral diseases which hamper its production. Apple growers have to face a lot of issues because of this mismanagement, which results in the low productivity of Apple and because of this, they have to suffer great economic losses. This is like how sport on the apple leaves have emerged as the biggest issue in apple cultivation. In addition from the environment, the fungal isolates enter into clinical spectrum.

Mycosis is a dangerous infection that requires treatment with antifungal agents such as amphotericin B, or isavuconazole. Amphotericin B, posaconazole, and isavuconazole are used. The uncontrolled use of antifungal agent may lead to development of resistance fungal isolates [27, 28]. Most common of them all is Marssonina blotch which results in premature defoliation in the leaves of Apple in the apple orchards this problem is mainly seen in areas like Jubbal, Rohru, mashobra areas of Himachal Pradesh, India even appeared as epidemic proportions during the 90s and other subsequent years as well. Of this disease are visible after the rains of monsoon and spring and even in the early summer months where dark brown spores of certain diameter appear on the leaves that turns yellow and then result in the dropping of the leave after few weeks the fungus even attacked the fruit by causing dark brown sports which is circular of different sizes on the fruit which results in reducing of the quality and hence affecting the commercial Apple cultivators of Himachal Pradesh. The development biology of fungal pathogens including Aspergilli is important to identify the virulent factors or mycotoxin producing organism [29]. Apart from the antifungal drug, several phytochemicals are studied to control the growth or colonization fungal pathogens on the leaves or fruits of the crops [30, 31].

Therefore collecting different soil samples from areas like Jubbal, Rohru, Mashobra of Himachal Pradesh in order to identify the type of pathogenic fungi and its kind in order to manage the fungus for better crop management not only for crops like Apple but other main crops that are grown in the state.

Materials and methods

Sample collection

A total of 10 different soil samples of different crops were collected from different areas of the district of Shimla in Himachal Pradesh. The samples were collected from locations like

Mashobra, Jubbal, and the Rohru region. Soil samples from areas growing wheat, onions, apples, tomatoes, etc are used for studying the fungi present in that particular location.

Figure 1-Soil samples from different locations of district Shimla

Isolation and identification of single colony fungal species

Isolation of different fungi from different samples of the soil that was collected was carried out with the help of direct plating method. First of all, all the soil samples from different regions were put in the petri plates. Then according to the sample that we are studying at that point in time the petri plate containing the soil samples from that particular region was taken and then serially diluted.

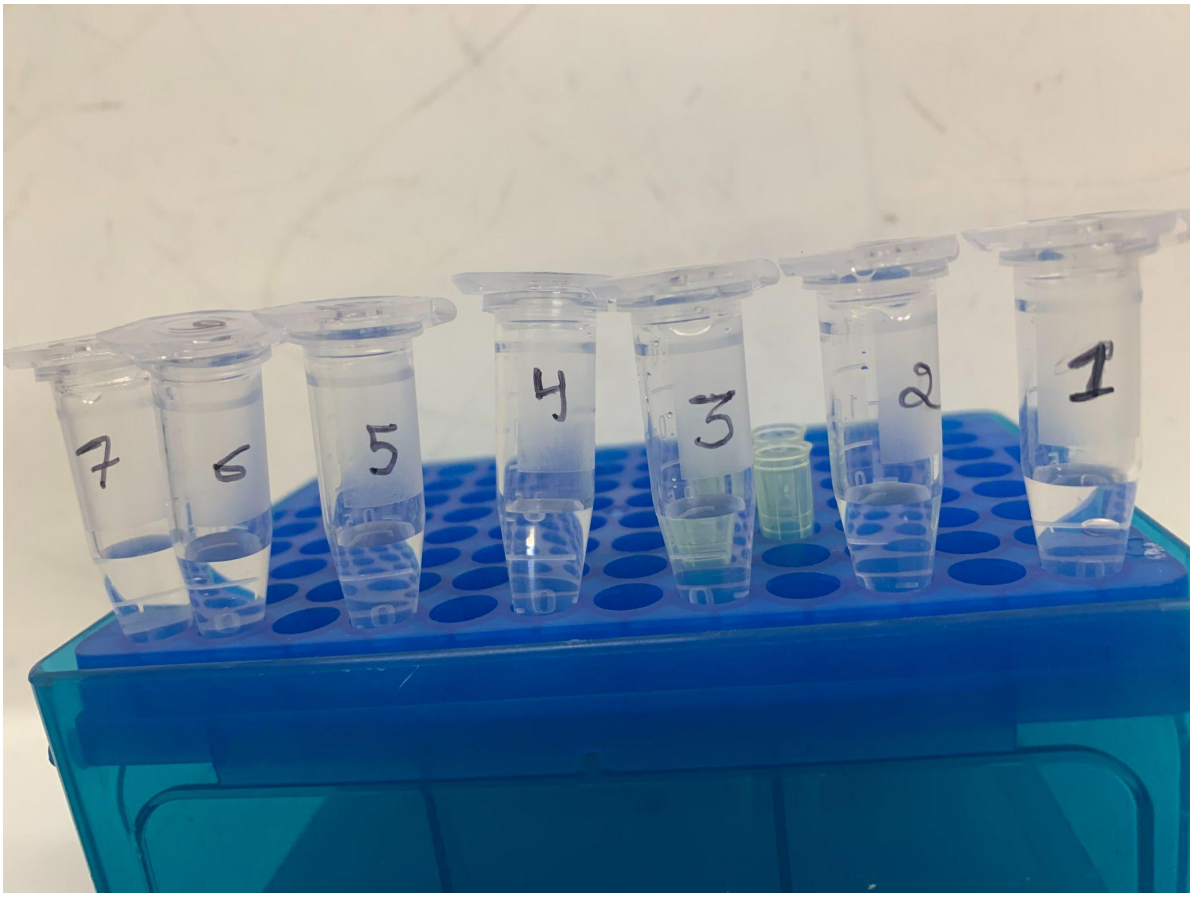


Figure 2- Serial dilution of the soil sample

For growing of the fungi, PDA medium plates were prepared. Then taking 100 mg of the soil sample in about 5mL of water and then serially diluting it for about 10 times and then inoculating the ideal dilution into the factory plate containing PDA medium for the growth of fungus. The petri-plate was then incubated at 37°C for 2 to 3 days, and then examined for fungal growth daily. Different colonies of fungus grow on the inoculated sample of that particular soil sample of the region. Repeating the same procedure for different soil samples from different regions in order to study the fungal pathogen in that particular area, after this

the plates were being studied for the identification of the fungus by looking at the different fungal colonies that were isolated.

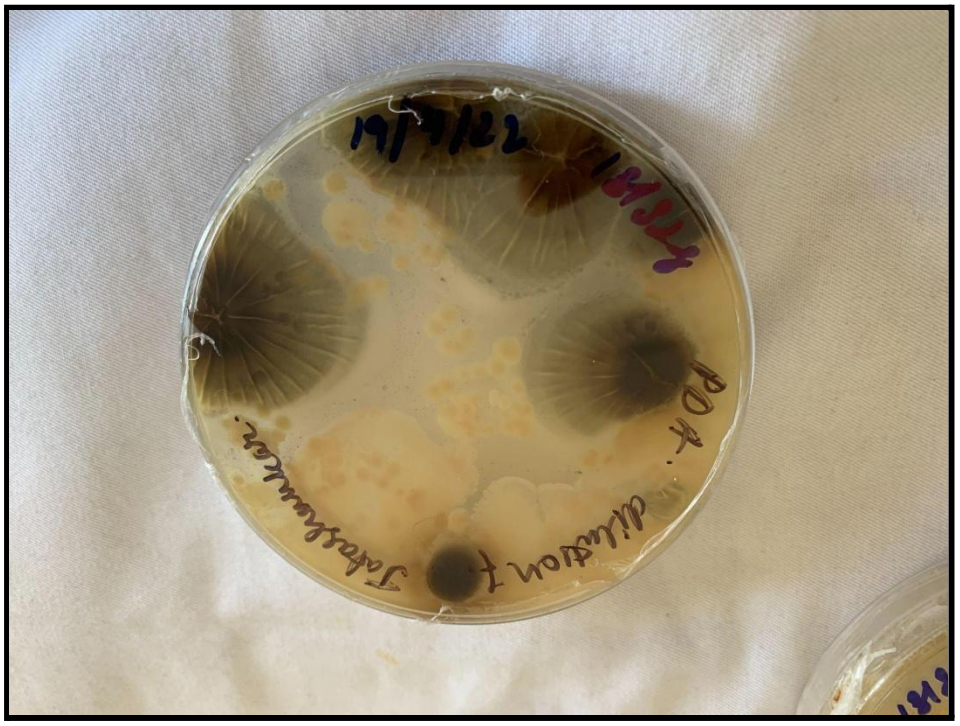


Figure 3- Fungal colonies from apple soil sample of Jubbal region at dilution 10^7 folds

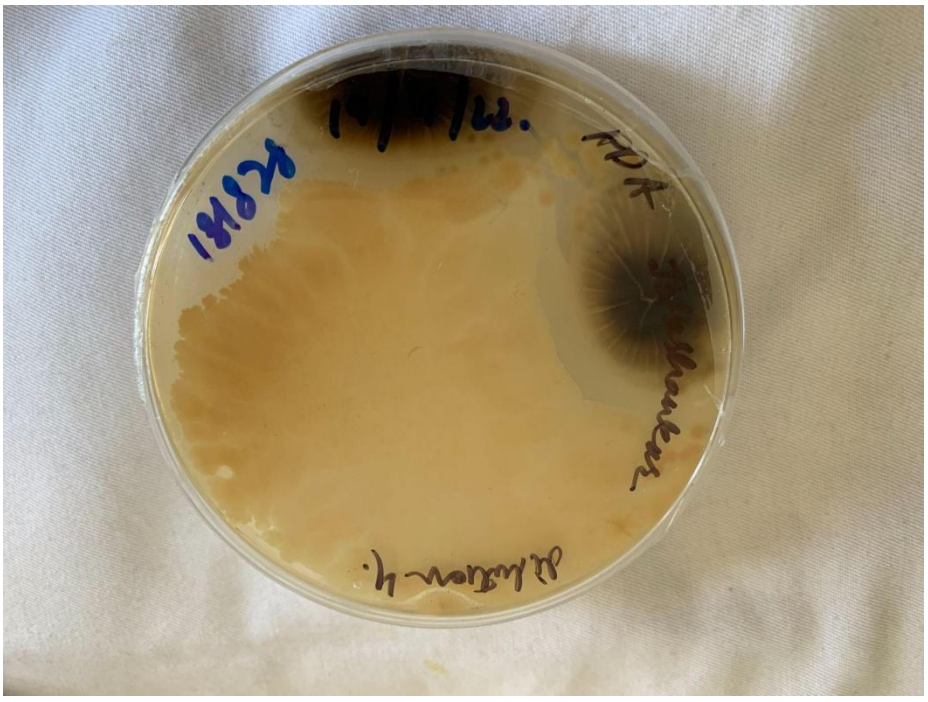


Figure 4- Fungal colonies from apple soil sample of Rohru region at dilution 10^4 folds.

Figure 5-Fungal colonies from apple soil sample of Mashobra region at dilution 10^6 folds.

Results and Discussion

Fungal species infecting crops of Himachal Pradesh were studied using the soil samples from different regions mainly Marssonina, Alternaria and lycopersici species. The abundance fungal species were observed in different geographical locations of upper Shimla region, however, needs molecular characterization. The fungal spore remains dormant inside the calyx of the fruit and later becomes active when the starch content of the fruits get converted into sugar. It is suggestive that suitable substrates such as polysaccharides or monosaccharides are when found, the colonization of spore followed by growth occurs. Hence, the post harvest crops are more prone for infection and diseases were often associated.

Further management of these pathogenic fungal species according to their occurrence in different areas is important in order to maintain the crop productivity and quality. The resistance patters among the fungal pathogen or mycotoxin producing microorganism could be assed in order to get better productivity and quality of the crops.

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