IDENTIFICATION AND ISOLATION OF FUNGI IN ABATTOIR AND POULTRY AMENDED PLOTS IN ILORIN, SOUTHERN GUINEA SAVANNA.

[*]Adegbite, M.A.[1], Sanda, A.R.[1], Ahmed, I.B.[1], Ibrahim, M.[1] andAdegbite, A.O.[2]

[1]*Department of Soil Science, Kebbi State University of Science and Technology Aliero.P.M.B.1144, Kebbi State, Nigeria.*

2*Paelon Memorial Clinic, No 6 Musa YaraduaOffKofoAbayomi Street, Victoria Island Lagos State, Nigeria.*

ABSTRACT

Traditional methods for soil remediation are often expensive and energy consuming and this has given rise to a new and ecologically safer method known as mycoremediation. A field experiment was carried out at the University of Ilorin Dam site to isolate and identify fungi present in organic amended plots which are capable of remediating heavy metal polluted soil of Asa River. Randomized Complete Block design in split plot arrangement was adopted using two treatments: Poultry Dropping (PD) and Abattoir Effluent (AE) at five levels i.econtrol, 1.3 t/ha, 2.6 t/ha, 1.3t/ha+NPK120KgN, 2.6t/ha+NPK120KgNfor abattoir effluent, and poultry droppings at control, 10t/ha, 15t/ha, 10t/ha+NPK120KgN and 15t/ha+NPK120KgN having three (3) replicates. Soil samples collected before and after planting were analysed for heavy metals (Mn, Fe, Pb, Zn, Cu, Co, Ni, Cr, Cd) using Atomic Adsorption Spectrophotometer. Result obtained showed that organic waste are effective in bioremediation of Asa River sediment with abattoir effluent having a reduction of 99.04% and poultry dropping 98.72 % ofheavy metal concentration in the soil which varied in the order of: Mn>Fe>Pb>Zn>Cu>Co>Ni>Cr>Cd. Result obtained also showed that eleven metal resistant Fungi were identified from abattoir effluent and poultry dropping amended plots i.e*Aspergillus niger*, *Trichoderma viride*,*Fusarium solani*, *Penicilliumnotatum*, *Aspergillus flavus*, *Trichoderma harzianum*, *Trichophyton verrusocum*, *Fusarium oxysporum*, *Stachybotryschartarum*, *Aspergillus ustus*and*Microsporumnanum. Aspergillus niger*was observedtohave the highest population (19% in poultry droppings and 19.6% in abattoir effluent).

Keywords: Mycroremediation, abattoir effluent, fungi, heavy metal and Asa river.

INTRODUCTION

Industrialization, global increase in population and indiscriminate disposal of waste has become a serious concern due to their release of heavy metals which has negative effect on the environment. Several toxic metals from industrial wastewater and other human activities are directly or indirectly released into the environment. Unlike organic contaminants, these pollutants from heavy metals are not biodegradable and able to travel up the food chain via bioaccumulation (45).

Several conventional methods have been used for the removal and treatment of heavy metalsin contaminated sites which include~~s~~ electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption (23). However, these methods are uneconomical due to high reagents and energy requirements, or ineffective at removing all metal contamination, and they can generate large quantities of toxic sludge (20).

More importantly, bioremediation offers a safer and more economical option for treating heavy metals incontaminated sites (21). The use of microorganisms and organic waste ~~has~~**have**several advantages, not least the highly efficient removal of heavy metals and cost effectiveness (15).

Some fungi are known to be particularly tolerant of heavy metals (18,9), and many species can adapt and grow under conditions of extreme pH, temperature, nutrient availability and high metal concentrations (3). Fungi tolerate and detoxify metals through various mechanisms involving valence transformation, extra- and intracellular precipitation or active uptake (31, 39). Scientists are currently exploring the use ofmicrobes and associated biota residing in ecosystems for the bioremediation of pollutants through degradation or accumulation (26), and numerous strains isolated from contaminated sites possess such abilities.

Fungal biomass proves to be advantageous in having a high percentage of cell wall materials which offer~~s~~ excellent metal binding properties (29,28). Fungal cell walls can act as a cation exchanger due to their negative charge originating from the presence of different functional groups, e.g. carboxylic, phosphate, amine or sulfhydryl, in different wall components (hemicelluloses, pectin, lignin, etc.). Cell walls of fungi are rich in polysaccharides and glycoproteins such as glucans, chitin, mannans and phospho-mannans. Metals and their compounds can interact with fungi in various ways depending on the type of metal, organism and environment (6). Moreover the use of fungal biomasses as biosorption materials is very convenient because of their inexpensive production methods based on simple fermentation

techniques (30). Fungi can also serve as an economical and constant supply of biomass for biosorption of heavy metals and they can also be grown using inexpensive media and unsophisticated fermentation techniques.

Many fungal species such as *Rhizopusarrhizus*, *Peniciliumspinulum*and *Aspergillus niger*have been extensively studied for heavy metals biosorption and the process mechanism seems to be dependent upon species (19,24).

Therefore, this research was aimed at using organic waste to bioremediate Asa River Sediment and also to identify the fungi biomass present in the amended plots.

MATERIALS AND METHODS

Site Description

The research was carried out during the dry season of 2015/2016 at the University of Ilorin Dam Site, Ilorin, Kwara State, Nigeria. The site was located in the Southern Guinea Savanna (SGS) belt at longitude N08^{0}28.049' and latitude E004^{0}39.798', approximately 344.7m above the sea level. The average temperature is 28^{0}C and an annual rainfall is 1100-1400mm per annum.

Experimental Layout

Randomized Complete Block design in split plot arrangement was adopted, using three (3) treatments: Rice Husk (RH), Poultry dropping (PD) and Abattoir Effluent (AE) at five (5) levels i.e control, 10t/ha, 15t/ha, 10t/ha+NPK and 15t/ha+NPK for rice husk and poultry dropping, while abattoir effluent were applied at control, 1.3 t/ha, 2.6 t/ha, 1.3t/ha+NPK and 2.6t/ha+NPK having three (3) replicate. The treatment combination followed a randomized arrangement.

Rice husk was obtained from Rice Milling Market at Iyana Oja Gboro, Ilorin, while abattoir effluent was collected from Ilorin Abattoir Center, Ipata market, Ilorin and poultry dropping from Unilorin Poultry Unit at the Teaching and Research Farm, Ilorin, Kwara State. All samples were collected in clean sacs and plastic containers as appropriate prior to analysis and field incorporation.

The area was mechanically ploughed and marking of the experimental plot was done manually. The treatments were spread uniformly over the plots and incorporated manually into the seedbed. One seed of Low Nitrogen Tolerant population white (LNTPw) maize (Early maturing variety) were planted per hill at a spacing of 25 cm x 75 cm and depth of 2 - 5 cm fourteen days ~~days~~ after incorporation of amendments. The experiment was carried out in dry season and water was supplied through irrigation at an interval of 3days.

Collection of Soil Sample

Soil samples were collected from six points on the field to form a composite sample and used to determine soil physical and chemical properties of the soil before amendment was applied. Soil samples were also taken from three points on each plot at a depth of 0 – 30 cm after harvest and bulked together to form composite samples. Soil samples were collected using soil auger.

Chemical Analysis of Sediment

The pH was determined by the method outlined by (10) using an electronically Jenway 3015 pH meter at ratio of 1:2.5 soil/water. Exchangeable acidity of the soil was determined by titration method using 1N KCl extract as described by (34). Exchangeable cations were extracted with an excess of 1M NH_4OAc (ammonium acetate) where sodium and potassium were determined using flame photometry, and atomic absorption spectrophotometer was used to determine the calcium and magnesium (4). Organic carbon in sediment was determined using wet oxidation method of (40), Available phosphorus was determined using Bray 1 (13) method. Total Nitrogen was determined using micro-Kjelhdal distillation method by (7).

Heavy metals analysis of soil

Heavy metals in soil (5g) was extracted with 1% EDTA as described by (8). Solutions were filtered through Whatman No. 1 filter paper and volume was made to 50ml by adding distilled water. Soil samples were digested in triplicates and analyzed for Fe, Mn, Zn, Co, Cd, Cr, Cu, Ni and Pb. For the determination of heavy metals the atomic absorption spectrophotometer was powered on and warmed up for 30 minutes. After the heating of cathode lamp, the air acetylene flame was ignited and instrument was calibrated or standardized with different working standards.

Sterilization of Apparatus

Petri plates, media bottles, distilled water, McCartney bottles and syringes were sterilized in autoclave. For sterilization purpose all apparatus were autoclaved for 40 minutes at 121°C. After autoclaving all sterilized material dried in oven at 95°C.

Media Preparation and Fungal Isolation

Potato Dextrose Agar (PDA) media was used for the isolation of fungi. Fungi were isolated on Potato Dextrose Agar (PDA) by soil dilution method (33).

Preparation of plates

Fungi were isolated on Potato Dextrose Agar (PDA) by soil dilution method (33).Poured the media in Petri-dishes and allowed to solidify for 24 hour. To suppress the bacterial growth, 30 mg/lit of streptomycin was added in the medium (33). Once the agar was solidified, and then put plates in an inverted position for 24 hours at room temperature(33).

Identification of fungi

The fungal cultures were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter and appearance of colony) and microscopic (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia, and presence of sterile mycelium) characteristics (41). Pure cultures of fungi isolates were identified with the help of literature (17,11).

RESULTS AND DISCUSSIONS

Table 1: Soil chemical analysis prior to planting

Chemical Properties	Soil	Abattoir Effluent	Poultry dropping
Nitrogen%	0.07	1.06	0.71
Available P (Unit ?)	0.046	24.52	11.92
Exchangeable Ca (Cmol/kg)	2.79	11.58	33.87
Exchangeable Mg (Cmol/kg)	0.14	2.61	17.85
Exchangeable K (Cmol/kg)	0.02	4.55	16.93
Exchangeable Na (Cmol/kg)	0.11	0.11	0.14
Organic carbon%	0.5		
Organic Matter %	0.87		
pH in H_20	9.3		
pH in KCl	8.9		
Acidity (Unit?)	0.4		
Sand	75.96		
Silt	11.28		
Clay	12.76		
Textural Class	Sand		
Cation exchange capacity	3.06		
Chromium mg/kg	25.3		
Cadmium mg/kg	0.5		
Nickel mg/kg	2.5		
Lead mg/kg	9.1		
Manganese mg/kg	350.5		
Iron mg/kg	25,250		
Copper mg/kg	9.65		
Zinc mg/kg	54.7		
Cobalt mg/kg	1.9		

The table 1 ~~**below**~~ shows the initial soil chemical properties of Asa River sediment, in which the sediments were observed to be sandy**. O**rganic matter content of the sediment was ranked low at (0.0.87%), while the nitrogen content of the sediment also ranked as low (0.07). The pH of the sediment showed 8.9 in KCl which means that the sediment was alkaline in nature and available phosphorus was also seen to be extremely low (0.046). Calcium was observed to be low, magnesium and potassium were also observed to be extremely ~~**very**~~ low and sodium also observed to have low value (0.11). Cation exchange capacity of the sediment was observed to fall below the range considered to be low. The chemical analysis of the sediment showed that the soil is low in terms of fertility. The heavy metal analysis of the sediment revealed that all metals ~~**fall**~~**fell** below maximum permissible limit as set by USEPA except manganese (350.5mg/kg). Table 1 also revealed that abattoir effluent has a high nitrogen content of (1.06) compared to poultry dropping of 0.71%. Table 1 further revealed that abattoir effluent had a higher phosphorus content (24.52%) than poultry dropping (11.92%), **and**potassium, calcium magnesium and sodium were observed to be higher in poultry dropping than abattoir effluent.

Table 2: Effect of organic waste on heavy metal availability in soil after cropping

Treat./Levels	Co (mg/kg)	Mn (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Ni (mg/kg)
Control	0.30	58.1	0.04	0.00	1.8	0.45	4.23	0.47	0.10
AE 1.6t/ha	0.18	46.9	0.05	0.01	1.53	0.57	3.00	0.48	0.067
AE 3.2 t/ha	0.24	44.0	0.04	0.01	1.47	0.59	2.37	0.51	0.03
AE 1.6t/ha+NPK120KgN	0.34	57.9	0.04	0.003	1.5	0.51	4.33	0.45	0.10
AE 3.2 t/ha +NPK120KgN	0.31	52.2	0.12	0.003	1.7	0.52	4.20	0.38	0.00
Control	0.30	58.1	0.04	0.00	1.8	0.45	4.23	0.47	0.10
PD10 t/ha	0.24	55.6	0.04	0.01	1.5	0.82	2.60	0.55	0.067
PD15 t/ha	0.31	47.0	0.07	0.003	1.77	0.86	3.73	0.62	0.067
PD10 t/ha+NPK120KgN	0.33	48.0	0.05	0.003	1.37	0.81	2.93	0.50	0.067
PD15 t/ha+NPK120KgN	0.24	40.5	0.04	0.00	1.37	0.78	4.13	0.42	0.067
SED	0.003	5.32	0.027	0.003	0.246	0.099	0.507	0.07	0.037
LSD(0.05)	ns	ns	ns	ns	ns	ns	*	ns	ns

Table 2 shows that there ~~are~~**were**no significant differences at <0.05 between the treatments at various levels of application except for Fe with the highest reduction in concentration observed at sole application rate of 3.3t/ha. Although they were no significant differences between the treatment**s** applied yet it was observed that the organic waste ~~were~~**was**very effective in reducing the concentration of heavy metals that were available in the soil. This result is supported by the studies of (42, 43)~~**who were both of the opinion**~~ that organic waste possesses large surface area, which~~**implies**~~**implied**a high capacity for complex**ing** heavy metals on their surface.Impact of organic amendments in bioremediation is effective and their effectiveness is related to the quantity applied (44).

Occurrence of fungi isolates in poultry droppings

Figure 1 shows the percentage of occurrence of the fungi in poultry dropping amended plots. Ten (10) fungal species were isolated. It shows that *Aspergilllusniger* had the highest ~~of~~ occurrence (19.1%), followed by *Aspergillus flavus*(14.6%), *Trichoderma viride*(14%), *Trichoderma harzianum*(12.3%),*Fusarumsolani* (12.1%), *Penicilliumnotatum*(10.3), *Trichoderma verrusocum*(6.4%), *Fusarumoxysporum*(5.3%), *Microsperumnanum*(3.8%)and *Aspergillus ustus*(2.1%).

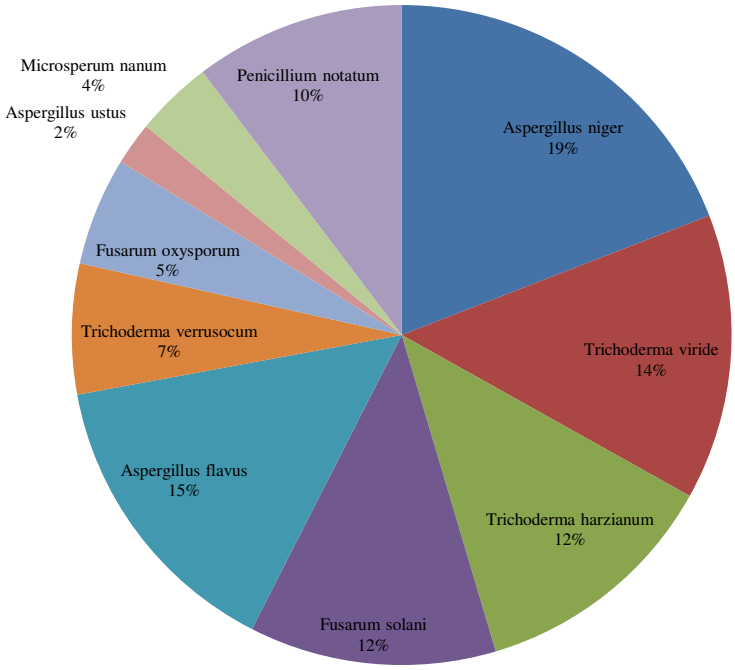

Figure 1: Percentage occurrence of fungi in poultry droppings

Occurrence of fungi isolates in abattoir effluent

Fig. 2 shows the percentage of occurrence of fungi in abattoir effluent. Eleven (11) fungal species were isolated. *Aspergillus niger*had the highest percentage of occurrence (19.6%), followed by *Trichoderma viride* (15.1%), *Fusarium solani*(11.2%), *Penicilliumnotatum*(10.1%), *Aspergillus flavus*(9.7), *Trichoderma harzianus* (7.8%), *Trichophyton verrusocum*(6.5%), *Fusarium oxysporum*(6.2%), *Stachybotryschartarum*(5.4%), *Aspergillus ustus* (4.6%) while *Microsporumnanum*had the lowest occurrence (3.8%).

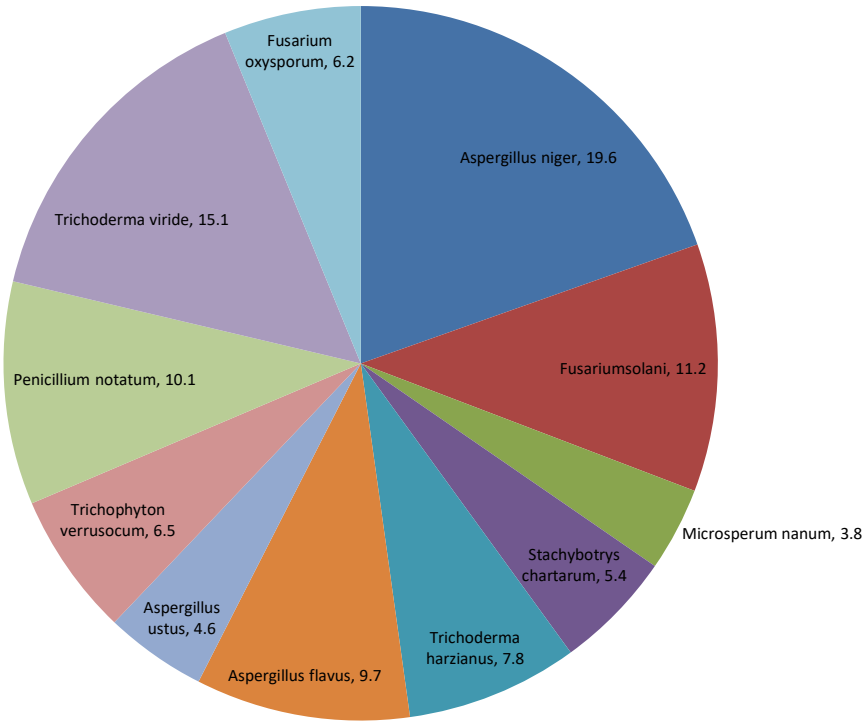

Figure 2: Percentage occurrence of fungi in abattoir effluent

Result ~~above~~shows that different fungi of Ascomycotina were isolated and identified in the amended plots and these fungi showedexcellent bioremediation capability for different heavy metals such as Co, Cd, Cu, Ni, Pb and Zn. These agrees with the findings of (12) who evaluated the efficiency of different fungi and found out that *Aspergillus niger*and *A. flavus*has high capacity in remediating Pb, Zn, Cr and Cd from refinery effluent. Furthermore, (5) in their study of Mycoremediation: utilization of fungi for reclamation of heavy metals at their optimum remediation conditions found out that *Aspergillus niger, A. flavus, A. fumigates and A. ustus*~~has~~**had**great potential in reducing heavy metals concentration in contaminated environment. This finding~~is~~ **was**similar to that of (1,32). Two *Fusariumsp*. were identified in which *Fusanriumsolani*was known to degrade Cr, Zn and Nikel (36), while *F. oxysporum*was also identified to be effective in reducing the concentration of Cd, Cu, Pb, and Zn. This is similar to the findings of (14) who discovered the *F. oxysporum*helps to biosorb Zn and Cu and *F. solani*removesCu(2). Similarly, two species of *Trichoderma* were identified; *T. harzanum*and *T. viride*. *T. viride* was identified to have a high accumulating potential for Ni, Cd and Cr (27), and (22) found out that *T. viride*hashigh uptake potential for chromium. While *T. harzanium* has been observed to have a high reducing capacity for Siddique *et al*., 2011, 35). Moreso, *P. notatum* was found to have capacity for reducing high chromium concentrations (37). Result further revealed that *Stachybotryschartarum*, ~~plays~~played an important role in reducing copper (Cu) in contaminated soil. In a study conducted by (16) to evaluate the tolerance capacity of *Stachybotryschartarum*in a copper based preservative, they found that the fungi ~~has~~had high tolerance level for copper. Furthermore,*Microsporumnanum*was found to play an important role onCd, Cr, Pb and this was supported by the findings of (12) who opined that *M. nanum*reduced the concentration of heavy metal from a refinery effluent.

It was also observed from Figure 1 and 2 that *Aspergillus niger*~~is~~wasthe dominant isolated specie from the two amended plots. *Aspergillus niger* has been known to be able to tolerate the heavy metals in organic wastes and the result obtained in this study confirm the earlier assertion by different authors who asserted that *Aspergillus sp.* ~~is~~wasthe dominant species in wastewater (25).

CONCLUSION

From the result of this study, it wasdeduced that organic ~~waste~~wastesare effective material for bioremediation, and their effectiveness was enhanced as a resultof the activities of certain fungi which ~~performs~~performed important functions coupled with their inherent potential to tolerate and reduce heavy metals. Fungi isolated and identified in abattoir and poultry amended plots includes *Aspergillus niger*, *Trichoderma viride*,*Fusarium solani*, *Penicilliumnotatum*, *Aspergillus flavus*), *Trichoderma harzianum*, *Trichophyton verrusocum*, *Fusarium oxysporum*, *Stachybotryschartarum*, *Aspergillus ustus*and*Microsporumnanum.Aspergillusniger*(19% in poultry droppings and 19.6% in abattoir effluent) was observed to have the highest population in all the amended plots.

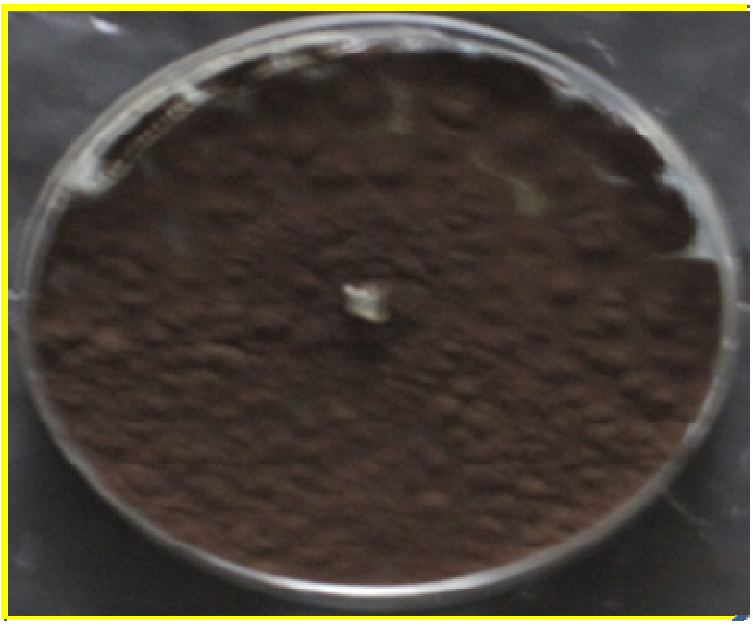

Plate 1(a): Colony morphology of *Aspergillus niger*

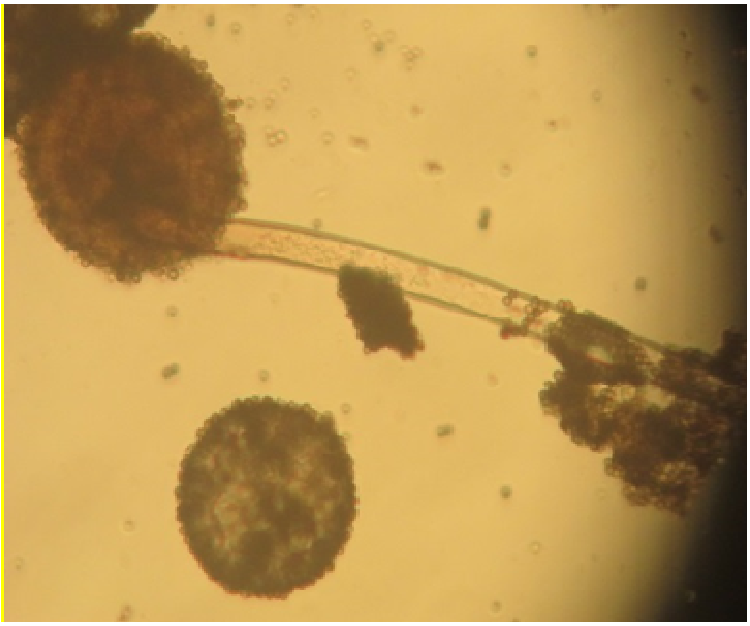

Plate 1(b): Microscopic structure of *Aspergillus niger*X100

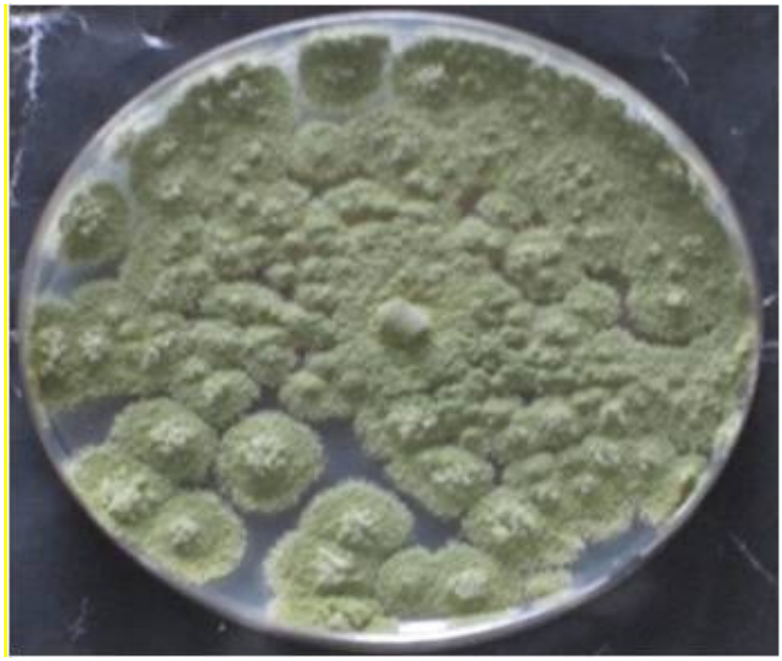

Plate 2(a): Colony morphology of *Aspergillus flavus*

Plate 2(b):Microscopic structure of *Aspergillus flavus*X100

Plate 3(a): Colony morphology of *Fusarium oxysporum*

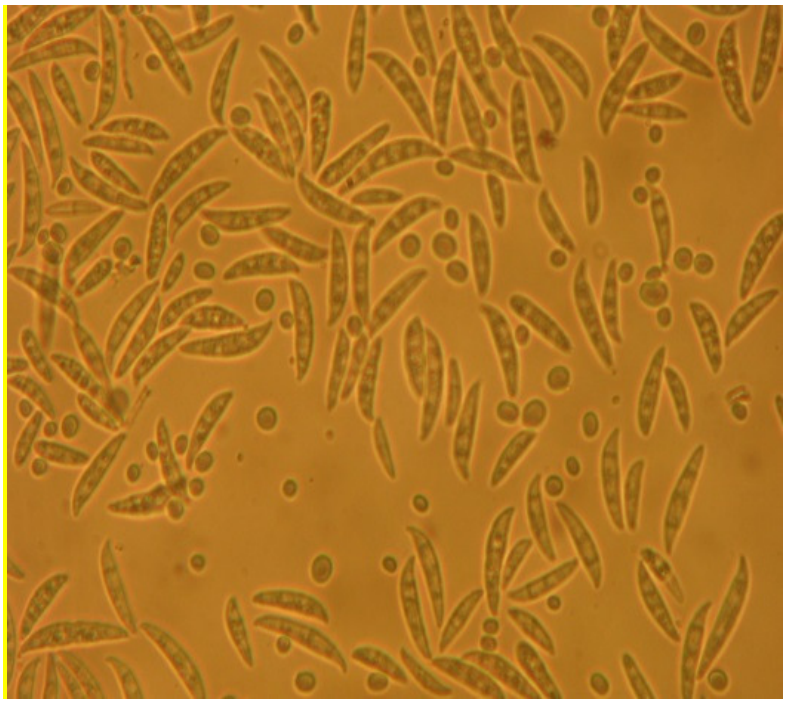

Plate 3(b): microscopic structure of *Fusarium oxysporum*X100

Plate 4(a): Colony morphology of *Trichoderma harzianum*

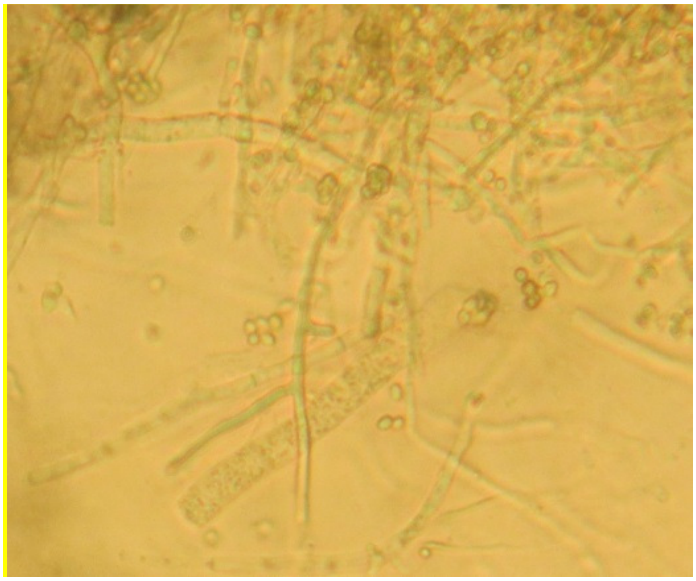

Plate 4(b): microscopic structure of *Trichoderma harzianum*X100

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