CELLULOLYTIC FUNGI FROM PALM PRESSED FIBER, EMPTY FRUIT BUNCH, AND PALM TRUCK

ABSTRACT

Cellulolytic fungi have shown potential applications in various industries such as pulp and paper, agriculture, biofuel production, and textile. In this study, palm biomasses (palm pressed fiber,empty fruit bunch, and palm truck)collected from Nigerian Institute for Oil Palm Research (NIFOR) mill.?Twenty(20) fungi strains were isolated and screened. Seven(7) isolates: 3 *Aspergillus species*, (2),*Trichoderma* spp. 1 *Rhizopus*spp. and 1 *Penicillium* spp.,had significant cellulolytic activity. *Trichoderma species*, C (from palm pressed fiber),showed the highest clearance zone of 11.5mm. Also, *Rhizopus species*(A) and *Trichoderma species*(B) (from empty fruit bunch and palm pressed fiber, respectively), showed high clearance zone of 8.5mm, and 6.5mm, respectively, with *Aspergillus species*(D and E)(palm truck and empty fruit bunch), having the most minor clearance zone of 1.0mm, each for the 3-5 days assayed. All the fungi isolates elicited high mycelia weight on day 4, with *Trichoderma species*(C) having the highest; 0.79g, followed by *Trichoderma species*(B)(0.58g) and *Aspergillus species*(D) (0.53g).*Aspergillus species*(E)(0.38g) showed the least mycelia weight. *Trichoderma species, Rhizopus species, and Aspergillus species* isolated and screened could be potential sources of cellulases for industrial application.

Keywords: Isolation, Fungi species, Cellulolytic, Palm biomass.

1.0 Introduction:

The term biomass is the collective term denoting all the organic materials found on earth, including terrestrial and aquatic plants and animals,and the organic wastes(Mc-Kendry, 2002).Generally the biomass encompasses plant-based woody biomass (mainly lignocelluloses), plant-based non-woody biomass (starch, sugar, and oils), and animal/human-based biomass (animal fats and proteins, slurry/slaughter wastes, household wastes, etc.) (Sajith*et al.,* 2016). Among the plant-based woody biomass, lignocellulosic biomass is a potential resource for renewable energy, but typically used for landfilling or burned off. Lignocellulose constitutes 60% of the plant cell wall and is made up of three biopolymers of sugars and their derivatives, viz., lignin, hemicelluloses, and cellulose (Sajith*et al.*,2016).

Cellulose is considered one of the most important sources of carbon on this planet, and its annual biosynthesis by both land plants and marine occurs at a rate of 0.85×10^{11}tonnes per annum (Nowak *et al*., 2005).Cellulose degradation and its subsequent exploitations are essential for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of deep research and industrial interest (Bhat and Bhat,1997).

The microbial hydrolysis of insoluble cellulose requires the action of multiple cellulases (endoglucanases, exoglucanases, and β-glucosidases) in a synergistically manner so that the complex polymer is converted to simple sugars (Sajith*et al*., 2016).Cellulolytic enzymes play an essential role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes, and protozoa.

In industry, cellulases are usedin the production of fermentable sugars, and ethanol, organic acids, detergents, and other chemicals, thereby providing a crucial opportunity for achieving remarkable benefits from biomass utilization (Wen *et al.*, 2005).Several microorganisms synthesis this enzyme among which fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). However, fungi are well-known agents of decomposition of organic matter in general and of a cellulosic substrate in particular (Lynd *et al*., 2002). Among the microorganisms, fungal cellulases are usually preferred by the industry, because they are extracellular adaptive in nature and usually secreted in large quantities during growth(Moore, 1996). Efficient cellulolytic fungi are represented by the species of *Aspergillus, Penicillium, Chaetomium, Trichoderma, Fusarium, Stachybotrys, Cladosporium, Alternaria, Acremonium, Ceratocystis, Myrothecium, Humicola,*etc.(Wood, 1985; Mehrotra, and Aneja,1990).

The oil palm industry in Nigeria generatesa lot by-products in the cause of palm oil production which includes the palm pressfiber (PPF), empty fruit bunch (EFB),etc.which are used in making fire for cooking in the industry. Due to the great interest in using cellulose waste as feedstock via fermentation processes, thus, converting low-cost starting materials into products of more excellent value, thus the need to isolate microbes that are responsible for its breakdown.In the study, the focus is to screen and isolate cellulolytic fungi from palm biomass.

2.0 Materials and Methods:

2.1 Source of Fungi and Initial Culturing: Samples of palm pressed fiber (PPF), decaying empty fruit bunch (DEFB), and decaying roots of the oil palm tree were collected from Nigerian Institute forOil Palm Research (NIFOR).The residual oil from the PPF removed. Potato dextrose agar (PDA) medium grew the initial cultures, where samples were cultured by serial dilution method. Chloramphenicol (antibiotic) was added to the PDA to prevent the growth of bacteria (deZaan Cocoa and Chocolate Manual, 2009)

2.2 Measuring of Cellulolytic EnzymesActivity: The activity of isolated fungi was measured qualitatively by Congo red method (Lekh*et al.*,2014). The isolated cultures were screened for ability to produce cellulase complexes following the method of Teather and Wood (1982). Czapek-dox medium was used: $NaNO_3$=2g, K_2HPO_4=4g, $MgS0_4$=0.05g, KCl=0.5g, $FeSO_4$=0.001g, carboxymethyl cellulose(CMC) =1%, agar=20g and distilled water=1L. The medium was autoclaved at 121ºC for 15 minutes. Wells of 5mm size were made on the plates (solidified medium). The plates were inoculated with fungal isolates and incubated at room temperature (28±2ºC) for 3 to 5 days to allow fungal growth. Congo red staining solution (1%) 10ml was added to the plates, agitated at 50 rev/min for 15 minutes and discarded.Exactly 10ml of 1N NaOH was added to the plates and agitated again at 50 rev/min for 15 minutes and discarded. The plates were examined and the clear or yellowish zone were measured.

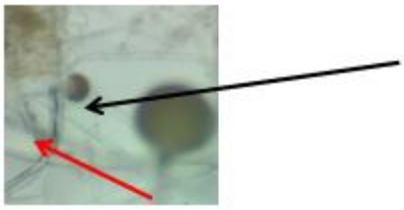
2.3 Measurement of the Mycellia: The fungal isolates growth rate were also assesed by measuring the weight of mycellia when grown on Czapek-Dox broth (Lekh, *et al.*,2014).Czapek-Dox broth was prepared amended with 1% cellulose and distributed into 100 ml conical flasks and the pH adjusted to 5.0. After sterilization, the fungal spores were inoculated into it and incubated at room temperature for 5 days on a rotary shaker at 120rpm. The mycelliawere harvested and weight taken (Lekh, *et al.*,2014).

3.0 Results and Discussion:

Screening of Isolates:A total of 7 fungi pure cultures were isolated from the palm biomass collected.

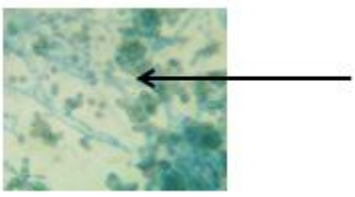
Identification of fungi: In order to identify the fungal colonies, colony colour, shape, border and spots were recorded. Microscopic features were examined under a microscope fitted with a camera (Motic B1 Digital camera) using the cover-slip method in which a little quantity of each culture was transferred onto the base of cover slips buried in potato dextrose agar (PDA). The fungal isolates were identified as described by Barnett and Hunter (1998)as shown in Plate 1 and date recorded and used for classification.Classification was based on microscopic observation of mycellia as well as reproductive structures such as spores and fruiting bodies, if present (Sivaramanan,2014).The fungal isolates were labelled A-G and identified to be three*Aspergillus species*,two*Trichoderma species*, one*Rhizopus specie*and one*Penicillium specie.*

A: Culture plate of *Rhizopus* sp. grown on PDA

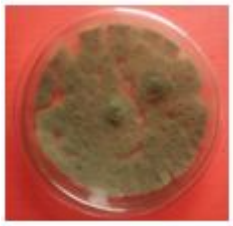


Photomicrograph of *Rhizopus* sp. showing mycellial (red arrow) and collumella (black arrow)

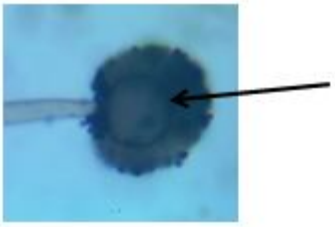
B&C: Culture plate of *Trichoderma* sp. grown on PDA



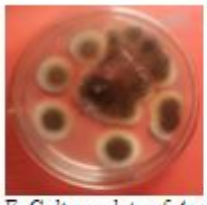
Photomicrograph of *Trichoderma* sp. showing clustered condia (black arrow)



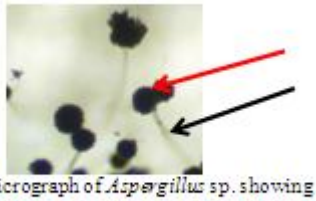
D: Culture plate of *Aspergillus* sp. grown on PDA



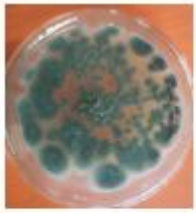
Photomicrograph of *Aspergillus* sp. showing conidia head (black arrow)



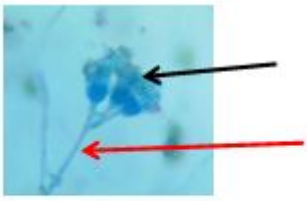
E: Culture plate of *Aspergillus* sp. grown on PDA



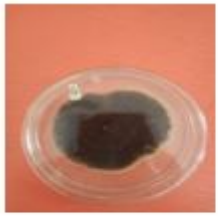
Photomicrograph of *Aspergillus* sp. showing mycellial (black arrow) and globose phialides (red arrow)



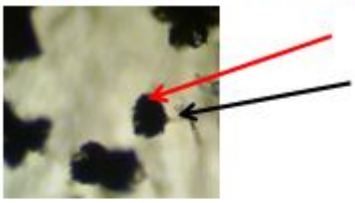
F: Culture plate of *Penicillium* sp. grown on PDA



Photomicrograph of *Penicillium* sp. showing mycellial (red arrow) and three metula (black arrow)



G: Culture plate of *Aspergillus* sp. grown on PDA



Photomicrograph of *Aspergillus* sp. showing mycellial (black arrow) and fused phialides (red arrow)

Plate 1: Macroscopic and microscopic identification of fungal isolates.

Cellulolytic activity: The results after screening the zone of clearance for 3-5 days (Fig.1) showed that; in day 3 organism C showed a highest zone of clearance (9.0mm) which is significantly larger compared to other fungi while organisms D and E have the least zone of

clearance of 1.0mm each which is smaller when compared to organism C. Nevertheless, organisms A, C, F and G showed zones of clearance of 6.5mm, 5.4mm, 2.7mm and 1.9mm respectively. However, on day 4, organism C also showed a high zone of clearance 11.5mm while D showed the least of 1.5mm. But organisms A, B, E, F and G showed reasonable clearance zone of 8.5mm, 6.5mm, 1.8mm, 3.2mm and 2.2mm respectively. These results tend to agree with the work of Mahdi, *et al*., (2011) where they showed that fungal isolates produced the highest zones of clearance at the fourth day.

In day 5, organism C showed the highest zone of clearance, 11.5mm while organism D all showed the lowest clearance zone of 1.6mm but not larger from organism E.

All the fungal isolates tends to show prime zone of clearance on day 4 with *Trichoderma species* (*C*) having the highest value of 11.5mm when compared to the other isolates followed by *Rhizopus species* (A) and *Trichoderma species* (B) at 8.5mm and 6.5mm respectively. Organisms A and C did not show any change in their zone of clearance at day 5 (8.5mm and 11.5mm respectively) but there was a slight increase in organisms D and G. Although, organisms E and F showed decrease in their zones of clearance. Changes in day 5 which may suggest that the fungi had used up the necessary nutrient and no other nutrient was avaible for its growth after day 4, as such may suggest "death" phase. This may be because the conditions used were more favorable to some. Hence showing that organism C have the highest ability of producing glucose from cellulose. These results also suggests that day 4 is the optimum day for cellulase production as earlier reported by Mahdi, *et al*., (2011).

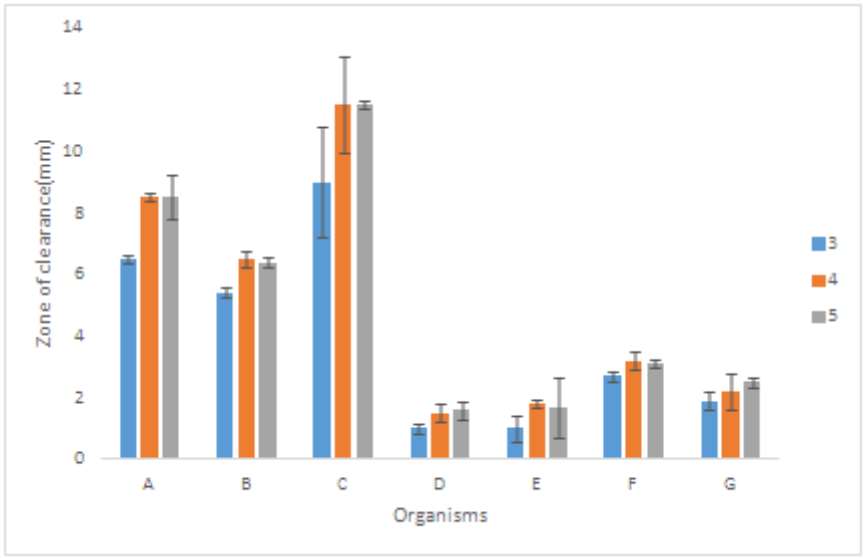


Fig. 1: Zone of clearance of the organisms at 3-5 days.

Key:

A *Rhizopus species*

B *Trichoderma species*
C *Trichoderma species*
D *Aspergillus species*
E *Aspergillus species*
F *Penicilliun Species*
G *Aspergillius species*

Mycellia Weight: Fig.2 shows the measured mycellia weight across 3-5 days of incubation in a broth. Fungi B and C showed high mycelia weight of 0.58g each, but organism C is higher than organisms E, F and G(0.38, 0.39 and 0.47g respectively). Organism E showed the least weight of 0.38g. Day 4 also shows organism C (0.79g) as having the highest mycelia weight higher than B (0.51g) and others, followed by organism D (0.53g). Organism E showed the lowest mycelia weight of 0.42g. In day 5, B showed highest mycelia weight of 0.51g, followed by organism G (0.49g), organism C showed a mycellia weight of 0.47g. Organism A (0.39g) have the least mycellia weight. All the fungal isolated showed high mycelia weight on day 4 suggesting it as the optimum day of incubation. But after that death phase sets in.
Day 4 showed the highest for both mycellia weight and clearance zone for all the fungi screened and C was the highest in both.

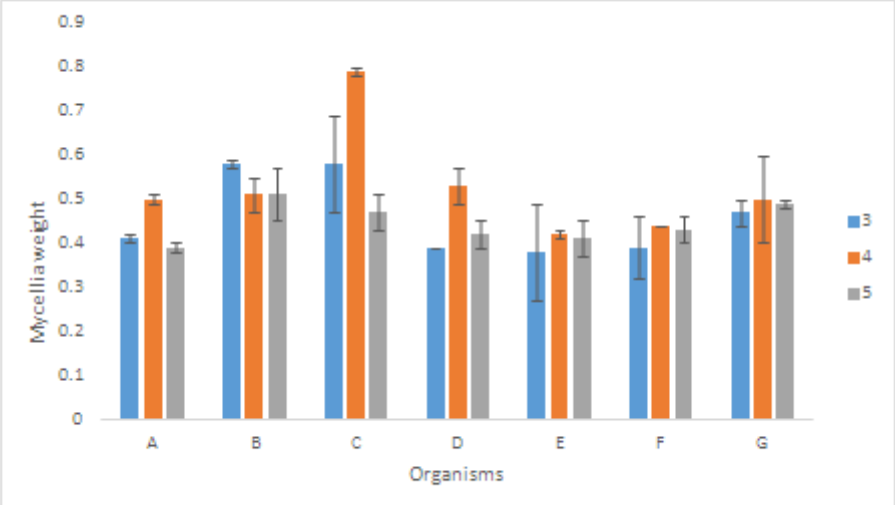


Fig. 2: The mycellia weight of the different organisms at 3-5 days.

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Key:

A *Rhizopus species*
B *Trichoderma species*
C *Trichoderma species*
D *Aspergillus species*
E *Aspergillus species*
F *Penicilliun species*
G *Aspergillius species*

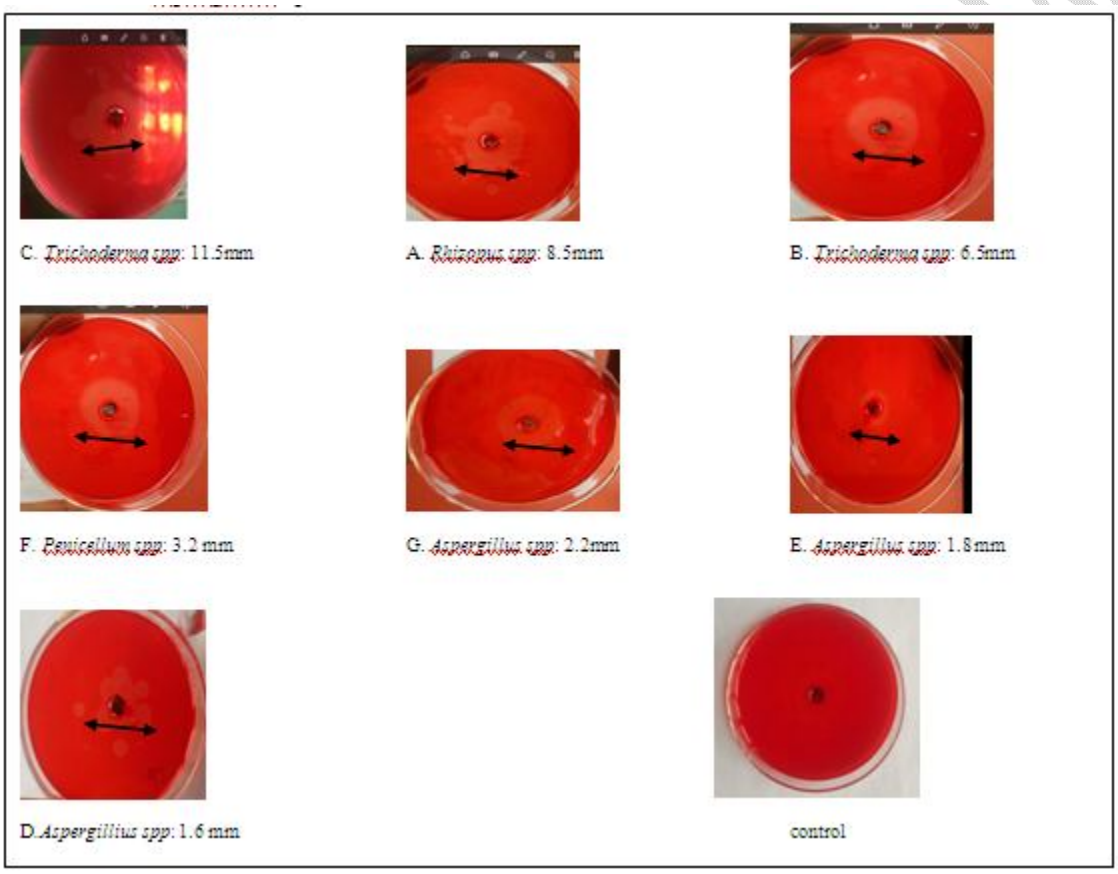

Plate 2: Plates screening of Congo red dye staining for displaying clearance zones.

Conclusion:

From this study, seven (7) species of the fungiisolated and characterized are potential cellulolytic species. *Trichoderma species* and *Rhizopus species*from PPF and empty fruit bunch respectively showed high cellulolytic activity by exhibiting high zones of clearance on reaction with Congo red on day 4. The results from the mycellia weight indicated that day 4 the highest production, thus suggesting that day 4 is the optimum day.

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