Quality Assessment of Fungal Synthesized Bio-Surfactants From Agro-Wastes(Cassava, Yam and Sweet Potato Peels)

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Research

Keywords: Agrowastes, Biosurfactants, Emulsion, Fungi, Hydrocarbon

Posted Date: September 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-70144/v1

Abstract

Background: Biosurfactants are produced extracellularly by microorganism on cell surface or extracellularly on media environments. They are known for their role in surface and interfacial tension reduction between molecules at the surface and interface, respectively. Thus, this study was conducted to determine and compare the capability of biosurfactant production from immediate agrochemical wastes (yam, cassava and sweet potato peels) using fungal isolates.

Materials and Method: The test fungi (*Aspergillus niger* and *Fusarium oxysporum*) were isolated from yam, potato and cassava peels showing advanced rottenness. Standardized production of biosurfactants using well researched protocol involving media and inclusion of agrochemical wastes in a fermentation broth containing the isolated fungi was carried out.

Results and Discussion: The highest biosurfactant activity value obtained was with *F. oxsporum* on yam peels with emulsification index (EI) of 94.2±0.20% on kerosene after 72 hours (EI72). While the lowest activity of biosurfactant was observed in *A. ni*ger grown on cassava peels broth with EI72 activity of 36.36±0.53% on diesel. The trend showed that emulsification index on diesel after 24 hours (E24) with biosurfactant from *A. niger* grown in yam peel broth gave the highest EI concentration (48.42±0.14%) followed by biosurfactant from *F. oxysporum* cultured in yam peels (48.27±0.99%). In comparison with kerosene emulsion formed: biosurfactant from *F. oxysporum* grown in yam peels showed highest EI (94±0.10%) followed by *A. niger* cultured in yam peels (78.72±1.08). The EI results showed that yam peel served as a better substrate for biosurfactant production while *F. oxysporum* is the better biosurfactant producing fungi. The EI values were slightly maintained for 48hrs (EI48) and 72hrs (EI72). The result of the oil spreading assay using engine oil revealed that biosurfactants produced by *F. oxysporum* grown on sweet potato peels broth displaced more area of expired engine oil in water by forming miscelles with oil displacement area (ODA) of 9.08±0.02cm^2 followed by biosurfactant from *A. niger* grown in sweet potato broth with ODA of 8.04**±**0.38 cm^2. In terms of substrate specificity the sequence of increment in EI was: Yam>Cassava>Sweet potato. However, considering the critical micellar level or the oil displacement ability of the product, the sequential increment was: Sweet potato>Cassava>Yam peels.

Conclusion: The biosurfactant produced shows more cleansing ability towards kerosene than it does for diesel. Conversely, it could be channelled toward clean-up of kerosene *viz a-viz* crude oil contaminated surfaces and environment.

Introduction

Biosurfactants are produced mainly by energy utilization of carbon sources by micro-organisms during their metabolic processes. These surface active agents have been known to have emulsification properties and thus act as biological detergents able to dislodge oil and petroleum hydrocarbon compounds deposited on materials or environments. They are increasingly studied as they currently have found usefulness in different industries mainly as cleansing agents. According to Banat et al. (2010),

varied micro-organism are known to produce a number of these surface active agents capable of surviving and thriving in different cheap substrates and thus helpful in putting to good use wastes agro-industry origin. Of recent, studies have shown that food and agro-industrial residues (orange peel, sesame peel flour, date molasses, corn steep liquor, peanut oil cake, sugarcane baggasse, tuna fish residue, banana peel, potato peel, cassava waste, moringa residues and other agro-industrial wastes) acted upon by various micro-organisms (*Bacillus licheniformis, Cunninghamella phaeospora, Candida tropicalis* and *Pseudomonas aeruginosa*) have the potential of converting to biosurfactants (Rubio-Ribeaux et al. 2017; Magalhaes et al. 2018). According to Sekhon-Randhawa and Rahman (2014), uses of biosurfactants produced by some companies in Europe are numerous, ranging from cleansing products, shower gels, shampoos, washing-up liquids, pharmaceutical (bioactive properties), cosmetics, bioremediation, pest control, skin care, sun-lotions hair care formulations, thickening polymers, rheological modifiers, natural gums and many more. Therefore, Africa with abundance of both microbial fauna as well as substrates especially of agricultural wastes, should begin to explore this important organic product of high commercial value.

Materials And Methods

Materials

Source of Carbon/Sample Collection

Yam and potato peels were obtained from a yam chip shop opposite Federal University Wukari, Taraba State Nigeria. Cassava root tubers for this research were harvested at a nearby farm site located along Federal University Wukari Road, in Wukari, Taraba State, Nigeria and the peeled portion properly washed with clean water for soil and dirt removal. The fresh peels were transported to the laboratory within 2 hours of procurement and washed with sterile distilled water. All the agro-waste peels were air dried for 7 days, separately grounded using mortar and pestle to obtain stock samples and thereafter stored in an airtight polythene bag.

Microorganism

The fungi used were obtained by isolation from decomposing agro-wastes and culturing in a prepared Potato Dextrose Agar (PDA) at the culture selection unit of the Department of Microbiology, Federal University Wukari, Nigeria. It was further characterized and sub cultured to yield pure strain of the organism.

Research Methods

Culture Media Preparation

The Potato Dextrose Agar (PDA) preparation was exactly according to the manufacturer recipe (39g in 1L of water). The autoclaved sterilization of media was at 121°C and 15 psi for 20 min; complete dissolution and homogeneity was achieved. Thereafter, it was cooled to 43°C followed by inclusion of a

chloramphenicol capsule to 500 ml of sterile cooled PDA (Green *et al.,* 1994). Solidified PDA was obtained by dispensing 15 ml of the media into each sterile petri dish of 8.6 cm (86 mm). Sterility was ascertained through incubation of the solidified media for 24h at 28°C before use (Cheesebrough, 1984).

Fungal Isolation

Onyike and Maduewesi (1985) described the isolation protocol employed in this study. Briefly, a small section of the cassava, yam and sweet potato peels showing advancing margin of rottenness were placed on the solidified agar. A section from each of the three samples were placed per plate. The incubation duration was 7 days at 27±2°C and fungi associated with the agro-wastes observed. Isolation frequency was as previously described by Okigbo and Ikediugwu (2000). Pure cultures were obtained by series of sub-culturing of individual isolates.

Fungal Isolates Identification

Fungal colonies were macroscopically and microscopically identified by direct low powered light microscope and identification atlas at the Department of Microbiology, University of Jos, Nigeria.

Biochemical Tests for Fungal Isolate Identification

Catalase Test: A small amount of the fungi was transfer to a clean, dry glass slide surface with the aid of a sterile teasing pin. Hydrogen peroxide (2 drops) were added, mixed and observed for bubble formation.

Indole Test: This test is based on the principle that Kovac's reagent can combine with indole and the solution turns from yellow to cherry red. The fungi were inoculated aseptically in a sterile test tube containing 4 ml of tryptophan broth and incubated at 37^0C for 24-28 hours after which 0.5ml of Kovac's reagent was added and observation for a ring formation after 20 mins. A red-violet colour ring at the top surface of the tube indicates a positive test while a yellow colour indicates a negative test. An un-incubated test tube served as control.

TSI Test: Triple sugar iron agar test is used to determine whether an organism utilize lactose and glucose or sucrose in fermentation or produce hydrogen sulphide. The isolated colony of fungi were introduced into the TSI with a sterile straight teasing pin. The medium was first agitated through the centre to the bottom and streaked with the fungi to the surface and kept in slanted position. The incubation time was 18-24hrs at 37^0C.

Citrate Utilization Test: This test detects the ability of micro-organism to utilize citrate as a sole source of carbon and energy. From 2% citrate, 5 ml was dispensed into various test tubes and covered with cotton wool and then incubated after inoculating with fungi. Colour variation from green to blue indicated a positive test.

Production of Bio-surfactant

Modified experiment by Michele *et al.* (2018) was conducted in a conical flask (250 mL) containing 25 mL of media culture (40 g/L of each agro-waste, 4% spent vegetable oil and 10 g/L yeast extract). The medium was autoclaved at 121°C for 15 minutes and inoculated after cooling. The conical flasks were incubated at 30^0C and manually vortexed (agitated) for 15mins 4 times daily for 21 days. An Erlenmeyer flask with 25 mL of culture medium and 4% vegetable spent oil (v/v), with no inoculation, was used as control. Afterward, the culture media was centrifuged at 3000 rpm for 10 minutes to obtain a cell free supernatant.

Bio-surfactant Quality Assessment

Emulsification Index (E24)

The emulsifying index test (E24) technique described by Cooper and Goldenberg and adapted by Kiran, *et al*. (2009) was used. The centrifuged supernatant (bio-surfactant) (2mL) was mixed with 2mL of diesel and kerosene separately (hydrocarbon compounds) in test tubes, and the mixture was agitated for up to 2 minutes in a constant tube agitator type vortex.

After 24 hours, the proportion of the emulsion formed was compared to the total volume of added hydrocarbon. The emulsification index was calculated by the following formula:

$$E_{24} = \frac{The\ height\ of\ the\ Emulsion\ Layer}{The\ Total\ Height\ of\ the\ Mixture} \times 100$$

E_{24} correlates to the biosurfactant concentration ie Emulsification Index after 24 hours (%).

Oil Drop-Collapse Qualitative Test

The test was conducted in 80 x 80mm Petri dishes containing 2mL of produced biosurfactant to which 3 drops of oil was added and observed for 0, 1, 5, 30 min, 1 and 72hrs. The result was regarded positive when the oil drop dispersed. Distilled water (2ml) and 2mL 1M sodium dodecyl sulfate (SDS) in oil surfactant solution were used as negative and positive control respectively.

Redox 2, 6-dichlorophenol indophenol (DCPIP) indicator Biodegradability test

Hanson *et al.* (1997) described the methodology used. Briefly, in test tubes, DCPIP concentrations were adjusted to 0.010g/mL from which DCPIP solution (2 ml), spent vegetable oil (2ml) and hyphae of fungi grown in wastes corresponding to 3 mm diameter, were added to each test tube and kept at (30°C). Medium discoloring-time measurements were taken after 48 hours. DCPIP in oil and without fungal strain was used as a positive control and DCPIP without oil and without strain was used as negative control.

Iodine Test: Iodine solution (4 - 5 drops) was added into a little amount of the formulated biosurfactant and gently agitated (Mahesh *et al.*, 2006). The bluish/dark colour formation was observed.

Saponification Test

NaOH 2% solution was added (2ml) to the small amount of biosurfactant and well agitated to observe for soap formation (Mahesh *et al.,* 2006).

Oil Displacement Assay

To the water (20ml) measure into a petri dish was added condemned engine oil (1 ml) and 2ml of synthesized biosurfactant. The diameters of the displaced engine oils were carefully measured and recorded. These were conducted in triplicate.

Statistical Analysis

All experiments were performed in triplicate and the mean and standard deviation were calculated. Also, an analysis of variance (ANOVA) test was performed to determine any significant difference ($p>0.05$). The analyses were all carried out using the Statistical Package for Social Sciences (SPSS) version 20.

Results

Table 1
Colony Morphology of the Biosurfactant Producing Fungi

FEATURE	Fungi A *(Aspergillus niger)*	Fungi B (*Fusarium oxysporum*)
Colony Type	Compact	Compact
Size	1 mm	1 mm
Shape	Irregular	Irregular
Hyphae	Aerial hyphae	Aerial hyphae
Colour	Dark brown	White to white violet
Texture	Cottony	Cottony
Surface	Hairy	Hairy
Opacity	Opaque	Opaque
Diameter	75 mm	75 mm
Pigmentation	Gray to dark blue	Pale dark to peach colour
Septation		3–5

Table 2: Biochemical Test for Identification of Fungi

TEST	Fungi A (*Aspergilus niger*)	Fungi B (*Fusarium oxysporum*)
Citrate Test	+	+
Catalase Test	_	+
Peptone/Covax Test	_	+
Triple Sugar Identification (TSI): Glucose	+	+
Lactose	_	_
Sucrose	_	_
Hydrogen Sulphide		_

The biochemical test for biosurfactant used in this work includes; iodine and saponification test as well as biodegradability test using a redox 2, 6-dichlorophenolindophenol presented in Table 3.

Table 3: Results of Iodine Test, Saponification Test and Biodegradability Test

Biosurfactant Source	Iodine Test	Saponification Test	Biodegradability Test Using 2,6-DCPIP
BAP	_	+	+++
BAC	_	+	+
BAY	_	+	+
BFP	_	+	++
BFC	_	+	+
BFY	_	+	+

BAP – Biosurfactant produced by *Aspergillus niger* in Sweet Potato broth

BAC - Biosurfactant produced by *Aspergillus niger* in Cassava broth

BAY - Biosurfactant produced by *Aspergillus niger* in Yam broth

BFP – Biosurfactant produced by *Fusarium oxysporum* grown on sweet potato broth

BFC – Biosurfactant produced by *Fusarium oxysporum* grown on cassava broth

BFY – Biosurfactant produced by *Fusarium oxysporum* grown on yam broth

The result of oil drop collapse assay is represented in Table 4 below

Table 4: Results of Oil Drop Collapse Qualitative Test

TIME	BAC	BAP	BAY	BFC	BFP	BFY
0mins	_	_	_	_	_	_
1mins	_	_	_	_	_	_
5mins	_	_	_	_	_	_
30mins	_	_	_	_	_	_
72hrs	+	+	+	+	+	+

The percentage emulsification index was analyzed using SPSS version 20 and the result is presented as Mean ± Standard Deviation in Table 5 below.

Table 5
Emulsification Index (%).

SAMPLES	Diesel (%E24)	Kerosine (%E24)	Diesel (%E48)	Kerosine (%E48)	Diesel (%E72)	Kerosine (%E72)
BAC	42.42 ± 2.83^{c}	62.86 ± 2.90^{c}	36.36 ± 0.49^{a}	64.7 ± 0.41^{d}	36.36 ± 0.53^{a}	64.70 ± 0.10^{d}
BAY	48.42 ± 0.46^{d}	78.72 ± 1.08^{d}	53.57 ± 0.45^{e}	78.72 ± 0.50^{e}	53.57 ± 0.55^{d}	77 ± 0.20^{e}
BAP	42.86 ± 0.26^{c}	50 ± 1.99^{a}	44.2 ± 0.20^{c}	51.28 ± 0.29^{a}	43.72 ± 0.12^{b}	51.28 ± 0.28^{a}
BFC	36.7 ± 0.99^{a}	59.37 ± 0.85^{b}	43.33 ± 0.14^{b}	56.25 ± 0.25^{b}	43.33 ± 0.73^{b}	56.25 ± 0.45^{b}
BFY	48.27 ± 0.99^{d}	94 ± 0.10^{e}	48.63 ± 0.10^{d}	94 ± 0.10^{f}	48.52 ± 0.18^{c}	94.2 ± 0.20^{f}
BFP	39.29 ± 1.03^{b}	57 ± 1.50^{b}	43.33 ± 0.73^{b}	59.5 ± 0.51^{c}	43.33 ± 0.34^{b}	59.5 ± 0.49^{c}

Result represent mean ± standard deviation of group result obtained (n = 3). Mean in the same column, having different letters of the alphabet are statistically significant ($p < 0.05$). BAC represent biosurfactant produced from *Aspergillus niger* grown on cassava broth, BAY represent biosurfactant produced from *Aspergillus niger* grown on yam broth, BAP signifies biosurfactant produced from *Aspergillus niger* grown on Sweet potato broth, BFC represent biosurfactant produced from *Fusarium oxysporum* grown on cassava broth, BFY represent biosurfactant produced from *Fusarium oxysporum* grown on yam broth, BFP represent biosurfactant produced from *Fusarium oxysporum* grown on sweet potato broth.

The engine oil area in square centimeter displaced by the biosurfactant was analyzed using SPSS version 20 and presented as Mean ± Standard Deviation below.

Table 6
Oil Displacement area (cm^2) test

BAP	BAC	BAY	BFP	BFC	BFY
8.04 ± 0.38^{e}	7.1 ± 0.11^{d}	4.9 ± 0.1^{b}	9.08 ± 0.02^{f}	5.31 ± 0.14^{c}	4.15 ± 0.15^{a}

Result represent mean ± standard deviation of group result obtained (n = 3). Mean in the same row, having different letters of the alphabet are statistically significant ($p < 0.05$).

Discussion

Three different waste (peels) samples consisting of yam, sweet potatoes and cassava showing progressive rottenness were used for fungal isolation. Two distinct fungal isolates; *Aspergillus niger* and *Fusarium oxysporum* were identified using the mycological atlas at the library of the Department Microbiology, University of Jos, Nigeria and in the Department of Microbiology, Federal University Wukari, Nigeria. This was identified after several microbiological (Figs. 1 and 2; Table. 1) and biochemical tests (Table 2). These two isolated and identified fungi, displayed biosurfactant producing ability to an appreciable extent. The potential of fusarium species to produce biosurfactant have been demonstrated by Santhappan and Pandian (2017). Muneer et al. (2013) showed that *F. oxysporum* produces biosurfactant of lipopeptide type. Also its related specie; *F. proliferatum* have been shown to produce enamide type (Bhardwaj et al., 2015). Priyam and Dinesh (2018) opined that few fungi genera including *Aspergillus spp* are good biosurfactant producers.

The Emulsification Index (EI) after 24 hours (Table 5) showed stable emulsion of produced biosurfactant with diesel depicting values with the following trends: 48.27 ± 0.99% (biosurfactant produced from *F. oxysporum* using yam peels); 48.42 ± 0.46% (biosurfactant produced by *A. niger* in yam peels broth); 42.86 ± 0.26% (biosurfactant produced from *A. niger* in sweet potatoes peels broth) and 39.29 ± 1.03% (biosurfactant produced from *F. oxysporum* in sweet potato peels). On the other hand, the stable emulsion formed with kerosene have the following E 24 values viz: 94 ± 0.10% (biosurfactant produced in *F. oxysporum* in yam peels broth); 78.72 ± 1.08% (for *A. niger* in yam peels synthesized biosurfactant) and 62.36 ± 2.90% (biosurfactant produced by *A. niger* grown on cassava peels broth) while that of *F. oxysporum* was 59.37 ± 0.85. The biosurfactant generated from *F. oxysporum* in sweet potato peels broth gave E24 value of 57 ± 1.50% whereas E24 value of *A. niger* in sweet potato peels broth biosurfactant in kerosene was 50 ± 1.99%. These values with no significant differences were maintained for 48 and 72 hours respectively.

The variation in EI may be due to variation in the level of active detergents constituents of carbon sources and bioconversion ability of fungi involved. It may also be due to the nature of the hydrocarbon from which the emulsification is being carried out and the degree of splitting of fat large globule structure in

which the produced biosurfactant may be of variance in lipolipid origin; thus effective against the specified hydrocarbons in variable degrees. The variation may also result from duration of emulsification and the pass rate of emulsification as well as active decrease of surface tension/viscosity in molecules of the petroleum hydrocarbons (kerosene and diesel). The decrease in the mean as seen in E48 (36.36 ± 0.49%) and E72 (36.36 ± 0.53%) values of biosurfactant synthesized by *Aspergillus niger* in Cassava peel broth (BAC) as well as E24 values of 36.7 ± 0.99% (biosurfactant of *F. oxysporum* in Cassava broth (BFC) and 39.29 ± 1.03% (biosurfactant of *F. oxysporum* in Sweet Potato (BFP) showed lower emulsification in diesel. This may be due to poor surface tension and poor lipopolypeptide catalysis of the biosurfactant on diesel. Apart from the above all other emulsification index were above 40% which is an indication of good solvent action and lipopeptide degrading ability of the biosurfactants. The emulsification index in this work showed a positive correlation with the concentration of the biosurfactant in solution. This is similar to the finding of Rahman (2002).

Oil drop collapse assay was effective by all the biosurfactants produced by 72 hours (Table.4). The oil displacement area (ODA) results showed that biosurfactant produced by *F. oxysporum* grown on sweet potato carbon source medium have the highest surface activity (with displaced area of 9.08 ± 0.02 cm^2), this value is attained with respect to the miscelles formation and dispersal; as large amount of the lipid of expired engine oil rearranged themselves in a spherical form in the aqueous medium. This is similar to the action of bile and gastric acid on lipases. However, *F. oxysporum* grown on yam substrate showed the least surface activity of about 4.15 ± 0.15 cm^2 displaced area. In terms of substrate specification, sweet potatoes based biosurfactants depicted better oil displacement than those of other carbon sources as seen in BFP (9.08 ± 0.02 cm^2**)** and BAP (8.04 ± 0.38 cm^2). The result of oil spreading or oil displacement test shows that the cell free supernatant of all isolates generates clear zones and oil displacement area as indication of biosurfactant production (Fig. 3).

The saponification and iodine test are biochemical assays. The entire biosurfactant tested negative with iodine. These reveal that the sole carbohydrate source utilized by the fungi isolates for biosurfactant production is not of polysaccharide constituent. The absence of blue or reddish brown complex indicates the absence of polysaccharide and possibly presence of di- or monosaccarides. On the other hand, in saponification test, the lipid layer present in the biosurfactant was saponified by NaOH. This therefore indicates the presence of lipid in the biosurfactant formed. Conclusively, it may be stated that components of the hydrocarbon bio-converted into biosurfactant are basically of lipid constituent and to a lesser degree of di- or monosaccharide origin. All the produced biosurfactant showed positive biodegradability of 2, 6-dichlorophenol indophenol (DCPIP) however, BFP showed very good biodegradability of DCPIP while BAP showed excellent biodegradability than the rest (Table 3).

Conclusion

This research highlighted the use of wastes from our immediate environment as an appropriate substrate for production of biosurfactant and therefore discovered that yam peels was able to produce a better and quality biosurfactant compared to cassava and sweet potato agro waste when viewed from the result of

the emulsification index action of the biosurfactants. However, in terms of the action on oil dispersal, biosurfactants from sweet potato had greater dispersal activity. From the method applied in the screening of biosurfactant production, the emulsification index and the oil displacement assay most especially indicate the production of biosurfactant from the fungi. Biosurfactant is a special surface active substance that attracts global market for safer environment. This research work revealed that biosurfactant produced by *F. oxysporum* in yam peels broth had the best emulsification activity while biosurfactant produced by *F. oxysporum* grown on sweet potato broth had the highest oil dispersal area. Thus, *F. oxysporum* is the ideal fungi for biosurfactant production generally from the result of this research.

The carbon sources (yam, cassava and potato peels) understudied are good substrates for biosurfactant production. *Aspergillus niger* and *Fusarium oxysporum* being fungi that utilizes carbon as its energy source can degrade them and release these surface active agents. Conclusively, the biosurfactant produced possess a substantial potential for cleansing oily wastes, especially, kerosene contaminated environment and possibly other similar petroleum hydrocarbons (PHCs).

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All the authors have given their consent through the corresponding author for this research work to be published under the Bioresources and Bioprocessing Journal.

Availability of data and materials

The data and materials collected were from the values and environment where the research was carried out. There is no dispute or ethical violation in both the data and materials used as recorded in the manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

This research work was not funded by any organization or institution. It is the sole efforts of the researchers.

Authors Contribution

CSE designed the study. SOO carried out the field/laboratory operations. Data analysis were by CSE and SOO. Supporting literature was by CSE. Preparation of manuscript was carried out by CSE, All authors read and approved the final manuscript.

Acknowledgement

The authors acknowledge the assistance of Laboratory Technologists of Biology, Biochemistry and Microbiology Departments of the Federal University Wukari, Taraba State, Nigeria. Also the inputs made by the personnel of the Microbiology Laboratory of University of Jos in identification of the fungi is hereby acknowledge.

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Figures

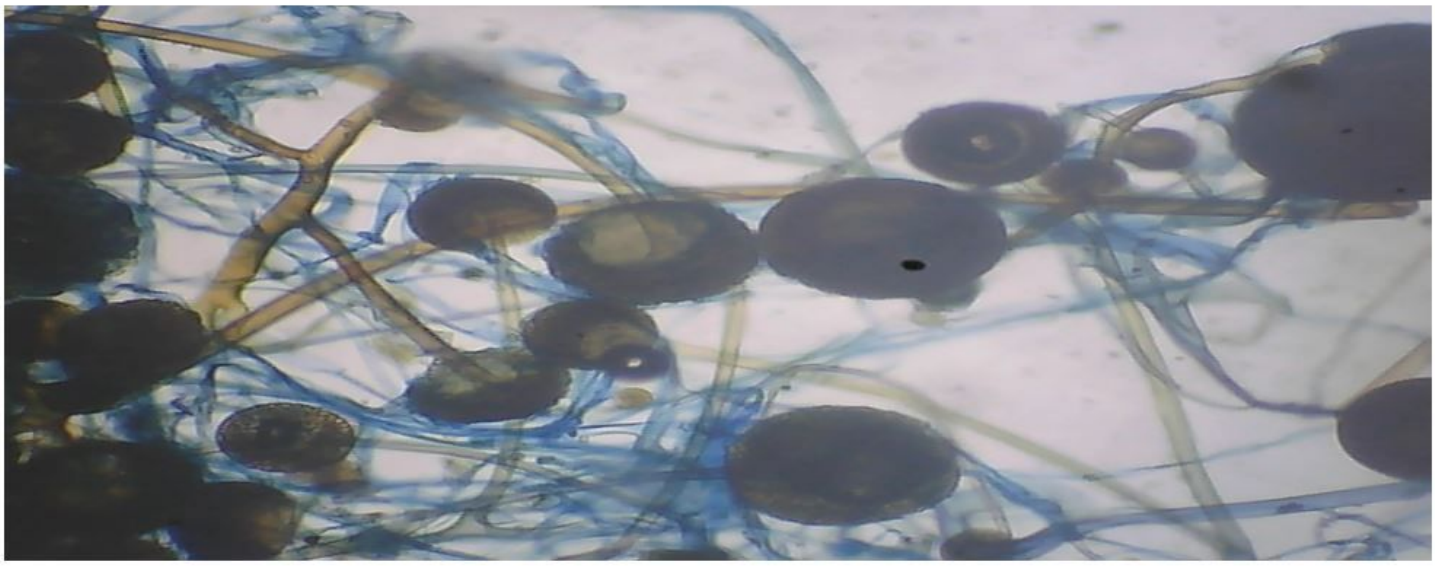

Figure 1

Microscopic photograph of Aspergilus niger

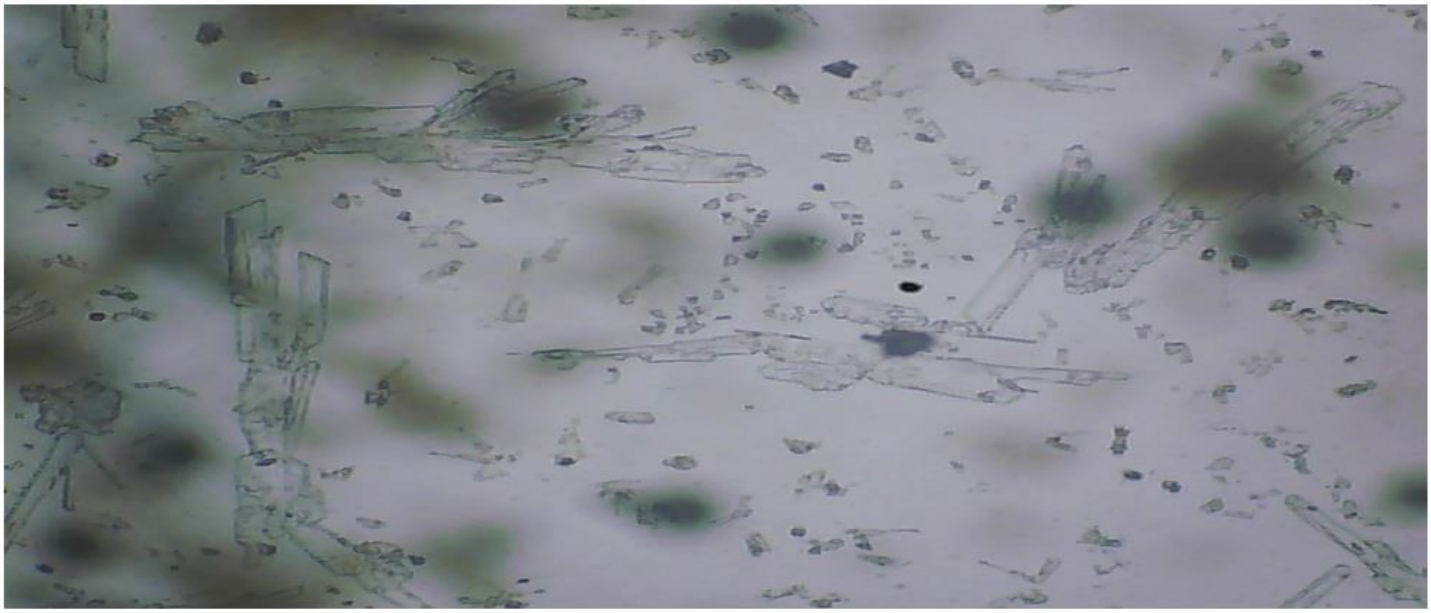

Figure 2

Microscopic photograph of Fusarium oxysporum

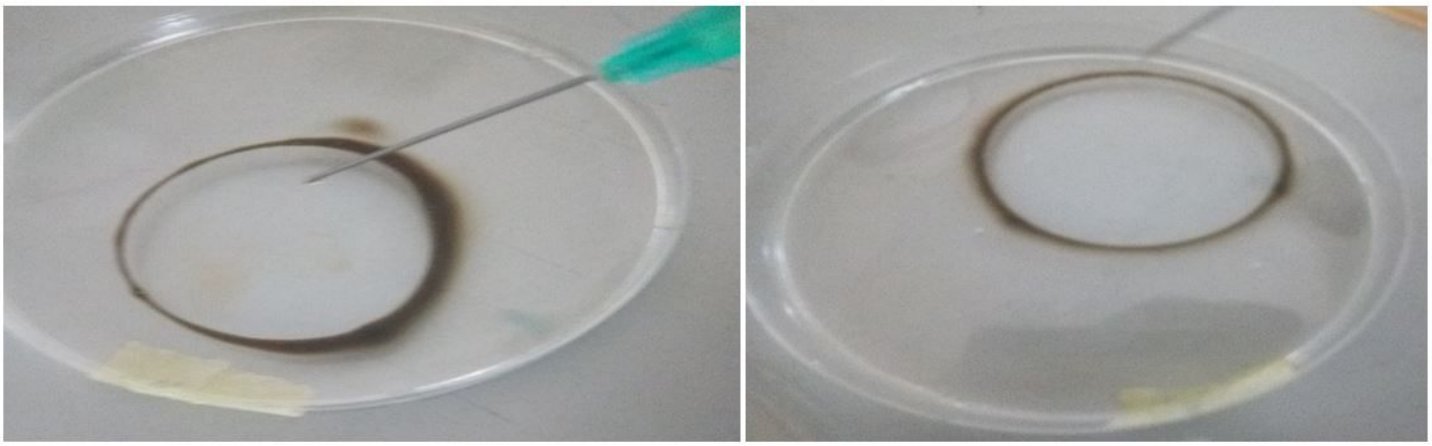

Figure 3

Oil spreading test using spent diesel oil

Supplementary Files

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