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Analysis of Bacteria, Helminthseggs and Heavy Metals in Tropical Mushrooms Sold in Selected Markets in Benin City, Nigeria

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Abstract: A study on bacterial, helminth parasites and heavy metal contamination was carried out on two edible mushrooms namely; the Sclerotia of *Pleurotustuberregium*(Fr.) Singer and the fruit bodies of *Lentinussquarrosulus*(Mont.) Singer sold at Ikpobahill and Ekiuwa markets in Benin City. Two species of helminth parasites*viz. Toxocaracanis* and Hookwormeggswere identified using test tube flotation method. The prevalence rates were 28.57, 4.76 & and 33.33% in Sclerotia and *Lentinussquarrosulus* for Ikpoba hill market and 40.00 & and 33.33 % in Sclerotia and *Lentinussquarrosulus* for Ekiuwa market, respectively. Three bacteria species, i.e. *Streptococcus* spp*, Bacillus* sppand *Lactobacillus* sppwere isolated. Out of the eight heavy metals analyzed for, only Zinc (Zn) and Iron (Fe) were indentified in this study. Zn had concentration values of 30mg/kg and 72mg/kg in Sclerotia and *Lentinussquarrosulus* respectively while Fe also had concentration values of 406 mg/kg and 478 mg/kg in Sclerotia and *Lentinussquarrosulus*, respectively.The presence of helminth parasites may be due to contamination with faecal matter from dogs and humans. Contamination by bacteria may have arisen from exposure and poor handling during harvest and in market places. The very high levels obtained for Zn and Fe were higher than WHO permissible limits and may have resulted from the mushroom ability to hyper accumulate heavy metals from soil.

Key words: Mushrooms · Bacteria · Helminth eggs · Heavy Metals

INTRODUCTION

In Nigeria, many people in both urban and rural areas are familiar with mushroom-forming fungi growing around their houses and farms, some of which they exploit for food and medicines [1]. They are also considered as rich food because they contain comparatively high level of protein, sugars, glycogen, lipids, vitamins, amino acids and crude fibers. Furthermore, some of these mushrooms are being used in the treatment of various human diseases and ailments [2,3].

In the southern part of Nigeria, edible higher fungi are considered as luxury food and important table delicacy especially among rural dwellers. This is because people living in the villages are more exposed to their natural vegetation (tropical rain forest) which supports the growth and fructification of mushrooms [4]. The collected edible species of mushrooms are usually sorted, cooked or sold in the local markets or may be hawked along local and major roads to attract the attention of buyers [4].

Mushroom growing processes are always been intruded and spoilt by bacteria, fungi and parasites. This normally leads to devaluation of quality and under-pricing of mushrooms by consumers [5].

Helminths are multicelluar eukaryotic animals that generally possessdigestive, circulatory, nervous excretory and reproductive system. Some are free living in soil and water while others are parasitic and are diagnosed diagonizedby examination of eggs and larva. They infect more than one-third of the world population [6]. Helminth infections differ from bacterial or protozoan infections because the worms do not usually decrease in number in the host. Symptoms are usually due to mechanical damage, eating host tissues, or competing for

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vitamins. When helminth eggs are consumed, they cause several zoonotic diseases such as visceral larva migrans, diarrhoea, nausea, vomiting, headache, weight loss, malnutrition and abdominal pain [6].

Presently, there is a dearth of information on bacterial and helminth parasite contaminants of wild edible mushrooms sold in Nigerian markets. Since mushrooms are bioaccumulators of heavy metals, it is also necessary to assess the level of heavy metals and to report possible contaminations that are potential health hazards.

The main objectives of this study was to isolate and identify bacteria, helminth parasites and heavy metal concentrations in Sclerotia of *Pleurotustuberregium* and fruiting bodies of *Lentinussquarrosulus* sold atIkpoba Hill and Ekiuwa markets in Benin City, Nigeria.

MATERIALS AND METHODS

Study Area: Benin City is the Capital of Edo State and lies between latitude 6°17' and 6°26' N and longitude 5°35' and 5°41' E. Annual rainfall ranges between 1850-2445mm and temperature 30-36.5°C. The soil is a compact literate which becomes flooded after heavy rainfall. In the peak of raining season (April - October), relative humidity is about 75% maximum, but drops to about 50 - 55% during the dry season (November - March). The City of Benin is about 80m above sea level and is located within the moist rain forest zone of Nigeria. The climate is tropical (Give reference).

Sample Collection: The two species of mushroom samples (Sclerotia of *Pleurotustuberregium* and fruiting bodies of *Lentinussquarrosulus*)used for this study were purchased randomly from two local markets namely*viz.*Ekiuwa and Ikpoba Hill markets from August to December 2010. These markets are located in Oredo and Ikpoba-OkhaLocal Government Areas in Benin City. The mushroom samples were placed in a clean, labeled polyethylene bags and were taken to the Laboratory for study.

Isolation of Helminth Parasites: Ten grams of each mushroom sample was weighed into a clean bowl containing 200ml tap water for the removal of parasitic ova, larva or cysts and allowed to stand for 5minutes after washing. The suspension we strained through a sterile sieve to remove undesirable materials.The filtratewas centrifuged at 5000rpm for 5minutes [7]. The supernatant was discarded and the sediment obtained was re-suspended in sufficient brine flotation fluid (NaCl solution). The flotation fluid was poured to fill the test tube up to brim and a cover slip was super-imposed on it. The cover slip was carefully removed and examined under the microscope using x10 and x40 objectives [8]. Eggs of helminth parasites observed were identified using the method of Thienpoint [9] and Soulsby [10].

Isolation of Bacteria: Ten grams of each mushroom sample was weighed and pulverized using a clean Laboratory mortar and pestle. The grounded sample was then transferred into 200ml clean beaker containing 100ml sterile distilled water and a sterile glass rod was used to stir the solution and 1.0cm^3 of it was transferred using a clear bulb pipette into a sterile test tube containing 9.0cm^3 of sterile distilled water. This process was repeated for other sterile test tube so that at the end dilution of 10^{-1}, 10^{-2}, 10^{-4}, 10^{-5} and 10^{-6} folds were obtained. Each dilution was then plated out by spread plate method on nutrient agar (NA)(Oxoid). A standard plate count was carried out on colonies obtained on nutrient agar using Electric Gallenkamp Model Colony Counter and the unit expressed in cfu/ml. All the bacterial isolates were streaked separately onto nutrient agar plates to obtain pure cultures and determine their colonial morphology, respectively. The plates were incubated at 37°C for 24 hours. The bacteria isolated were subjected to gram's reaction, catalase, oxidase, coagulase and other standard biochemical tests based on the methods of [11]. Bacterial identification and characterization were carried out by comparing the results obtained with the characterization definitions of Bergey's Manual of Systemic determinative Bacteriology (2001).

Heavy Metal Analysis: One gram each of Fungi(Sclerotia of *Pleurotus tuberregium* and fruiting body of *Lentinus squarrosulus*) were weighed in digestive flask and treated with 5ml of concentrated nitric acid (HNO_3). A blank sample was prepared applying 5ml of HNO_3 into empty digestion flask [12]. The flask was treated for two hours on an electric hot plate (HP 220, UTEC Product Inc. Albany N.Y. USA) at 80 - 90°C and then the temperature was raised to 150°C in order to make the samples boil. Concentrated HNO_3 and 30% hydrogen peroxide (H_2O_2) were further added to the samples (3-5ml of each was added occasionally) and digestion continued until a clean solution was obtained. After cooling, the solution was

filtered with Whatman No. 42 filter paper and < 0.45µm Millipore filter paper. It was then transferred quantitatively to a 25ml volumetric flask by adding distilled water. Working standard solution of Fe, Mn. Zn, Cu, Cr, Ca, Cd, Ni and Pb were prepared from the stock standard solutions containing 1000ppm of element in 2N HNO_3 (nitric acid). Calibration and measurement of element were done on atomic absorption s

RESULTS

Two species of helminth parasites were identified from the mushroom samples bought from Ikpoba Hill market. They include *Toxocara canis* and Hookworm egg. The prevalent rates were 6(28.57%), 7(33.33%) and 1(4.76%) in Sclerotia of *Pleurotus tuberregium* and *Lentinus squarrosulus*, respectively. Only *Toxocara canis* with prevalent rates 6(40.0%) and 2(33.33%) was identified in samples from Ekiuwa market as shown in Table 1. The most prevalent helminth parasite observed in this study was *Toxocara canis*.

Total viable bacterial count and an average colony count of 2.3 and 2.6cfu/ml for Sclerotia and *Lentinus squarrosulus,* respectively was recorded in this study (Table 2). Based on cultural, morphological and standard characterization of the isolates, three bacterial species were identified. They were *Streptococcus* spp, *Bacillus* spp *and Lactobacillus* spp as shown in Table 3.

The results obtained for heavy metal analysis showed thatiron(Fe) had concentration value of 406mg/kg in Sclerotia and 478mg/kg in *Lentinus squarrosulus* while zinc (Zn) was 30mg/kg in Sclerotia and 72 mg/kg in *Lentinus squarrosulus* (Table 4).

DISCUSSION

The burden of intestinal helminthiasis has remained a public health problem worldwide. The global estimate shows that about 2 billion people harbor soil-transmitted helminthes (STH), mostly by*Ascarislumbricoides*, *Trichuristrichiura* and hookworms [13]. In this study, the prevalence of *Toxocara canis* eggs was recorded in two species of mushroom samples examined (Table 1). A report by Luis *et al*. [14] showed that the presence of this helminth parasite egg is an indication of soil contaminated with dog feces. This helminth parasite was reported as the causative agent of Visceral Larva migrans in humans. According to Camparoto *et al*. [15], the most commonly affected organs are the liver, lungs and eyes.

Toxocara canis have also been reported by Chattha *et al*. [16] as one of the most common parasites living in the intestine of domestic and stray dogs. The adult parasites produce prodigious number of eggs that are passed with the feces. Humans get infected by eating food contaminated with *Toxocaracanis*eggs such as unwashed vegetables and fruits and most people consume raw mushrooms which they eat as salads Chatthaet *al*. [16]. A report by Chio*et al*. [17] shows the possibility of fresh vegetables as agents for the transmission of protozoa cysts and helminthes eggs and larvae. The mushrooms examined in this study may have been contaminated with eggs of *Toxocara canis* and Hookworm eggs through indiscriminate defecation by stray and hunting dogs and humans and also through dumping of excreta in bushes and farmlands. These faecal materials are then transported by water during heavy rains.

Table 1: General Prevalence Rate of Helminth Eggs in all Mushrooms Sampled at Ikpoba Hill and Ekiuwa Market

Market	Mushroom	Number of sampling units examined	Sample units found infected with helminth eggs	Helminth eggs recorded	Prevalence (%)
Ikpoba Hill Market	Sclerotiaof Pleurotus tuberregium	21	6	Toxocara canis	28.57
	Lentinus squarosulus	21	7	Toxocara canis	33.33
Ekiuwa Market		21	1	Hookworm eggs	4.76
	Sclerotiaof Pleurotus tuberregium	15	6	Toxocara canis	40.0
	Lentinus squarosulus	6	2	Toxocara canis	33.33

Table 2: Total Viable Bacteria Count (TVBC) at 37°C for 24-48 hours

Mushroom	Dilution	Average Colony Count (Cfu/ml)
Sclerotia ofPleurotus tuberregium	10^{-3}	2.3
Sclerotia ofPleurotus tuberregium	10^{-3}	2.6

World Health Organization (WHO)/FAO standard limit for total viable count for food products and water is 1.0 ×10cfu/ml.

Table 3: Characteristics of Bacteria Isolates from Mushroom Samples

	Cultural						Morphological		Biochemical								
Isolate code	Size	Elevation	Shape	Surface colony	Margin	Colour	Cell arrangement	Cell type	Gram Reaction	Oxidase	Catalase	Citrate	Coagulase	Glucose	Mannitol	Lactose	Bacteria Suspected
Xa	0.8cm	flat	round	dry	rough	cream	in chains	Short rod	+	-	+	+	-	-	+	+	Bacillus spp
Xb	0.8cm	flat	Round	dry	rough	cream	In chains	Cocci	+	-	-	+	-	+	+	-	Streptococcus spp
Xc	0.9cm	flat	Round	dry	rough	cream	in chains	Short rod	+	-	-	+	-	+	+	+	Bacillus spp
Ya	0.6cm	flat	Round	dry	Rough	cream	Singly	Short rod	+	-	-	+	+	-	+	-	Lactobacillus spp
Yb	0.4cm	flat	Round	dry	Rough	cream	in chains	Cocci	+	-	-	+	-	-	+	+	Streptococcus spp
Yc	0.4cm	flat	Round	dry	Rough	cream	in chains	Cocci	+	-	-	-	+	-	+	+	Streptococcus spp

X(a,b,c) =Sclerotia of Pleurotus tuberregium andY(a,b,c) =Lentinus squarrosulus.Whereas,+ = Positive and- = Negative.

Table 4: Heavy Metal Content of Sclerotia of Pleurotus tuberregium and Lentinus squarrosulus

	Heavy metals (mg/Kg)							
Mushroom	Fe	Mn	Zn	Cu	Cr	Cd	Ni	Pb
Sclerotia of Peurotus tuberregium	406	ND	30	ND	ND	ND	ND	ND
Lentinus squarrosulus	478	ND	72	ND	ND	ND	ND	ND

ND = Not detectedWorld Health Organization (WHO) standard limit for Iron in food per day = (10-50)mg/kg.World Health Organization (WHO) standard limit for Zinc in food per day = (10-15)mg/kg.

This study also recorded the presence of Hookworm egg, Nwosu and Anya [18] noted that 88% of hookworm infestations in Southern Nigeria are due to *Necator americanus,* this is due to the ability of the eggs to withstand adverse conditions. Hookworm is prevalent in areas where there is poor hygiene, although the eggs were not readily observed in this study.Mushroom like other vegetables and fruits are widely exposed to microbial contamination through contact with soil, dust, water and by handling during harvest, transportation and marketing [19]. Therefore, they harbor a diverse range of microorganisms including plant and human pathogens. *Streptococcus* spp was observed to be predominant. The occurrence in this study may be as a result of bad habit (sneezing and coughing) by handlers of the mushroom as asserted by Adebayo-tayo *et al.* [20]. The frequency of occurrence of *Bacillus* spp in this study is shown in Table 2, agrees with the research work of Corlett and Brown [21]. They implicated these species of organisms as one of the major bacterial contaminants of Laboratory cultures. The prevalence of their spores in the environment may also have accounted for their dominance in this study. Vanderzant and Splittstoesser [22] also reported that *Bacillus* spp are part of the natural flora of vegetables and are among the most common vegetable spoilage bacteria. Though some *Bacillus* spp (*B. cereus*) are capable of causing food borne illnesses and can affect humans.

Ajmal and Ahmed [23] reported that these organisms are often associated with decaying of plant and animal matter, feces, vegetables and fruits. Therefore, the presence of this species of bacteria in this study may be from the substrates where the mushrooms grew.

Heavy metals are essential to the human body in trace amount but in excess could leadto varying degrees of illness based on acute or chronic exposures [24]. Several studies have been carried out to detect and explain the presence and distribution of several heavy metals in edible mushrooms, particularly arsenic, cadmium, caesium, copper, iron, lead, manganese, mercury, selenium, rubidium and zinc [25]. Latif *et al*. [26] reported an iron concentration range of 100-1216 ug g^{-1} in mushroom. While Turkekul *et al*. [27] reported 568-3562 mg/kg $g^{-1}$of the same in mushroom samples from Torat, Turkey.

The values obtained in this study for iron fall within the range of values obtained in other literatures as 116-835 mg/kg [28], 180-407 mg/kg [29] and 211-628 mg/kg [30], respectively. The maximum iron level permitted for food by WHO is 15 mg/kg while that of Zinc is 60mg/kg [31]. Although the values obtained in present study for both Fe and Zinc were above the WHO's permissive values for food which could pose health hazard. However, the high values may be from the environment where the mushroom samples were collected and coupled with their uptake ability. This is in agreement with the report of Frangkun *et al*. [32].The values obtained for Zinc in this study were 30 and 72mg/kg in Sclerotia and *Lentinussquarrosulus* respectively. However, other studies reported the values of Zinc to be 29.3 - 158 mg/kg, [29], 33.5 - 89.5 mg/kg [28] and 40.3-64.4 mg/kg [33], respectively. Therefore, the values obtained in this study fall within the ranges in earlier reports.

Thus the contamination of mushroom sold at Ikpoba Hill and Ekiuwa markets with pathogenic intestinal helminthes parasite may pose a health risk to consumers.

Local health and environmental authorities should educate the public on the health hazards of unwashed fresh wild edible mushroom and the importance of proper washing before consumption. The result of heavy metal concentration in this study also showed a high concentration of Zinc and excessively high concentration of iron above WHO permissive limits in foods. This study therefore underscores the need to conduct a more extensive study of their nature with a view towards having a better understanding of the status of edible and medicinal mushrooms harvested from the wild.

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