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ORIGINAL ARTICLE

Investigation of A New Local Isolate of Penicillium Lanosocoeruleum That Produces The Antifungal Griseofulvin

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ABSTRACT

Penicillium (140) isolates were isolated from different sources to investigate penicillium species that produced griseofulvin. Penicillium species were identified according to taxonomic keys. P.lanosocoeruleum was isolated from wheat grains that produce the extrolites, tryptoquialanins, griseofulvin, and viridicatumtoxin.

P.lanosocoeruleum have been also diagnosed molecularly by detection of the presence of the Internal Transcribed Spacer (ITS) in fungi using PCR technique. After the fungus was diagnosed, recorded in the NCBI as a new isolate LC635342.1. Several methods have been used to detect the production of the antifungal griseofulvin of P.lanosocoeruleum, Potassium dichromate detection, dichloromethane detection, extraction of griseofulvin industrially, and bioactivity test against dermatophytes fungi (Trichophyton mentagrophytes).

INTRODUCTION

Coinciding with the technical development of analytical devices and great advancements in biological sciences, it became necessary to change the study patterns from a reductionist approach to a comprehensive approach for filamentous fungi understanding (Kluger et al., 2015). Filamentous fungi are ever-present in the natural world, with a significant role in maintaining the ecosystem's situation through degradation of organic substances, nutrient recycling, and interaction of organisms (Ferreira et al., 2020).

Penicillium is a type of ascomycetous mold that is a portion of the mycobiome which is of great importance in the naturalist environment, also in food and drug manufacture (Houbraken et al., 2020). Several members of the penicillium produce penicillin or its derivatives, and is used as a medications, which inhibits the development of certain types of microorganism that can cause disease.. (Alberti et al., 2017).

P.lanosocoeruleum was previously known as p.veridicatum or p.expansum, today known as p.lanosocoeruleum or p.aethiopicum (Frisvad, 1995 and Houbraken et al., 2012). The first isolation of p.lanosocoeruleum was from barley in Ethiopia, after that repeatedly isolated from various commodities such as peanuts, rice, soybeans, cashews, and other commodities (Pitt and Hocking, 1997)**.**

P.lanosocoeruleum is very similar to P.chrysogenum in terms of its weekly growth at 37°C, it is similar to P.expansum in their production of fasciculate colonies, both species produce conidiophores with sleek-walled stipes. The most important distinguishes P.lanosocoeruleum from other species is colonies formation a wavy yellow color when growing on Czapek Yeast Autolysate Agar (CYA), Penicilli are tighter and compact than P.chrysogenum, while the brown pigment is less than p.expansum (Pitt and Hocking, 2009b). P.lanosocoeruleum outputs three structurally motivating and biologically efficient polyketides: viridicatumtoxin-like tetracycline, antifungal representative griseofulvin or Dechlorogriseofulvin, and Tryptoquialanine is a tetrapeptide shapely identical to tryptoquivaline, which is recognized as tremorgenic (Chupakhin et al., 2019).

Griseofulvin is inhibits microorganism growth and is utilized for the treating of mycotic maladies of human, cultivate, and veterinary systems (Fig. 1). It is synthesized by numerous species of penicillium and other microorganisms (P.griseofulvum, P.janczewskii, P.nigricans, p.urticae, P.raistrickii, P.albidum, P.Coprophilum, Carpenteles brefeldianum, Khauskia oryzae, and Nigrospora musae) (Paget and Walpole, 1958; Venkata and Panda, 1999; Bills and Gloer, 2016 and Hao et al., 2020).

Griseofulvin is an antifungal medicine utilized to cure several kinds of dermatophytoses which involves fungal infections of the fingernails and the skin of the head. It is taken orally ("Griseofulvin," 2019). The genes clusters (gsf) responsible for griseofulvin production in p.lanosocoeruleum (Chooi et al., 2010).

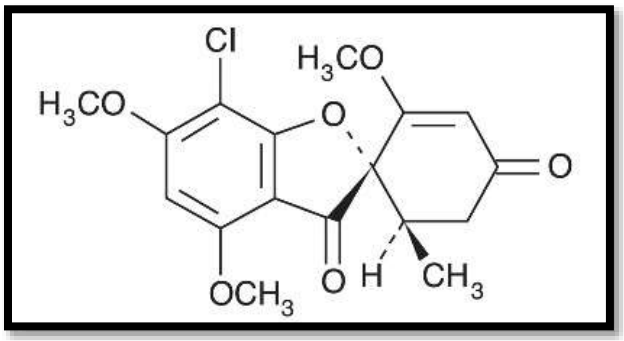

Fig 1: Griseofulvin Structure

Griseofulvin is less effective than terbinafine in the treatment of scalp infection by Trichophyton fungi, but it is more effective in treating scalp infection by Microsporum fungi (Chen et al., 2017). The medication relates with tubulin, meddlesome with microtubule job, consequently preventing mitosis. It relates with a fibrous protein forming the main structural constituent of hair (keratin), in keratin cells make them resistant to fungal infections. The medication reaches its location of activity when hair or epidermis is changed by the keratin-griseofulvin combination ("Griseofulvin," 2016).

MATERIALS AND METHODS

Fungi isolation

Isolation from fruits: Several infected fruits in Penicillium were brought to the laboratory. They were cut into small pieces and placed in a sterile solution (NaOCl) for 1-2 min, after which they were picked with a sterile tong and washed with sterile distilled water (DW) several times, and transferred to filter papers to dry.

The dry pieces had been transferred to Petri dishes containing potato dextrose agar (PDA) medium and incubated at 25 °C for 5 days (Thiyam and Sharma, 2013).

Air-borne fungi: To obtain the spores from the air, Petri dishes containing (PDA) medium were exposed to air 0.5 hr and incubated at 25 °C for 5 days (Duncan et al., 2010).

Isolation from soil: Soil quantity (50-100) g took after raising the top layer from soil (10-15) cm. The samples were placed in sterile plastic boxes and transferred to the laboratory.

A-Direct method: It was taken small quantities of the soil and distributed in Petri dishes containing (PDA) medium and incubated at 25 °C for 5 days (Warcup, 1950).

B-Dilution plate method: Soil samples 25 g were taken and placed in a glass container, then added 250 ml to it and mixed well to obtain 10^{1-} dilution, 1 ml of the suspension solution was withdrawn and transferred to a tube containing 9 ml of a sterile DW to obtain10^{2-} dilution, the previous process was repeated until obtaining 10^{4-} dilution. One drop was taken by a loop from each dilution and spread on a petri dish containing a (PDA) medium. The petri dishes were incubated at 25 °C for 5 days (Barron, 1971).

Isolation of fungi from wheat seeds: Wheat seeds were collected randomly from local markets 100 g from each local, seeds were superficially sterilized with (NaOCl) then washed with sterile DW to remove traces of the sterilizer. Wheat seeds (5 seeds) placed in each Petri dish contains (PDA) medium and incubated at 25 °C for 5 days (Rajput et al., 2005).

Diagnosis of the species of Penicillium: Penicillium Species have been diagnosed according to its growth on the three diagnostic mediums at different temperatures (5, 25, and 37) °C.

1 Czapek Yeast Extract (CYA).
2 Malt Extract Agar (MEA).
3 Glycerol Nitrate Agar 25% (G 25 N).

Fungal isolates of Penicillium spp. were grown on (PDA) medium and incubated at 25 ^{0} C for 5 days, the suspension method was used to inoculate media. The suspension was placed in a 2.5 ml of Eppendorf tubes containing semisolid medium, composed of 7 g agar and 10 g sucrose dissolved in 1000 ml of DW.

Semisolid medium inoculated with a suspension of fungi spores by using a sterile needle, then the three diagnostic media were inoculated and incubated at (5, 25 and 37)°C, three replications for seven days.

The diameters of the growing colonies were measured with a ruler electronic on the back of the plate and the results were recorded and compared with the taxonomic keys of the genus penicillium (Pitt and Hocking, 2009).

Extraction of DNA from the fungi: The Genomic DNA mini kit supplied by Geneaid Company was utilized and included the following (Mutib et al., 2014)**:**

1 Add 100 mg of fungi to a ceramic mortar, then add liquid nitrogen to it and grind for 3 sec.
2 The powder was transferred to a 1.5 ml glass tube, added 400 µl of GP1 and 5 µl of RNase then mixed well by using a Vortex apparatus.
3 The tube was incubated in the oven at 60 °C for 10 min, turning the tube every 3 min.
4 Add 100 µl of GP2 Buffer solution and mix with a Vortex device and incubate in ice for 3 min.
5 The mix was transmitted to a Filter Column tube and centrifuged for one min at a speed of 1000 revolution per min (rpm), the precipitate was discarded and the filtrate was transmitted to a 1.5 ml tube.
6 Supplement 1.5 ml of GP3 solution and combine immediately and leave for 5 sec.
7 Then, transfer 700 µl of the solution to the GD Column tube and centrifuge it for 2 min.
8 Then the filter was discarded and the GD Column was reinstalled into the collector tube.
9 Add 400 µl of W1 solution, centrifuged for 30 sec, the filtrate is discarded, and the GD Column is re-fixed to the collection tube.
10 Add 600 µl of Wash Buffer Solution and centrifuge for 30 sec at upper speed, neglecting the filtrate and a centrifuge for 3 min again at upper speed.
11 The GD column is fixed in a 1.5 ml tube, added 100 µl of Elution solution to it, and laid aside for about 3 min to permit it to relate with DNA.
12 Finally, centrifugation is carried out at an upper speed for 30 sec to remove the GD column. The sediment is taken and kept at 20-°C until utilized.

Agarose gel preparation and DNA electrophoresis: DNA relay and detection was done by an agarose gel which is prepared at a concentration of 1%, to obtain this concentration, 0.5 g of agarose powder is dissolved in 50 ml of X1 TBE and added 3 µl of red safe dye, using heat source with continuous stirring until boiling and left to cool.

The gel solution is poured into the tank of the relay tray, after the special comb is fixed to form the wells at the edges of the gel, the pouring is quiet to avoid the formation of bubbles, and if they are formed, they are removed using a pipette, then leave the gel to solidify.

Migration specimens are prepared by mix-up 5 µl of the DNA specimen with 3 µl of the loading solution, the relay is operated at 5 v/cm, and takes time (1.5-2) hr, After that the gel is photographed under UV rays using a gel documentation device to be able to see DNA bundles as well as the product of the PCR reaction (Dilhari et al., 2017).

DNA extraction from gel: The beams produced from the PCR reaction were removed from the gel, to be clarified and sent for a nucleotide sequencing test, found on the test kit equipment by Geneaid (Parayre et al., 2007).

1 The section of agarose gel is cut off utilizing a sterile fleam while removing the largest amount of gel that surrounds the bundle
2 A cut gel is placed in an Eppendorf tube of 1.5 ml, then buffer solution DF 500 µl is added and mixed utilizing the Vortex apparatus.
3 The tube is incubated at a temperature (55-60) °C for 15 min, the tube inverted every 3 min through the incubation time and the tube is laid aside to cold at laboratory temperature.
4 Transfers 800 µl of the mixed sample to the DF column, then the filtrate was removed using a centrifuge at 16000 rpm for 30 min.
5 Reinstall the DF column again to the collecting tube, add 600 µl of lotion solution, and lay aside one min.
6 Using a centrifuge with the same force and time at 16000 rpm for 30 min, this process repeats again, then re-centrifuge for 3 min to assure a dry column DF.
7 The collecting tube is neglected and the DF column is carried to a new tube of 1.5 ml.
8 Add a solute solution (Elution buffer) 50 µl to the column, then leave for two min to make certain absorption of the solute solution
9 Finally, dissolved DNA was obtained after 2 min centrifugation at 16000 rpm.

PCR reactions: The DNA concentration in all study samples was adjusted by dilution by TE buffer solution to obtain the required concentration for PCR reactions of 50 ng/µl for each sample. The master reaction mixture was prepared for each PCR reaction by mixing DNA sample and special primer for each gene with components of the Pre-mix inside an Eppendorf tube 0.2 ml, supplied by Biolaps company.

The mixture is placed in a Microfuge for a period (3-5) sec to ensure reaction components mixing, reaction tubes were inserted into the thermocycler to conduct the multiplication reaction by using the special program for each reaction. then added 2% of the volumetric guide DNA Ladder supplied by Biolaps company in one of the holes, that the samples relay by the electrophoresis device for a period (60-70) min, the gel is photographed using the gel Documentation device (Erlich, 1989).

Detection of the presence of the internal transcribed spacer (ITS) in fungi using PCR technique: The gene of P.lanosocoeruleum fungi was detected by adding 4 µl of template DNA and 1 µl (10 picomols) of each gene-specific primer to master mix contents (Table 1). The reaction tubes were inserted into the thermo cycler to carry out the multiplication reaction using a special program for the reaction (Table 2).

3-12 Determination of nucleotide sequences for amplified pieces using DNA sequencing: The sequence of nitrogenous bases of the P.lanosocoeruleum under study was determined, the PCR reaction products were sent with the primers of the resulting package, the genes were read based on the 3130 Genetic Analyzer device supplied by Hitachi company.

The sequences of genes were matched with the sequences of the genes documented in the National Center for Biotechnology Information (NCBI), and the results were analyzed using the BLAST program (Wheeler et al., 2006).

Table 1: Nitrogenous bases for the primers used in the DNA amplification process

primer	Sequence
Forward	TGAATCATCGACTCTTTGAACGC
Revers	TTTCTTTTCCTCCGCTTATTGATAT

Table 2: special program to multiplication reaction

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	denaturation	95	45 sec.	35
3.	Annealing	55	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1

Methods of griseofulvin compound detection: The griseofulvin reference standard was obtained from Sigma company, Spain, at 98% purity. Griseofulvin compound was confirmed before using it in the study experiments by an IR device.

1- Potassium dichromate detection: Preparation of the standard solution: dissolved 5 mg in 1 ml of H_2SO_4 and added 5 mg of potassium dichromate. Preparation of the test solution: equal amounts of chloroform solvent and filtrate of P.lanososcouloruom broth 5 ml each one was mixed well for 5 min, then put the mixture in a centrifuge 3000 rpm for 5 min, then the broth medium was discarded.

The chloroform solvent was placed in a glass tube and completely evaporated at 50°C, then added 0.5 ml of H_2SO_4 and 1 mg of potassium dichromate (Rhodes et al., 1961; Cartwright, 2016).

2- Dichloromethane (CH_2CL_2) detection: P.lanosocoeruleum was grown on Czapek's agar medium for 5 days at 25 °C, the mycelium of fungus was collected from the surface of the medium by skimming.

Mycelium 5 g was added to a CH_2CL_2 solvent 20 ml a ratio (1W:4V), leave The solution at 4 °C for 15 min to homogeneous and removing impurities, after that decolorized with charcoal, then filtered through several layers of gauze and filtered again by a Buchner funnel using filter paper (Whatman No.2) under vacuum.

The solution was evaporated at 50 °C to concentrate 6.6% of its original volume and cooled at 0°C (Řezanka and Spížek, 2005).

3- Extraction of griseofulvin industrially: Griseofulvin is produced industrially by the following steps (Zaidi, 2014):-

1 Add 20 ml of acetone to a glass container (flask) containing mycelium of P. lanosocoeruleum 4 g at a ratio (1W:5V).
2 Decolorization by calcium hydroxide 50 g/ L.
3 Add aliphatic hydrocarbon 5 ml such as hexane.
4 Add alkaline water 15 ml as a precipitation agent.
5 Add Methanol 10 ml as washing solvent.

4- Bioctivity test for secondary metabolites of P.lanosocoeruleum against Trichophyton mentagrophytes: Prepare the culture medium for the production of griseofulvin by dissolving (gm/L):NaCl 0.8, K_2HPO_4 0.1, $CaCl_2$ 0.04, $MgSO_4.7H_2O$ 0.2, KCl 0.1, NH_4Cl 1, $ZnSO_4$ $7H_2O$ 0.04, $CaCO3{\cdot}2H2O$ 1.0, glucose 10 and yeast extract 3, in a liter of distilled water and pH 6.5(Venkata et al., 2002).

The medium (antibiotic-producing broth medium) was distributed in flasks (100 ml each flask), then closed and placed in the autoclave, after sterilization left flask to cool, thereafter inoculated each flask by 1 ml of conidial suspension of P.lanosocoeruleum (1V:100V) and incubated on a rotatory shaker at 25 ^{0} C, 150 rpm for 10 days.

T. mentagrophytes was grown on sabouraud dextrose agar (SDA) medium at 30 °C for 15 days (Frías-De-León et al., 2020), took one disk from growing colonies of fungi 9 mm diameter, and placed in the tube containing 10 ml of nutrient broth medium and shaken well, took 1 ml from the last spore suspension and added to a tube containing 9 ml of nutrient broth shake well too, then took 0.2 ml from diluted spore suspension by loop and spread it by swab well on Muller Hinton agar medium with 2% glucose (MHA 2%), and left for 15 min to dry.

Agar diffusion test of holes was followed in T.mentagrophytes sensitivity test for secondary metabolites of P.lanosocoeruleum. The method included making dimensions equal holes 5 mm diameter in (MHA 2%) and filled each hole 30µl of secondary metabolites of P.lanosocoeruleum, other holes were filled also 30 µl with secondary metabolites of Penicillium fungi, which are known not to produce griseofulvin as P.chrysogenum, and P.expansum as bioactivity test. All Petri dishes were incubated at 28 °C, PH 5.6 for 15 days and with three replicates (Khadka et al., 2017).

RESULTS AND DISCUSSION

Fungi isolation: Penicillium (140) isolates were isolated from different sources to investigate penicillium species that produced griseofulvin. Penicillium species were identified according to taxonomic keys (Pitt and Hocking, 2009), 17 species were diagnosed, as shown (Table 3). The phenotypic characteristics of Penicillium colonies varied in their shapes on the diagnostic media (fig. 2 and fig. 3).

P.lanosocoeruleum was isolated from wheat grains, in former studies, the fungus was isolated from soil, corn, walnuts, and air (Wells and Cole, 1977; Cruickshank and Pitt, 1987; MaŃKa et al., 1991; Shi et al., 2017).

Table 3: Penicillium fungi isolated from different source.

Penicillium spp.	Resources	Isolates number
Penicillium verrucosum	Wheat grains.	9
Penicillium oxalicum	Soil and Air.	6
Penicillium expansum	Soil.	21
Penicillium viridicatum	Peaches, Wheat grains and Air.	10
Penicillium crustosum	Soil.	9
Penicillium lanosocoeruleum	Wheat grains	1
Penicillium chrysogenum	Wheat grains, Apple, Air and Soil.	22
Penicillium sclerotiorum	Peaches and Soil.	2
Penicillium thomii	Soil and Air.	10
Penicillium digitatum	Orange, lemon and Air	15
penicillium fellutanum	Soil and Wheat grains.	2
Penicillium roqueforti	Soil and Wheat grains.	4
Penicillium simplicissimum	Wheat grains.	1
Penicillium italicum	Pomegranate and Apple.	4
Penicillium citrinum	Air and Wheat grains.	5
Penicillium janthinellum	Air and Wheat grains.	2
Penicillium paxilli	Air.	2
Penicillium purprugenum	Peaches.	1
Penicillium glabrum	Air.	1
Penicillium implicatum	Soil	3
Penicillium aurantiogriseum	Soil	1
Penicillium solitum	Soil	3
Penicillium corylophilum	Soil	4

P.lanosocoeruleum and P.aethiopicum are conspecific (fig. 4). This is supported by molecular information, phenotypic distinguishing, and extrolite information. Both species have ellipsoidal conidia (Peterson and classification, 2000), and produce the extrolites, tryptoquialanins, griseofulvin, and viridicatumtoxin (Frisvad and Samson, 2004 and Chooi et al., 2010).

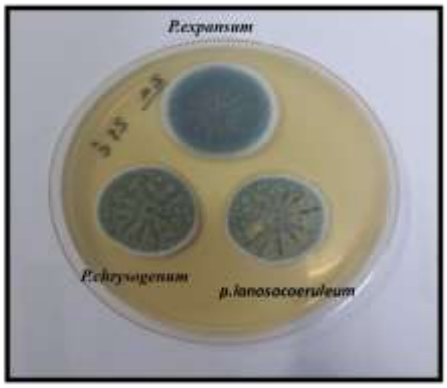

Fig 2: p.expasum, p.chrysogenum and p.lanosocoeruleum growth on CYA medium.

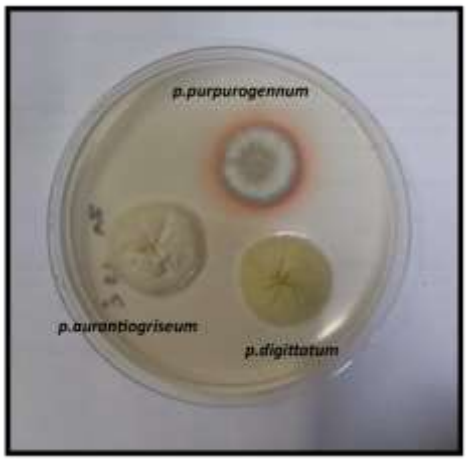

Fig 3: p.purpurogennum, p.digittatum and p.aurantiogriseum growth on CYA medium.

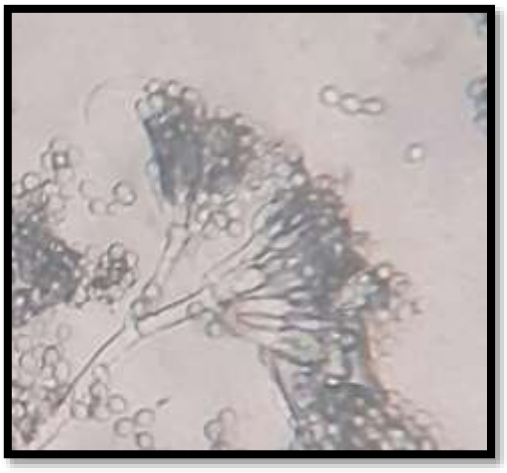

Fig 4: p.lanosocoeruleum under microscope (40 X).

4-2 Molecular diagnosis of P.lanosocoeruleum depending on (ITS): P.lanosocoeruleum have been also diagnosed molecularly by detection of the presence of the Internal Transcribed Spacer (ITS) in fungi using PCR technique (Fig. 5).

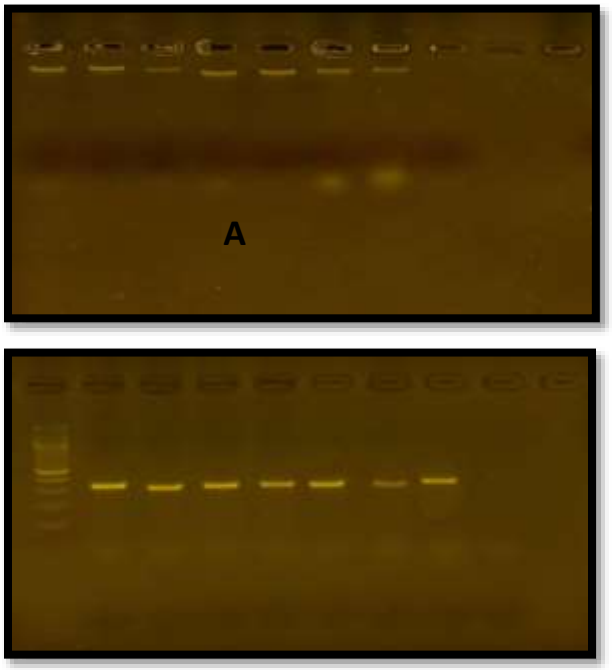

Fig 5: A Genome extraction from fungus.
B PCR reaction outputs of fungus at size 330bp.

The PCR reaction products were sent with the primer of the resulting package, the genes red based on the 3130 Genetic Analyzer device supplied by the Hitachi company (Fig. 6).

CGGGGGGCNTGCCTGTCCGAGCGTCATTGCTGCCCTCAAGC
ACGGCTTGTGTGTNGGGCCCCGTCCTCCGATCCCGGGGGAC
GGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGA
GCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGN
GCTTGCCGATCAACCCNAATTTTTATCCAGGTTGACCTCGGAT
CAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGG
AGGAAAAGAAAA

Fig 6: genes sequences of P.lanosocoeruleum

The sequences of the genes were matched with the sequences of the genes documented in the National Center for Biotechnology Information (NCBI), and the results were analyzed using the BLAST program (Fig. 7). After the fungus was diagnosed, recorded in the NCBI as a new isolate LC635342.1 (Fig. 8).

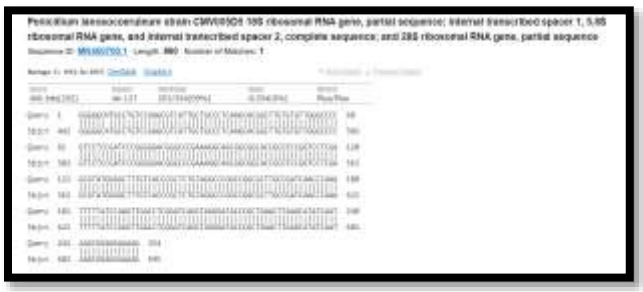

Fig 7: the sequences of the genes documented of p.lanosocoeruleum in (NCBI).

Fig 8: A new local isolate of P.lanosocoeruleum from wheat grains

In a study to update classification methods, P.lanosocoeruleum was isolated and identified from groundnut in south Africa using PCR technique, based on ITS of fungi (Schoch et al., 2020). A total of 65 qPCR tests, included an enzyme that cleaves the nucleotide chains of nucleic acids into smaller units, diagnosed species of Paecilomyces, Aspergillus, and Penicillium have relied upon (ITS) (Haugland et al., 2004). In another study, which included the distinction between the medically important species of Aspergillus and opportunistic molds and yeasts, the species were distinguished based on the variance (ITS) region of ribosomal DNA (de Aguirre et al., 2004).

Results of detection griseofulvin compound: The griseofulvin reference standard was diagnosed by using the IR device for confirmation (fig. 9).

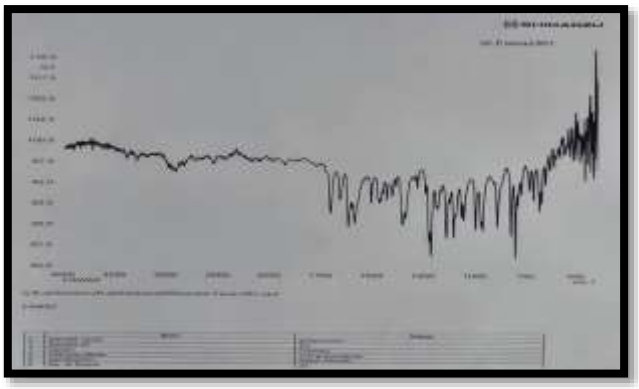

Fig 9: Confirmation of the diagnosis of griseofulvin by IR

1- potassium dichromate detection: The griseofulvin was extracted by chloroform solvent and evaporating it, leaving traces of griseofulvin on the test tube wall, added 0.5 ml of H_2SO_4 to give yellow color, then added 1 mg of potassium dichromate which showed a dark red color as a positive result (Fig. 10), yellow color acquired by griseofulvin in H_2SO_4 relies on oxonium salts of griseofulvinic acid. The dark-red color generates by the oxidation of potassium dichromate by orthoquinon (Auterhoff and Kliem, 1976). In a study, limitation of Griseofulvin in leavening Samples, griseofulvin was extracted by several solvents (Butyl acetate, Methyl Cellosolve, Diniethylforrnaniide, Methanol, Ether, Chloroform and Benzene), detected by using sulfuric acid and potassium dichromate (Ashton and Brown, 1956). Another study concerned the ability of B.subtilis to biodegrade Griseofulvin, the last was diagnosed by the same chemical reaction (Ahmed and Bayoum, 2014).

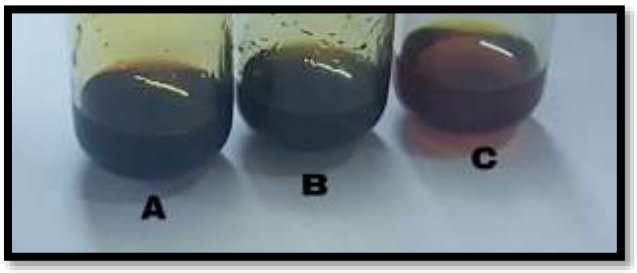

Fig 10: A Griseofulvin reference standard
B Test tube griseofulvin extraction (positive)
C Test tube without griseofulvin extraction (negative)

2- Dichloromethane (CH_2CL_2) detection : Griseofulvin was extracted by dichloromethane and evaporated at 50 ^{0} C to 6.6 % of the original volume and cold to 0^0 C, it has been observed griseofulvin's Chrystal formation (Fig. 11) (Řezanka and Spížek, 2005).

Dichloromethane is commonly used to extract antifungal compounds such as ketoconazole and griseofulvin (Odhiambo et al., 2010). Dichloromethane was also used to increase the dissolution average and oral sucking of griseofulvin which is a poorly water dissoluble drug, by forming a substance that tends to reduce the surface tension of a liquid in which it is dissolved-containing micro-particles (Wong et al., 2006). In another study, dichloromethane and aqueous sodium chloride were used as an extract to determine the doses of griseofulvin tablets or capsules (Townley and Roden, 1980).

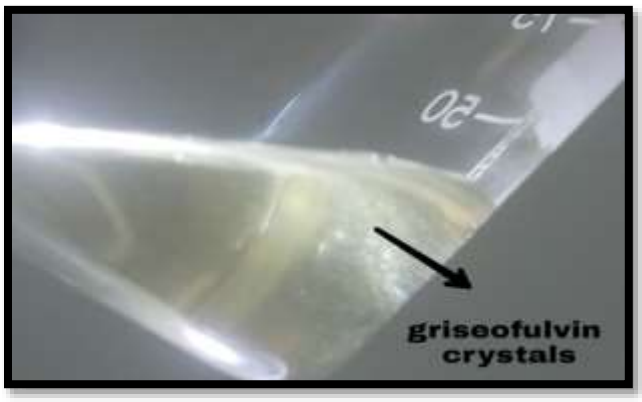

Fig 11: griseofulvin's Chrystal formation in Dichloromethane at 0 ^{0} C

3 acetone detection: Griseofulvin extraction was done by cold acetone, utilized as an extraction factor. The extraction may happen at high temperature or in the cool, but in general, cold acetone is preferred over hot, because the latter has some defects that negatively affect the precipitation of griseofulvin. Thus they choose to extract in the cool when acetone is utilized as an extraction factor. Then the extract improved by addition 50 g/L of calcium hydroxide (VasudhaUdupa et al., 2021).

The impurities taken away by washing the extract with a dissolvent (hexane) make extract immiscible, and also griseofulvin is unsolvable, griseofulvin precipitated from the dissolvent extract by addition alkaline water (drops of ammonia + water, pH about 8.5) which is more efficient for elimination colored defects existing in griseofulvin's crystals, the precipitate is mostly made better by a wash with a methanol dissolvent (Fig. 12). After deposition, the griseofulvin may oftentimes alter in color from white to brown and it may be desired to offer the precipitate to more purification.

In other studies, acetone also was used as one of the solvents to the enhancement of the ability to be dissolved, especially in water and degradation of griseofulvin by nanocrystallization (Venkata and Panda, 1999).

Fig 12: griseofulvin's crystals formation by acetone extract.

4- Bioctivity test for secondary metabolites of P.lanosocoeruleum against Trichophyton mentagrophytes: The inhibitory effect of P.lanosocoeruleum against T.mentagrophytes is due to the presence of griseofulvin compound. As bioactivity test, Inhibitory effect of P.lanosocoeruleum against T.mentagrophytes compared with non-producing fungi of griseofulvin compound (p.chrysogenum and p.expansum), it was observed P.lanosocoeruleum was the only fungus that showed inhibitory activity against T.mentagrophytes (fig. 13).

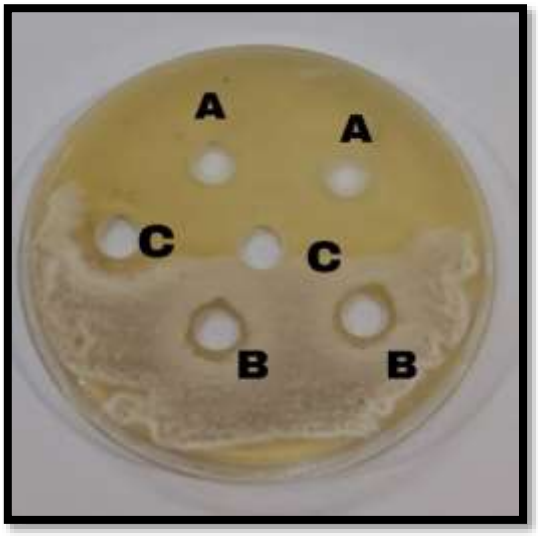

Fig 13: A P.lanosocoeruleum. B) p.chrysogenum. C) p.expansum.

P.lanosocoeruleum is one of the most important fungi that produce antifungal griseofulvin after P.griseofulvum fungus (Valente et al., 2020). There is a study that investigated the secondarymetabolites of a group of Penicillium species (P. griseofulvum, P. coprophilum, p. persicinum, P. chrysogenum and P. lanosocoeruleum). Analysis of compounds of secondary metabolism based on HPLC and UV-VIS, the results showed that both species of Penicillium (P. griseofulvum P. lanosocoeruleum) produced griseofulvin (Wang et al., 2004).

CONCLUSIONS AND RECOMMENDATIONS

1 P.lanosocoeruleum fungus was isolated for the first time from wheat grains

2 Recording a new local isolate of P.lanosocoeruleum in the NCBI that can produce the antifungal griseofulvin.

3 Paying attention to local fungal isolates and comparing them with global isolates of fungi in terms of their production of antibiotics.

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