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Biodegradation Of Propanil by Fusarum Oxysparum from Rice Farm

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Abstract
Propanil is a widely used aniline contact herbicide. It has been reported that poor handling and application of propanil have adverse effects on non -target organisms and can cause water pollution through runoff, persistence of propanil and its by–by-products, human health problems, and reduced crop yields Propanil is a widely used aniline contact herbicide. It has been reported that poor handling and application of propanil have adverse effects on non-target organisms. It can cause water pollution through runoff, persistence of propanil and its by–products, human health problems, and reduced crop yields. The propanil degraders were further characterized with molecular typing using 16s rRNA sequencing. The biodegradation study was conducted for fourteen days and the residual concentration of propanil was determined using gas chromatography. The microbial examination of contaminated propanil soil showed the presence of Fusarium sp and a preliminary test showed Fusarium oxysporum. The study showed that the application of propanil to agricultural fields is safe as there are indigenous microorganisms that can degrade it and the consumption of rice grown on propanil-contaminated soil will not be hazardous to human health as the residual propanil concentration is much below the level stipulated by regulatory authorities.

Keynotes: *Propanil, Fasurum sp., Molecular, and Rice Farm.*

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I. Introduction

Substance is biodegraded to an environmentally acceptable extent, it means that its undesirable properties have been eliminated. Loss of properties are referred to as biological transformation by (Arbeli, 2007) .Propanil, also known as "Propacare" or "extonet pip-propanil," is an aniline compound resulting from the formal condensation of the carboxyl group of propanil acid with the amino group of 3,4-dichloroaniline. It is a post-emergence herbicide with no residual effect. propanil is most effective when applied to susceptible grasses and broadleaf that are small and actively growing under favorable soil moisture and weather conditions. Controling weeds using propanil removes weed competition, conserves soil moisture and generally contributes to increased crop yield. Aside from rice fields, propanil is also used for grass weed control in potato, wheat and cotton fields. Classified of Propanil is as moderately toxic (toxicity class II) due to its potential to irritate the eyes and skin. In the soil, Propanil biodegradation releases 3,4-dichloroaniline (DCA), which is further converted by microbial peroxidase into 3,3',4,4'-tetrachloroazobenzene (TCAB) and other azo products in the soil. TCAB and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) may accumulate in growing soils and leach into groundwater (Arbeli, 2007). Investigating on the influence of environmental factors on pH affect on the degradation of propanil by the isolates is crucial. The knowledge help identifing the optimal conditions that promote efficient propanil degradation, allowing for the development of tailored remediation strategies. Various industries, organic compounds are used as insecticides, herbicides, antibiotics, lubricants, or flame retardants. Pesticides, in particular, are chemical substances used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). (Droz *et al*., 2021) can be biological agents, such as viruses, bacteria, antimicrobials, or disinfectants that deter, incapacitate and kill pests . Pesticides employed in public health to eradicate disease vectors like mosquitoes, as well as in agriculture to eliminate pests that damage crops. Based on their target organisms, mode of action, duration of effectiveness, or chemistry, pesticides are categorized as insecticides (targeting insects), bactericides (targeting bacteria), fungicides (targeting fungi), herbicides (targeting plants/weeds), nematocides and rodenticides (targeting rats, mites, squirrels, woodchucks, chipmunks, nutria and beavers) (Olivera *et al*., 2015).

The aim of this study was to-biodegradate propanil by fusarum oxysparum from rice farm -in Enugu State, Nigeria.

II. Materials And Methods

Preparation of Propanil

Propanil, commonly known by its commercial names Proper care, Proper force and propanil Model, was purchased from Enugu Main Market in Enugu State. The market is a reputable supplier of agricultural chemicals, ensuring the availability and quality of the propanil products needed for the study.

Collection of Propanil Contaminated Soil Samples

Selection of Rice Farms

The soil sample was collected from Ugboawka in Nkanu East in Enugu State. The farm represented different agricultural regions and potential propanil contamination conditions.

Each selected rice farm was divided into five portions to ensure representative sampling from various areas within the farm. This division allowed for a comprehensive assessment of propanil pollution and microbial diversity within the study sites.

Triplicate soil cores were collected from each portion of the rice farms using aseptic techniques to minimize contamination. These soil cores were taken from a sufficient depth to capture the propanil-contaminated layer and were collected in sterile polyethylene bags to maintain sample integrity.

The collected soil samples underwent necessary preparation steps to ensure their suitability for subsequent analyses. The samples were sieved using a 2mm mesh to remove large debris and obtain a uniform particle size. Air-drying of the soil samples was performed to eliminate excess moisture, preventing microbial growth and preserving the physical and chemical properties of the soil..

To maintain sample quality and integrity, the soil samples were immediately transferred to the Applied Microbiology Laboratory, Enugu State University of Science and Technology (ESUT) using sterile polyethylene bags. During transport, appropriate care was taken to minimize sample disturbance and ensure that the soil samples remained representative of their original condition. Upon arrival at the laboratory, the samples were stored in suitable conditions (cool, dark place) to preserve their properties until further analysis.

Gas Chromatographic Profile of propanil before degradation:

The unused propanil was analyzed by Gas Chromatograph-Buck M910 Gas Chromatography equipped with Electron Capture Detector that allowed the detection of contaminants even at trace level concentration (in the upper µg/g and µg/kg range) from the matrix. The GC condition used were Capillary HP 88 Capillary Column (100m x 0.25µm film thickness) CA, USA.

Isolation of Heterotrophic Microorganisms from Soil Samples collected from rice farms treated with propanil using the method described by Nwankwegu and Onwosi (2017)

One gram of the sieved soil was transferred to separate test tube containing 9ml of sterile distilled water. The soil and water were vigorously shaken for 15 minutes to ensure thorough mixing and suspension of microorganisms present in the soil.

Serial dilutions of the soil suspensions were prepared to facilitate the isolation of individual microbial colonies. The soil suspensions were subjected to 10-fold dilutions in test tubes, labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} 10^{-5}…….

The serially diluted soil samples were inoculated onto media suitable for the growth of target microorganisms. Nutrient agar and MacConkey Agar were used as the primary media for bacteria and Sabouraud Dextrose Agar (SDA)was used as the primary media for fungal isolation Each diluted sample was inoculated onto the respective media in triplicates to enhance the chances of obtaining pure cultures.

The inoculated Petri dishes were incubated at an appropriate temperature (25°C for Fungi) for the growth and formation of visible microbial colonies. Incubation periods varied depending on the microbial group being targeted, typically ranging from 7 to 14 days (14 days for Fungi).

After incubation, individual colonies representing distinct morphological characteristics were picked using sterile techniques. The selected colonies were transferred onto fresh culture media, such as sabouraud dextrose agar for fungi , to establish pure cultures. Agar slants were prepared for long-term maintenance of the isolated microorganisms, with regular sub-culturing intervals to ensure viability

Biochemical test on Fungus was carried out

Morphological Identification of Fungi

Lactophenol cotton blue staining

The method as described by Geddeyya *et al*(2009) as cited by Sheehan *et al, (*2012) was used. The fungus was prepared for microscopic examination by mounting it in a drop of lactophenol cotton blue on a glass slide. The slide was carefully observed under a microscope to examine the morphological features of the fungus. Various characteristics were considered during the examination, including the shape and size of the spores, the presence or absence of septa, and the type of hyphae. Morphological features such as spore size,

shape, and coloration, as well as the structure of hyphae and the presence of septa, were carefully documented. To ensure accurate identification, the morphological features of the fungus were compared to those in a reference collection of known fungi. This comparison allowed for a detailed analysis to determine similarities and differences, aiding in the accurate identification of the fungus. Upon completion of the examination and comparison process, the fungus was identified based on its morphological features. The results were reported, documenting the key characteristics that led to the conclusive identification of the fungus in question.

III. Preliminary Test For Propanil Utilization

Preparation of Growth Media with propanil as the Sole Carbon and Energy Source:

To assess the ability of the isolates to utilize propanil as the sole carbon and energy source, the following steps were followed for the preparation of growth media: using the method described by Benslama and Boularouf, (2013). In a conical flask, 1ml of propanil was added in 99ml of mineral salt medium. A controlled medium, mineral salt medium was prepared without propanil and inoculated with the isolates singly and as consortium. Also isolates were inoculated in the mineral salt propanil medium singly and as consortium. The bacterial isolates were incubated at 37˚C for 24_- 48 hours while the fungi isolates were incubated at 30˚C for 7 days to observe growth. The utilization of propanil was observed based on the growth of the isolates on the culture media plates.

Note:

1. The mineral salt medium contained the following nutrients:
a. NaNO3: 1 g/L
b. KH2PO4: 0.5 g/L
c. MgSO4·7H2O: 0.2 g/L
d. CaCl2·2H2O: 0.01 g/L

pH: (This was done using the method described by Olowomofe *et al*, (2017).

The pH of the mineral salt propanil media was adjusted using hydrochloric acid for acid and sodium hydroxide for bases to achieve desired pH conditions. The pH levels typically ranged from acidic to alkaline, covered a wide range of values of 3,5,7,9 ,11 . The pH meter was used to monitor and confirm the accuracy of the adjusted pH levels. The isolates were inoculated into the mineral salt propanil media with adjusted pH levels. The cultures were then incubated at the specified pH conditions, maintaining the pH throughout the experiment. The incubation period lasted for 2-7 days

TABLE 1 Morphological Identification of Propanil degrading fungi

Cultural characteristics	Morphological characteristics	Suspected genera
1. Colonies are woolly cotton with cream to white aerial mycelium with cluster of conidia	Gray coloured colonies with velvety to cottony surface with septate hyphae. It has lilac to purple colour stroma.	*Fusarium Sp.*
2. White and dark purple mycelium on SDA. The spores were oval	Macroconidia were slender with three septate with a curved apical cell and notched basal cell	*Fusarium Sp.*
3. Colonies appear fibrous, compact, cottony and whitish to grayish in colour	Broad, oval-shaped dark-brown conidia with rod ends showing two nuclei. The conidia were attached to the conidiphores at the pointed end of their basal cell. The hyphaeare freely branched and septate	*Colletotrichum Sp.*

Table 2: preliminary test of Isolates of propanil utilization

Mineral salt media without propanil	Mineral salt media with propanil	Level of utilization	Suspected Organism
Fungi			
+++	-	-	*Penicillum Sp.*
	+++	Heavy	*Fusarium Sp.*
-	++	Moderate	*Fusarium Sp.*
	+++	Heavy	*Colletotrichum Sp.*
	+++	Heavy	Mixed culture

Key: - = No growth, no utilization
+ = Slight growth, slight utilization
++= Moderate growth, moderate utilization
+++ = Heavy growth, heavy utilization

Table: 3 ; Effects of pH on the degradation of propanil by the monocultures and the mixed cultures on fungi

Fungal count					
A. niger	1.26×10⁶	2.31×10⁶	2.28×10⁶	1.08×10⁶	1.02×10⁶
A. niger	2.10×10⁶	2.0×10⁶	2.19×10⁶	1.20×10⁶	1.23×10⁶
F. Scirpi	1.23×10⁶	1.71×10⁶	1.6×10⁶	1.40×10⁶	1.02×10⁶
F. Oxysporum	1.01×10⁶	1.98×10⁶	1.75×10⁶	1.70×10⁶	1.30×10⁶
C. Siamense	1.50×10⁶	2.21×10⁶	2.18×10⁶	2.50×10⁶	2.30×10⁶
Table 4 : Effects of pH on the degradation	*1.23×10⁶*	*2.32×10⁶*	*1.80×10⁶*		

Effect of moisture content on the degradation of propanil by the monoculture and mixed cultures.

Concentration (ml)+1ml of propanil	*Microbial count (cfu/ml)*				
	S1	*S2*	*SC*	*F1*	*F2*
100	*3.51X10⁶*	*2.24X10⁶*	*3.36X10⁶*	*3.84X10⁶*	*3.53X10*
90	*3.42X10⁶*	*2.01X10⁶*	*3.07X10⁶*	*3.62X10⁶*	*2.95X10*
80	*2.83X10⁶*	*1.75X10⁶*	*2.51X10⁶*	*2.91X10⁶*	*2.64X10*

SC – Mixed Culture
F1 – *Aspergillus niger*
F2 – *Aspergillus niger*
F3 – *Fusarium Scirpi*
F4 – *Fusarium Oxysporum*
F5 – *Colletotrichum Siamense*
FC – Mixed culture

IV. Results

Monitor of the degradation of the propanil by Fusarum oxysporum

Benslama and Bolahrouf method was used. Each isolated microorganism was individually inoculated into different test tubes containing 10 mls sabourand dextrose broth for fungi.

The broths were incubated for 3-5 days thereafter 0.1ml aliquot of 10^{-4} dilution factor was collected from each tube and put into different 250ml Erlene Meyer flasks.

The flask containing 99ml of Msm with 1ml (63.16µg/ml) of propanil; 98.5ml of Msm with 1.5ml of propanil and 98ml of Msm with 2mls of propanil individually.

Furthermore they were incubated at 150rpm in rotary shaker for 14days at 30˚C. After 14days the residual particles were determined.Mixed culture degradation: using Benslama and Boularouf method 2013.

All of fungal isolates were also inoculated into one test tube containing 10 ml of SDA broth and incubated for 72 hrs. A 0.l ml aliquot of 10^{-4}dilution was collected from individual culture tubes (fungi) and put into two different250ml flask containing 99ml of Msm with 1m(63.16µg/ml) of propanil. They were incubated along with the monoculture for 14days in rotary shaker at 150rpm at 30˚C. After 14 days both result s were sent for gas chromatography.

Determination of Residual propanil after Degradation by monoculture and mixed cultures.

The extent of degradation of propanil by the isolates was studied by performing a Gas chromatography –Mass Spectrometer (GC-MS) analysis of the residue from cultures that were grown for 14 days.

Preparation of Standard

A 10µl aliquot of ACC standard was injected in the chromatographic instrument and the retention time compared with retention time compared with retention time of standard.

Gas Chromatographic-conditions for propanil determination

Gas Chromatographic-Buck M910 scientific gas chromatography equipped with Electron even at trace level concentrations from the matrix.

The Gas conditions used were capillary column HP 88 capillary column (100m × 0.25µm film thickness) CA, USA.

The injection and detector temperature were set at 25˚C and 29˚C respectively. The oven temperature was programmed as 110˚C held for 10 min, ramp at 10˚C/min to 200˚C held for 5 min and ramp at 10˚C/min to 320˚C. Helium was used as carrier gas at a flow rate of 1.0ml/min and detector make-up gas of 29ml –min-1 whereas the total run time sample for a sample was 48min.

Quantification of Pesticide residue.

The residue levels of pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincided with the standards were extended to their corresponding calibration curves to obtain the concentration.

Determination of residual propanil degradation of the harvested rice grains

Harvested rice grains from the experimental plots were collected, 10g of the rice grains were prepared by removing contaminants/ impurities and transferred to a suitable container for extraction.

Solvent extraction methods were used to extract propanil from rice grain. Half of the concentrated extract was subjected to gas chromatograph-mass spectrometry (GC-Ms) analysis.

V. Discussion

Pesticides play a crucial role in modern agriculture by controlling pests and increasing crop yields. (Abdullahi et al 2018) also used in crop protection, soil treatment and post-harvest storage. Propanil, an herbicide widely employed on rice cultivation has been detected on surface water and. soil. The usage of propanil in rice farms has led to weed suppression, promoting healthy rice growth and increasing crop yield (Singh et al 2020). (Akpan and Chukwuma 202) said propanil has helped rice plants to get access to essential resources like sunlight, water and nutrients thereby increasing their productivity. Present study also isolated other fungi propanil degraders as *Fusarium oxysporum* ,*Fusarum scirpi* , and *Colletotrichum siamese*. Normally fungi are nutrient cyclers, pathogens and multualists that associate with plants and other organisms. They are also decomposers (Lahlali et al 2022). (Abdei-megeed et al 2010) studies have shown that the presence of *Aspergillus niger* increases the degradation rate of propanil contaminated soil thereby aiding in bioremediation efforts. According to Li and Zhany (2018), *Aspergillus niger* secrets enzymes that break down propanil into less toxic compounds. The degradation of propanil by *Aspergulus niger* helps in mitigating the adverse effects of this herbicide on non target organisms including the beneficial soil microorganisms and plants (Fazi, S., Amalfitano et al 2015). *Aspergillus niger* has fast growth rate and can produce significant biomass quickly.can thrive in various environmental conditions such as wide range of pH levels and temperature (Meyer 2008).

Previous studies reported *F.oxsporum* to colonize the rice root and had the highest growth rate in crude oil (Nunez - cano et al 2023).

Table 2 showed the preliminary test of other isolates that can utilize propanil. *Fusarum* species have shown potential in degrading propanil, this agreed with the work of Wang et al (2023) that demonstrated *Fusarum sp* as metabolizer of propanil through specific enzymatic pathways. Key enzymes involved are amidases and dioxygenases degrade propanil into less harmful substance (Wang et al 2023). Additionally, This metabolic flexibility allowed the fungus to thrive in environment where propanil is present, making it an effective agent for bioremediation in agricultural soils (Gomez - De et al 2023). *Fusarium oxysporum* forms

synergistic relationship with other soil microorganisms, creating a microbial consortium that enhanced the overall degradation efficiency of propanil (Ellis et al 2023; Zhelezova et al 2023).

VI. Conculusion:

Fungi play major role in breakdown and mineralization of contaminants introduced into the environments. Some fungi possess the natural ability to degrade propanil and Convert it into less toxic metabolites. This study has suceeded in isolating and identifying the fungi that are able to breakdown propanil introduced in rice Soil is Enugu State, Nigeria.

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