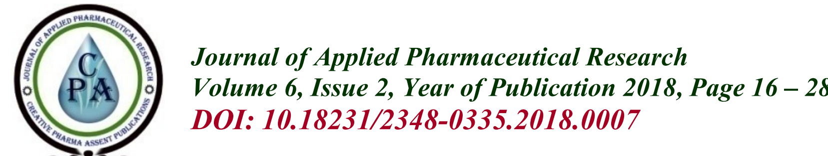

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MYCOTIC LOADS' DETERMINATION OF NON-STERILE PHARMACEUTICALS IN LAGOS STATE AND 16S rDNA IDENTIFICATION OF THE FUNGAL ISOLATES

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ABSTRACT
Pharmaceuticals are medicinal products used in the prevention, treatment, and diagnosis of diseases. As such, the presence of microorganisms' especially fungal toxins can reduce or eliminate the product's therapeutic activity and constitute a potential danger to patient health. This study attempts to determine the fungal loads of liquid preparation used as medication in Lagos State and its environs and identify the fungal isolates. 252 different types of oral liquid drugs (200syrups and 52 suspensions) which included paracetamol syrup, cough syrup and antibiotics suspension manufactured in five different pharmaceutical industries in Nigeria were methodically sampled and analyzed for fungi contamination using standardized method. The isolated fungi were identified using morphological characterization as well as 16SrDNA sequencing. 13% of the sampled syrups were found to be contaminated with fungi where thecolony forming units for paracetamol syrup, cough syrup and antibiotics suspension ranges from $2.0x10^1$ - 9.6×10^4; $1.0x10^2$- $7.5x10^4$ and $1.0x10^1$- 8.8×10^4cfu/ml respectively while87% yielded no growth.Thirteen (13) morphologically different species of fungi were identified which included *Aspergillus niger strains, A. tamari strains, A. japonicas, A. flavus, A. awamari, A. ellipticus, A. tubingensis, Meyerozyma aaribbica, Candida carpophila and Eurotiomycetes spp,* The presence of microorganisms in oral liquid samples might explain the treatment complicacy of the diseased children. Therefore, microbiological quality of such drugs is thus suggested.

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INTRODUCTION

Pharmaceutical drug or medicinal product according to United State Food and Drug Administration [1] is any chemical substance formulated or compounded as single active ingredient which may be in combination with other pharmacologically active substance intended for external or internal use in the medical diagnosis, to cure, treat or prevent diseases. The use of pharmaceutical drug to eradicate an array of diseases in human have been in existence for a very longtime and the possibility of microbial contamination such as viruses, bacteria, fungi and actinomycetes cannot be over emphasized due to impaired manufacturing condition [2]. Oral liquid preparations have been earmarked as one of the pharmaceutical formulations that possess a greater chance of microbial contamination than any other drug [3,4] and unless they are carefully and tightly closed immediately after opening, oral liquid preparations are highly liable to be contaminated by air borne bacteria and fungal spores [3]. Moreover, microorganisms such as fungi, yeast, bacteria, actinomyces and viruses have been found to play a major role in the contamination of pharmaceutical products leading to changes in the physical characteristics, such asfermentation of syrups, breaking of emulsions, thinning of creams, appearance of turbidity or deposit, and changes in colour and odour [5, 6]. The contamination of the raw materials used for the manufacturing of oral drugs with microbes may be associated with the unexplained treatment complications been experienced in patients [7, 8]. As such, the presence of contaminating microflora, especially when it is in excess of the acceptable limit of >10cfu/ml in oral drugs, result to a major threat in public health measures [9].

The presence of certain fungi especially molds in pharmaceuticals are harmful due to the production of metabolites that may be toxic to consumers and cause rapid deterioration of the product. This invariably results in biodegradation of the different components of formulations arising from the production of toxins[10,4]. Some fungi genera especially Aspergillus and Mucor which have been confirmed to produce toxins such as *A. flavus, A. parasiticus* and *A turingensis* should not be found in pharmaceutical products [6].Moreover, some of the dosage forms of oral drugs, if stored in favourable environment, can serve as substrates for fungi and other microorganisms [11,12]. Mugoyela and Mwambete [13] stated that moisture and high amount of sugar in the oral liquid drugs in particular, can support the microbial growth. Nirmala *et al*. [14] concluded that improper storage, defects in handling, presence of sweetening agents, and reconstitution methods of oral liquid drug formulations such as aqueous solutions, suspensions, syrups and emulsions used for pediatrics are at a greater risk of microbial contamination during consumption.

Drug manufacturing industry is one of the dynamically growing and expanding production sectors in Nigeria, and the quality of medicine available domestically varies significantly as they are mostly retail oriented. Adeshina *et al*. [15] felt that an anarchy situation could prevail in the marketing of drugs, due to a large number of illegal and unlicensed drug stores selling poorly manufactured pharmaceuticals. However, in Nigeria, there is paucity of report or research studies on microbial load of fungi of finished pharmaceutical products as well as the molecular studies on possible fungal isolates implicated in pharmaceutical product contamination. Therefore, intermittent examination of microbiological quality of the pharmaceutical or medicinal products especially non-sterile preparation consumed mostly by the children is highly important for their well-being. Though the knowledge of these according to Hossain, [11] and Moniruzzaman *et al*. [16] does not prove to be sufficient in predicting the safety of the sold oral liquid drugs, but contribute significantly in the area of prevention.

MATERIALS & METHODS

Sampling methodology

Paracetamol syrup, cough syrup and antibiotics were sampled from five different pharmaceutical industries; E, F, M, S and V located in Lagos State. Samples were collected from pharmaceutical shops and directly from the production companies. Drug collection was done for two years which was limited to products manufactured within one year as shown by manufacturers. The pharmaceutical products were collected in sterile Ziploc bags from one location to the other. All the Ziploc bags used for sampling were methodologically labelled with necessary information concerning the product. Information such as date of manufacture, expiry date and batch number were recorded and transported to the Laboratory under aseptic condition in order to minimize environmental exposure. All the samples collected have NAFDAC registration numbers clearly written on the packs and bottle labels.

Culture technique

The total viable fungiwere estimated using serial dilutions.Pour plate method was used from the dilution 10^{-1}, 10^{-2}, $10^{-3}$and $10^{-4}$of each sample.1ml of dilution was aseptically transferred to disposable sterile petri-dishes (15cm diameter at a depth of 4.0mm). Potato Dextrose Agar (PDA) (Oxoid) (supplemented with streptomycin) was used for culturing. The agar was sterilised by autoclaving at 121˚C for 15 minutes and allowed to cool to approximately 45°C before pouringtwenty millilitres (20 ml) of the liquefied agarinto each petri-dish. All the plates were incubated at 30°C for 4 – 7 days. Moreover, plating of each sample was done in duplicate and the average values obtained were multiplied by the dilution factors to get the total colony forming unit per millilitre (cfu/ml) count.For those plates that showed growth, re-culturing was carried out three months after sampling and a month before expiration of the product [17].

Identification of the Fungal Isolates

Morphological, biochemical and molecular methods were used for the identification of the isolates. The plates were examined for the presence of noticeable growth and oncethis is observed, the texture, pigmentation and topography of each specific type of colony was noted for proper and accurate identification. A little portion of the growth colony was teased with an inoculating needle and mount on the slide with a drop of Lactophenol blue, covered with a cover slip. The preparation was examined under a light microscope with an attached camera (Motic Mc digital coloured camera) connected to a computer for the microscopic photography of the fungi. The essence of this was to observe the exact arrangement of the conidiophores and the way the spores are produced. The identities of these fungi isolates were certified using cultural as well as morphological methodologies by comparing the isolates with confirmed representatives of different fungalspecies in relevant texts before molecular identificationfor proper confirmation [18, 19].

DNA Extraction using modified CTAB protocol

DNA extraction was done according to the modified method of Umesha *et al.*[20]. Ten millilitre (10ml) of isolation buffer (10x CTAB) containing 80µl of mercaptoethanol in 50ml blue cap tube was pre-heated in 65°C water bath. One gramme (1g) of freshly scrapped pure culture plates of each fungal isolate was added to the preheated isolation buffer in each tube. The mixture was then incubated at 65°C for 15 minutes. Ten millilitres (10ml) of SEVAG (24:1 chloroform: Isoamyl alcohol), then mixed gently but thoroughly.

The cap of the tubes containing the mixture was then opened to release gas, which was then re-tightened and homogenized using an orbital shaker (100rpm) for 60minutes. After rocking, the tubes were spinned at 4000rpm and 25°C for 20minutes. The mixture gave a clear and colourless aqueous solution at the top containing the DNA, which was then removed into another set of tubes with the aid of a plastic transfer pipette. Two third volume of isopropanol was added to the aqueous solution. The mixture was then mixed gently and stored in a freezer at -20°C for 24hours in order for the inherent DNA to precipitate. The mixture was then spinned in a centrifuge at 3000rpm for 5minutes, followed by the addition of 3ml of 70% ethanol to dislodge the pellet and facilitate washing. The mixture was spinned again at 3000rpm for 5minutes, after which the liquid was discarded and the alcohol was allowed to evaporate by leaving the tubes open on its side. The DNA was re-suspended in 1.5ml of water and stored at (-20°C). The samples were run on 1% Agarose Gel Electrophoresis to verify the presence of DNA in the samples, prior to PCR and DNA sequencing.

DNA Amplification and 16SrDNA Sequence Determination of the fungal isolates

Polymerase Chain Reaction (PCR) assay was performed using the method stated byGonzalez-Mendoza et al. [21]. PCR amplification reaction was carried out using ITS1 F (TCCGTAGGTGAACCTGCGG) and ITS4 R (TCCTCCGCTTATTGATATGC) primers. Amplification was done in 0.2-ml tube and reaction mixture containing 2.5 µL of 80–100 ng of genomic DNA, 1 µL of 20 pmol of each primer, and 20 µL of Dream Taq Green PCR master mix (Containing: 0.25 mM each dNTP, 2 mM $MgCl_2$ and Taq DNA polymerase).

The PCR was carried out in a master gradient thermal cycler (LABNET, NJ, USA). The following conditions were used for PCR: initial denaturation was done at 94 °C for 5 min; 30 cycles of denaturation for 1 min at 94 °C, annealing was performed at 52 °C for 1 min, initial extension was done at 72 °C for 1 min while final extension was carried out at 72 °C for 10 min, followed by cooling at 4 °C until the samples were recovered. The amplified DNA was confirmed through gel

electrophoresis using 1 % agarose gel. The generated sequence data from the DNA extraction were sequenced by Inqaba Biotechnology, South Africa. The 16SrDNA gene sequences obtained were compared with the NCBI database [22].

RESULTS & DISCUSSIONS

A total of two hundred and fifty-two (252) samples were collected, out of which thirty three samples (33, 13%) showed growth while two hundred and twenty samples (220, 87%) did not show any noticeable growth on incubation (Fig. 1). The colony forming units for Paracetamol syrup, cough syrup and antibiotics suspension ranged from 2.0×10^{1}-9.6×10^{4}; 1.0×10^{2}-7.5×10^{4} and 1.0×10^{1}-8.8×10^{4}cfu/ml respectively (Table 1a, 1b &1c). Paracetamol syrup from Pharmaceutical companies VM, VI and EI produced the highest contaminations while FM, MP and SM did not showed any noticeable growth at all.

Cough syrups from pharmaceutical company VI produced the highest growth on incubation while FM and SMshowed no growth. Antibiotics suspensions from production companies EM and VI gave the highest contaminations while that of FM and SMshowed no appreciable growth (Fig. 1).The thirty three (33) samples produced thirteen (13) morphologically different species of fungiwhich were identified using cultural, morphological and molecular methods.

Figure 2 shows the Electrophorogram of the extracted DNA of the thirteen isolates using modified CTAB protocol. BLAST (Basic Local Alignment Search Tool) analyses of the 16S rDNA genes nucleotide sequences of the fungal strains showed a range of 95-99% similarities(with their respective accession number)to:*A. niger* strains A(KJ881377.1), *A. niger* strains B(KF305758.1), *A. niger* strains C(KF305742.1); *A. tamari* strain A(KP784375.1) *and A. tamari* strain B(KF221089.1); *A.japonicas*(KC128815.1),*A. flavus*(KF221065.1), *A. awamari*(KF154413.1), *A. ellipticus* (EU821329.1)*A. tubingensis* (KC020122.1), *Meyerozyma aaribbica* (KP674752.1), *Candida carpophila* (KP131683.1) and *Eurotiomycetes* sp. (JX174154.1)(Fig. 3and 4). Furthermore, out of the four fungal genera isolated, the genus Aspergillus gave the highest percentage occurrence of 76% while the genera Candida, Meyerozyma and Eurotiomycetes gave 8% respectively (Fig. 5).

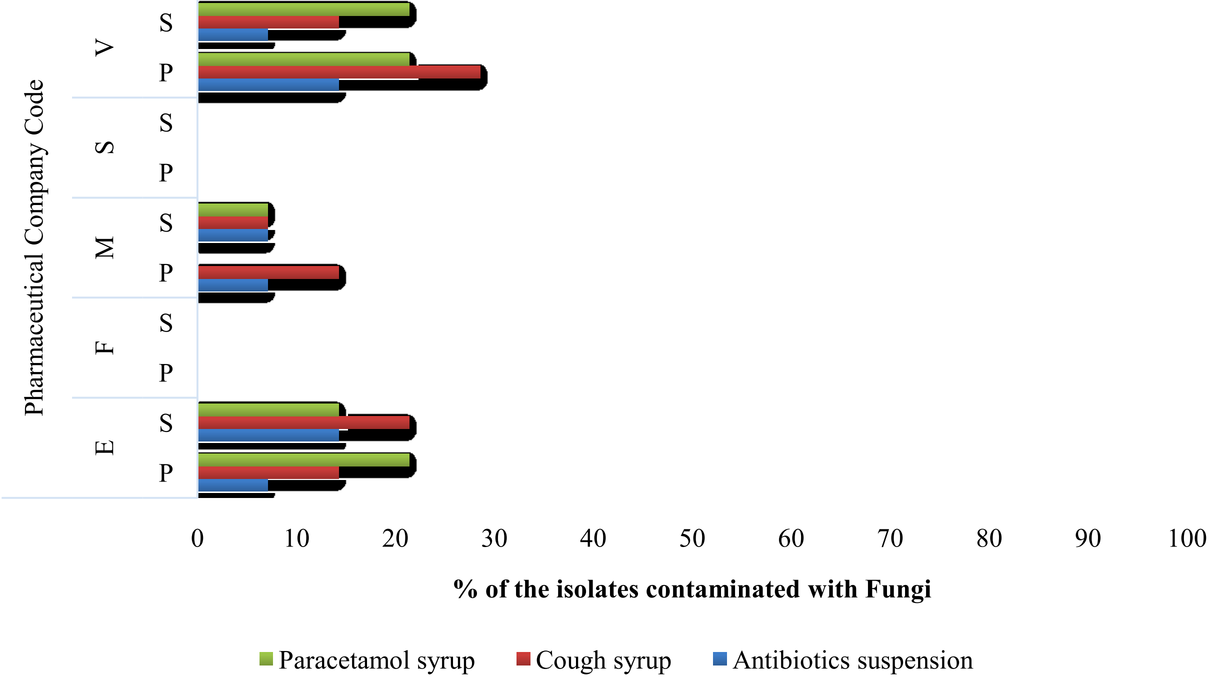


Fig. 1: Percentage occurrence of fungi isolates from different Pharmaceutical companies

Key: E, F, M, S and V indicate pharmaceutical Companies codes. Sub S and P indicate samples collected from pharmaceutical shops and production companies respectively.

Table 1a: Fungi Load of the Paracetamol Syrups Sampled

Sampling code	Sampling Location	TFCIMAS (cfu/ml)	TFC3M (cfu/ml)	TFCBE (cfu/ml)
EP(P)1	DF	3.7×10^2	6.7×10^3	9.6×10^4
EP(P)4	DF	4.0×10^2	7.1×10^2	6.5×10^4
ES(P)1	PS	4.0×10^2	4.7×10^3	6.8×10^4
ES(P)5	PS	$3.0x10^2$	$3.7x10^4$	$5.7x10^4$
MP(P)2	PS	$5.0x10^1$	$5.7x10^4$	$7.5x10^4$
MP(P)3	DF	$4.0x10^1$	$4.7x10^2$	$5.0x10^4$
MS(P)2	PS	$2.5x10^2$	$4.0x10^3$	$6.7x10^4$
VP(P)1	DF	4.0×10^2	6.0×10^4	7.6×10^4
VP(P)12	DF	$2.0x10^1$	$3.0x10^2$	$4.7x10^4$
VP(P)13	PS	$2.0x10^2$	$3.7x10^4$	$4.0x10^4$
VS(P)11	PS	4.0×10^2	4.4×10^3	5.1×10^3
VS(P)3	PS	4.4×10^2	3.7×10^4	5.7×10^4

Table 1b: Fungi Load of the Cough Syrups Sampled

Sampling code	Sampling Location	TFCIMAS (cfu/ml)	TFC3M (cfu/ml)	TFCBE (cfu/ml)
EP(C)5	PS	$1.0x10^2$	$2.5x10^4$	$5.0x10^4$
EP(C)9	DF	$2.5x10^2$	$3.0x10^4$	$5.5x10^4$
ES(C)12	PS	4.4×10^2	5.0×10^3	5.0×10^4
ES(C)3	PS	4.0×10^2	5.4×10^3	5.4×10^3
ES(C)4	DF	$3.0x10^1$	$5.0x10^4$	$6.6x10^4$
MS(C)13	DF	4.4×10^1	4.0×10^4	6.0×10^4
VP(C)1	PS	$2.2x10^2$	$4.4x10^4$	$5.4x10^4$
VP(C)6	PS	$3.5x10^2$	$4.2x10^4$	$5.5x10^4$
VP(C)8	DF	4.7×10^2	5.4×10^3	6.6×10^4
VS(C)2	PS	$3.2x10^1$	$5.5x10^3$	$6.0x10^4$
VS(C)3	PS	$4.0x10^2$	$5.0x10^3$	$7.5x10^4$

Table 1c: Fungi Load of the Antibiotics Suspension Sampled

Sampling code	Sampling Location	TFCIMAS (cfu/ml)	TFC3M (cfu/ml)	TFCBE (cfu/ml)
EP(A)10	PS	$2.0x10^2$	$3.7x10^3$	$6.7x10^4$
EP(A)2	PS	$1.0x10^1$	$3.0x10^3$	$6.7x10^4$
ES(A)4	PS	$3.0x10^2$	$4.4x10^3$	$4.0x10^4$
ES(A)5	DF	$1.0x10^2$	$2.5x10^3$	$4.0x10^4$
MP(A)13	DF	3.7×10^2	6.4×10^3	8.8×10^4
MS(A)3	PS	$2.0x10^2$	$3.5x10^3$	$6.7x10^4$
VP(A)3	DF	3.7×10^2	6.3×10^3	7.8×10^4
VP(A)4	DF	4.0×10^2	7.5×10^3	8.5×10^4
VS(A)2	DF	$1.0x10^2$	$4.0x10^3$	$7.0x10^4$
VS(A)7	PS	$3.7x10^1$	$4.4x10^3$	$6.0x10^4$

Key: *TFCIMAS=Total fungi count immediately after sampling; TFC3M= Total fungi count 3 months after sampling; TFCBE= Total fungi count a month before expiration; DF=Directly from Factory; PS= Pharmaceutical shops; cfu /ml= colony forming unit per ml*

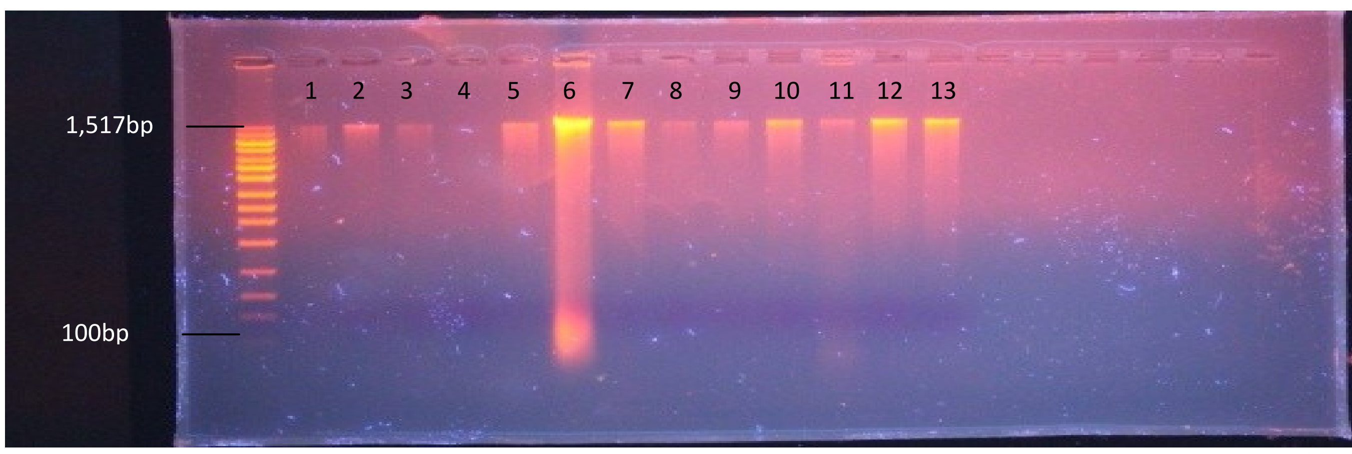
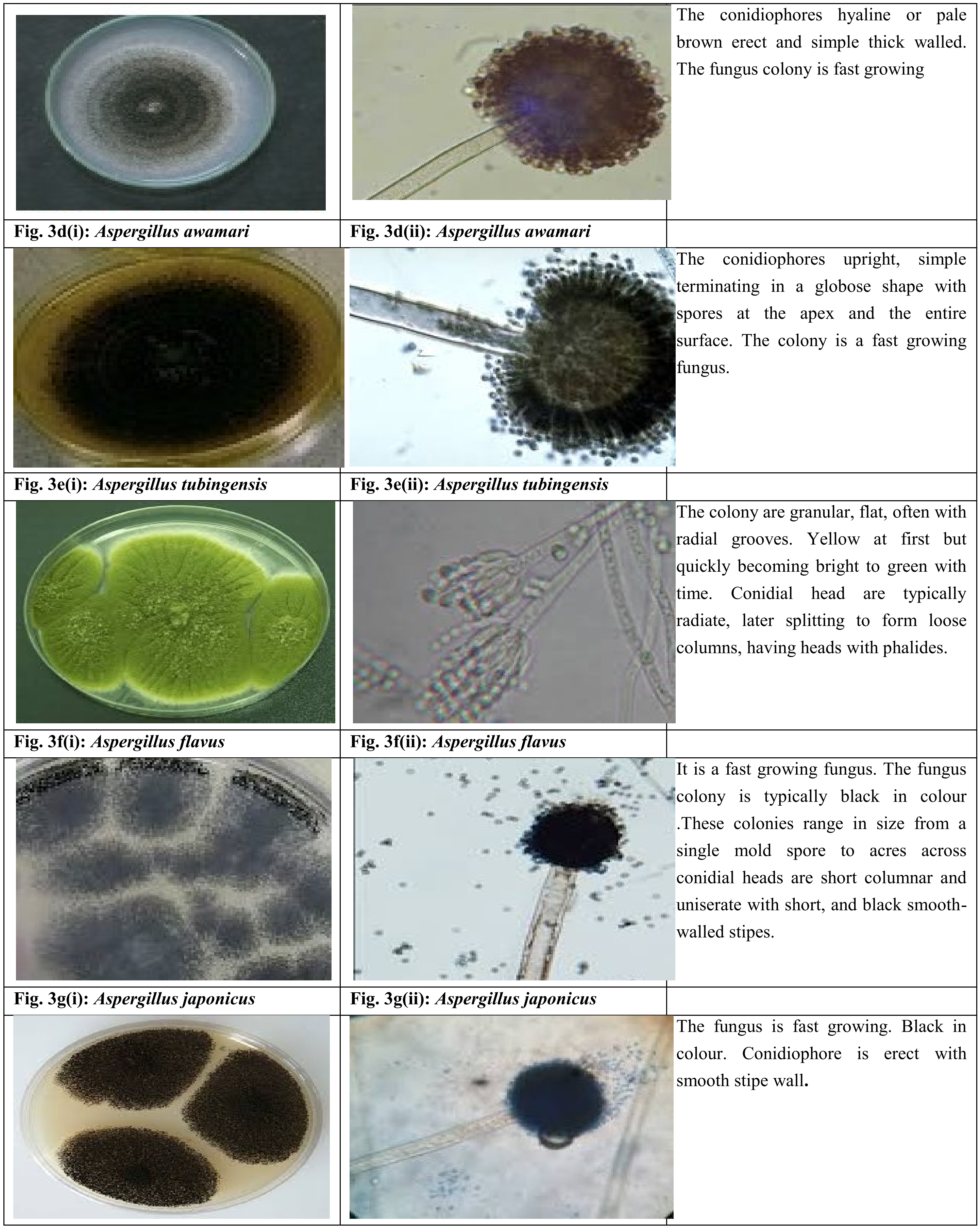


Fig. 2: Electrophorogram of extracted DNA samples for the 13 morphologically different fungal isolates using modified CTAB protocol. EP(P)1, MP(P)2, ES(P)1, VP(P)12, VP(C)1, VP(C)6, VS(C)2, VS(C)3, EP(A)2, ES(A)5, MP(A)13, VS(A)7, MS(A)3 respectively

Culture plates of the isolates	Photomicrograph of the isolates	Characteristics of colonies
		Colony is a fast growing one, black in colour. Conidial head is short. Conidiophores is erect, simple with thick wall
Fig. 3a(i): ***Aspergillus niger strain A***	**Fig. 3a(ii):** ***Aspergillus niger*** **strain A**	
		The colony is a fast growing one formed in groups, black in colour. Conidial head are short. Conidiophores is erect, simple with thick walled.
Fig. 3b(i): ***Aspergillus niger*** **strain B**	**Fig. 3b(ii):** ***Aspergillus niger*** **strain B**	
		The conidial head are compact with phalides borne directly on vesicle. This fungus colony is typically black in colour. Conidial heads are long columnar and biserated with long and thin blue smooth-walled stipes.
Fig. 3c(i): ***Aspergillus niger strain C***	**Fig. 3c(ii):** ***Aspergillus nigerstrain C***	



		The conidiophores hyaline or pale brown erect and simple thick walled. The fungus colony is fast growing
Fig. 3d(i): ***Aspergillus awamari***	**Fig. 3d(ii):** ***Aspergillus awamari***	
		The conidiophores upright, simple terminating in a globose shape with spores at the apex and the entire surface. The colony is a fast growing fungus.
Fig. 3e(i): ***Aspergillus tubingensis***	**Fig. 3e(ii):** ***Aspergillus tubingensis***	
		The colony are granular, flat, often with radial grooves. Yellow at first but quickly becoming bright to green with time. Conidial head are typically radiate, later splitting to form loose columns, having heads with phalides.
Fig. 3f(i): ***Aspergillus flavus***	**Fig. 3f(ii):** ***Aspergillus flavus***	
		It is a fast growing fungus. The fungus colony is typically black in colour .These colonies range in size from a single mold spore to acres across conidial heads are short columnar and uniserate with short, and black smooth-walled stipes.
Fig. 3g(i): ***Aspergillus japonicus***	**Fig. 3g(ii):** ***Aspergillus japonicus***	
		The fungus is fast growing. Black in colour. Conidiophore is erect with smooth stipe wall.

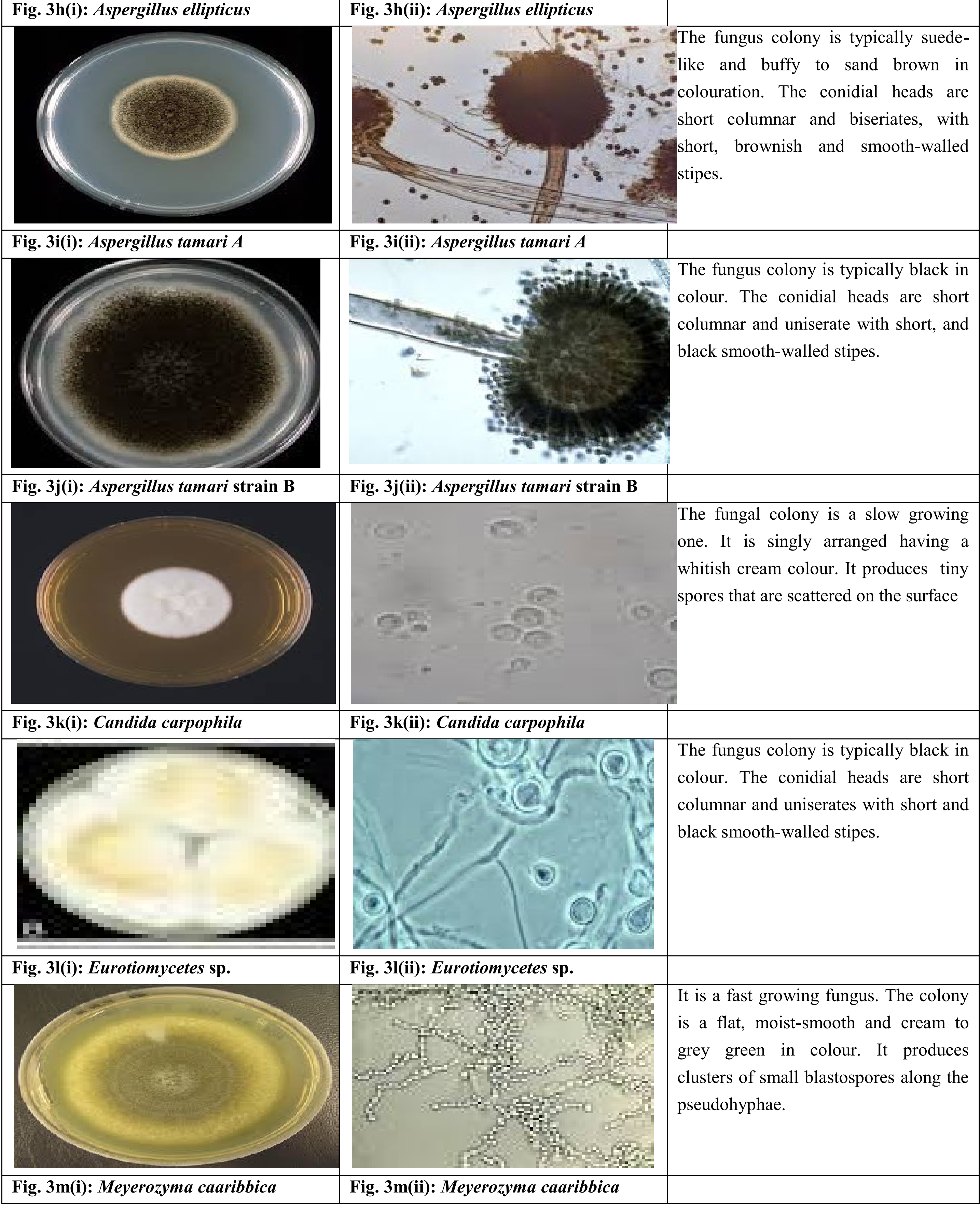


Fig. 3h(i): ***Aspergillus ellipticus***	**Fig. 3h(ii):** ***Aspergillus ellipticus***	
		The fungus colony is typically suede-like and buffy to sand brown in colouration. The conidial heads are short columnar and biseriates, with short, brownish and smooth-walled stipes.
Fig. 3i(i): ***Aspergillus tamari A***	**Fig. 3i(ii):** ***Aspergillus tamari A***	
		The fungus colony is typically black in colour. The conidial heads are short columnar and uniserate with short, and black smooth-walled stipes.
Fig. 3j(i): ***Aspergillus tamari*** **strain B**	**Fig. 3j(ii):** ***Aspergillus tamari*** **strain B**	
		The fungal colony is a slow growing one. It is singly arranged having a whitish cream colour. It produces tiny spores that are scattered on the surface
Fig. 3k(i): ***Candida carpophila***	**Fig. 3k(ii):** ***Candida carpophila***	
		The fungus colony is typically black in colour. The conidial heads are short columnar and uniserates with short and black smooth-walled stipes.
Fig. 3l(i): ***Eurotiomycetes*** **sp.**	**Fig. 3l(ii):** ***Eurotiomycetes*** **sp.**	
		It is a fast growing fungus. The colony is a flat, moist-smooth and cream to grey green in colour. It produces clusters of small blastospores along the pseudohyphae.
Fig. 3m(i): ***Meyerozyma caaribbica***	**Fig. 3m(ii):** ***Meyerozyma caaribbica***	

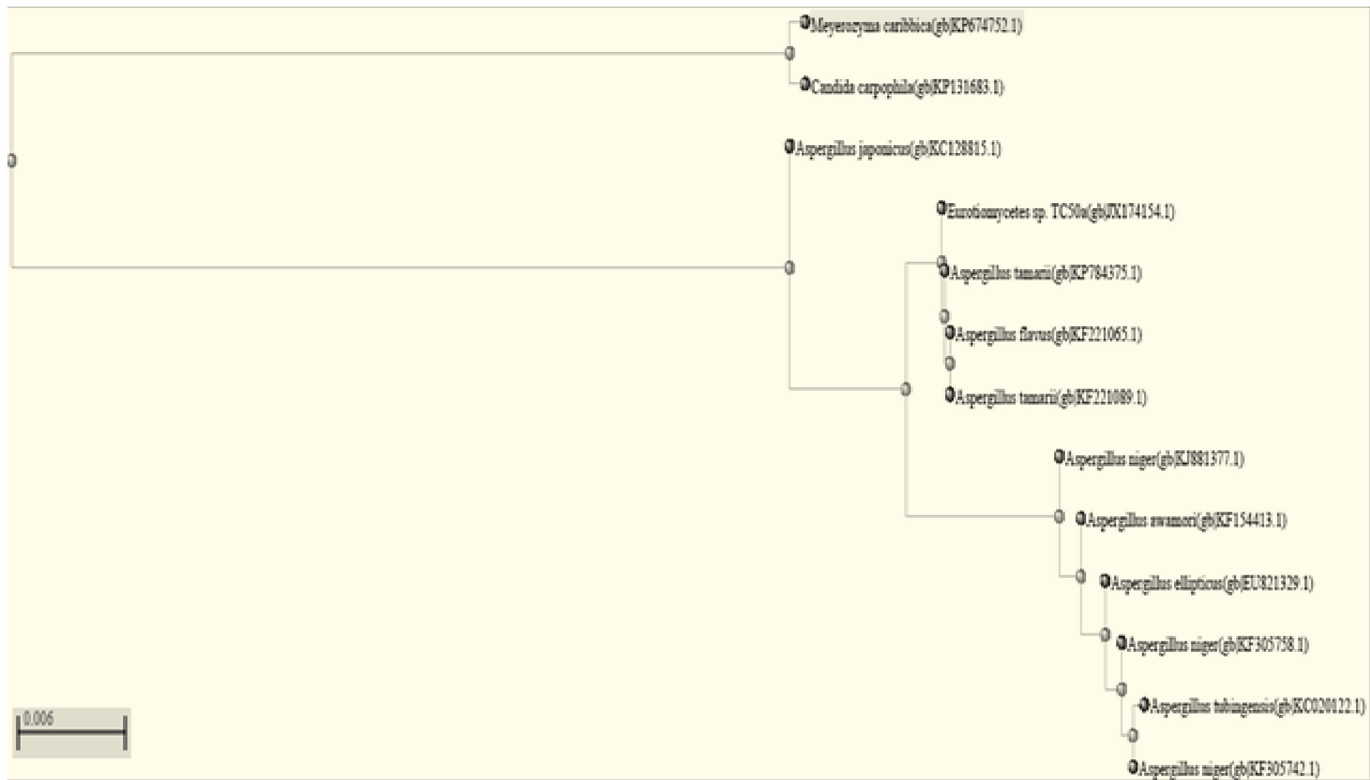


Fig. 4. Molecular Phylogenetic analysis by Maximum Likelihood method of the isolates

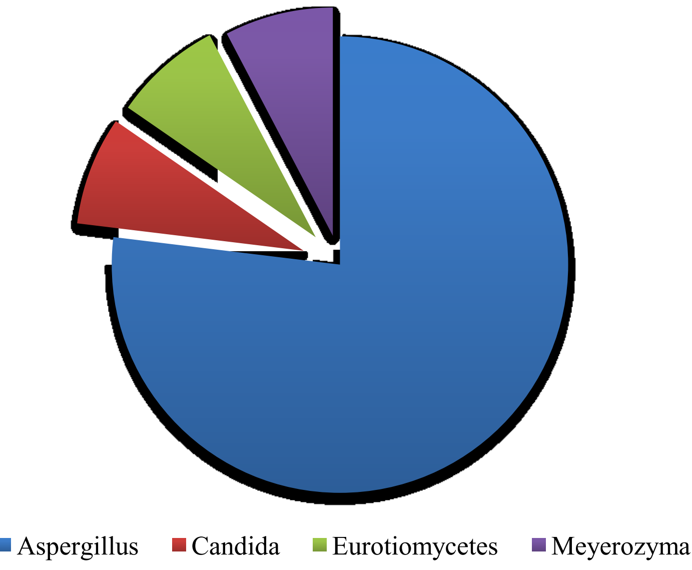


Fig. 5: Percentage occurrence of different fungi genera isolated. With Aspergillus having 76.9% occurrence while Candida, Eurotiomycetes and Meyerozyma have 7.7% respectively

Non-sterile liquid drugsare characterized by their viscous consistency and sweet taste with active medicaments. Theses pharmaceutical syrups constitute the most convenient dosage for babies, children and adult [23]. The deleterious effect of ingesting microbiologically contaminated non-sterile pharmaceutical is more significant as the patients involved are already diseased. As such, it is very important to carry out all round examination on the potency of these drugs before useas medication especially in babies and little children.Mahboob *et al*. [24] and Emejuru *et al*. [25],observed that the addition of high amount of sweetening agent as well as preservatives could enhance mycotic growth in pharmaceutical products.The World Health Organization (W.H.O), European Pharmacopoeia (EP),United States Pharmacopoeia (USP), and other recognized regulatory agencieshave guidelines to ascertain the quality assurance of pharmaceutical products [26]. National Agency

for Food and Drug Administration and Control (NAFDAC), the body assigned with the responsibility of monitoring the quality assurance of consumable food items including pharmaceutical products in Nigeria, published a handbook in 2000 [27], where the standard microbiological specifications for the certification of syrup oral suspensions are well outlined. Thus, it was typically stated that total viable fungal counts must not exceed $1.0x10^2$CFU/ml which is far below the observed mycotic loads in some of the analyzed samples. The European Directorate for the Quality of Medicines & HealthCare [28] as well as United State Pharmacopoeia [9] standards for the Total Yeast and Mould Count (TYMC) for the assessment of the microbiological quality of non-sterile pharmaceutical is 10^2cfu/ml or cfu/g which is in line with NAFDAC standard [29, 30]. Furthermore, high contaminations of the sampled antibiotics from our study were not unexpected as the drug is formulated to inhibit the growth of bacterial infections and not necessarily fungi. Emejuru *et a l*[25] reported a fungal growth in 16 out of the 24 selected oral liquid pharmaceuticals in Southeastern geopolitical zone of Nigeria. Gad *et al*.[6] observeda range of 10^1 - 10^3 cfu/ml contamination of the paediatrics drugs he sampled from Egyptian market with very high prevalence of major fungi genera.Moreover, the observed high fungal loadsfrom this work were similar to the findingsof Al-Kaf*et al*. [31] on his research on microbial and physicochemical assays of paracetamol in different brands of analgesic and antipyretic syrups sold in Sana'a City of Yemen. However, Khanom *et al*. [32] who carried out microbiological analysis of liquid oral drugs available in Bangladesh, contradicted this finding where his total fungal count of the oral suspensions were found to be in the range of 10^1 to 10^2.Fatema *et al*. [33]report on the assessment of microbiological quality of the pediatric oral liquid drugs in Dhaka city, Bangladesh, was similar to our finding. He observed that fungi presence were not significant during the initial assessment of sampling, however, most of the samples were found to support fungal growth after 21 days with the CFU ranging from 10^1 – 10^3which is similar to our finding.

As observed from this study, Aspergillus accounts for about 70% of the total fungal isolates (Fig. 4). The genus Aspergillus is one of the most well researched fungi genera and more than 180 officially recognized species have been identified which included 20 human pathogens as well as beneficial strains used in the production of foodstuffs and important industrial enzymes [34]. In particular, the genera Candida, Rhodotorula, Penicillium,Aspergillus, Rhizopus, Alternaria, and Mucor have been reported to be a great contaminant of pharmaceutical products especially syrup, suspension and cosmetics materials [35]. Moreover, Elmorsy and Hafez, [36] observed that fungal isolates from cosmetic preparation were 30% *Aspergillus* spp., 30%*Rhizopus* spp., 18% *Candida* spp., 15% *Trichoderma* spp.. and 7% *Penicillium* spp. Timberlake and Marshall [37] described Aspergilli as ubiquitous group of filamentous fungi which have been in existence for over 200 million years witha great impact onhuman health and society. Atkinson and Brojer, [38] described the ubiquitous nature of Aspergillus has been due to their saprophytic feeding habit as well as their ability to grow in a wide range of temperatures. Mugoyela et al. [13] noted that the presence of potentially pathogenic opportunistic microbes especially Aspergillus in pharmaceutical drugs cannot be overemphasized because of their significancein the deterioration of the health status of patients particularly infants with an immature immune system and immune-compromised individuals. Furthermore, the species of Aspergillus frequently isolated include *A. fumigatus, A. flavus, A. nidulans, A. niger* and *A. terreus*[38]. Aspergillus has beenidentified as the causative agent of Aspergillosis infection which is a non-contagious disease that affect humans, mammals and mostly wild or domestic birds [39].Perfect et al.[40] categorized the various Aspergillus infections as: invasive aspergillosis, chronic necrotizing aspergillosis, allergic broncho-pulmonary aspergillosis and fungus ball or aspergilloma. Wattier and Ramirez-Avila, [41]identified *A. fumigatus* and *A. flavus* as one of the causative agent of Invasive Aspergillosis (IA)which happened to be one of the most common and serious infectious complications occurring in immune-compromised patients.

The presence of moulds in any pharmaceutical products especially consumables like syrup and suspension should be considered as harmful since they produce metabolites that may be toxic to consumers. Gad *et al*. [6],Wu *et al*. [42] and Ratajczak *et al*. [35] reported that moulds and some species of Aspergillus such as *A. flavus and A. parasiticus* can produce mycotoxins which can be carcinogenic and mutagenic in nature. Furthermore, they emphasized that these mycotoxins can cause allergies, acute and chronic poisoning, liver damage, and diseases of the respiratory and digestive systems. Raw materials, ingredients, water supply, unhygienic environmental condition and lack of aseptic handling has been identified as

major factors that can significantly contribute to high microbial growth in pharmaceutical products [43, 44]. In developing countries like Nigeria, the possibility of disease incidence is very high due to poor hygienic practices, consumption of contaminated food and water, and unstable environmental condition due to pollution [45]. These give room for the infectious and virulent nature of opportunistic pathogens when resistance mechanisms are impaired either by severe underlying disease or by use of immunosuppressive drugs [46].

CONCLUSION

The finding from this research shows that fungal loads are present in some of pharmaceutical products sampled in the quantities that are not acceptable according to the standard provided by local (Nigeria) and international drug regulatory bodies. Some of the isolated fungi may not be pathogenic but their presence can interfere with the functionality of the drug through biodegradation of the active component of such syrup. Fungi are also known to produce toxins which can be carcinogenic in nature, as such their presence in any pediatrics' drug should not be taken lightly. Usually most patients are potentially immune compromised when they are taking drugs which accelerate the chances of diseases acquired by opportunistic pathogens. Therefore, the presence of any microorganism should be considered undesirable for all drugs. The compliance sector in Nigeria's Pharmaceuticals should strictly deal with microbial stringency within the manufacturing which should include packaging, distribution and storage of pharmaceutical products, added preservatives, sweeteners, and production environment. Furthermore, microbial loads of non-sterile pharmaceuticals can be reduced to barest minimum by preventing the possibility of spoilage organisms and by adding well-researched antimicrobial agents orchemical preservatives. Unhygienic environmental condition coupled with improperhandling of raw materials, ingredients and products must also be checked. Finally, good manufacturing practice is non-negotiable and must be strictly adhere to at all times if the microbial contamination is to be totally eradicated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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