

SCREENING AND CHARACTERIZATION OF POTENTIAL CYPERMETHRIN-DEGRADING BACTERIA VIA PHENOTYPIC AND MOLECULAR TECHNIQUES

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Abstract The need for sustainable management of cypermethrin, a widely used synthetic pesticide with significant ecological impact, calls for urgent concerns among environmental stakeholders. Therefore, in this study, the potential cypermethrin-degrading (PCD) bacteria were screened and identified from the soil samples collected from the Cowpea farm of Teaching and Research farm of Kwara State University, Nigeria. The bacterial strains were screened and isolated through enrichment techniques and identified using the standard phenotypic and molecular techniques. The potential for cypermethrin utilisation was determined in the cypermethrin-treated mineral salt medium. The three PCD bacterial strains isolated were PCD1 (*Lysinibacillus fusiformis*), PCD2 (*Bacillus sonorensis*), and PCD3 (*Achromobacter* sp.). Molecular characterization confirmed the identities and revealed high sequence similarity with known species. The accession numbers for these strains are MF973057 (PCD1), MF973058 (PCD2), and MF973059 (PCD3). The growth-dependent utilization of cypermethrin using the optical density values (OD 600 nm) showed the highest OD value on day six (PCD2: 0.60 and PCD3: 0.65) and day nine (PCD1: 0.80) respectively. The observed increases in optical density (OD 600 nm) affirmed their potential for cypermethrin degradation. While the strains exhibit promise in metabolizing cypermethrin, further research is needed to assess their suitability for bioremediation.

Keywords: Cypermethrin, Phenotypic, enrichment; *Lysinibacillus fusiformis*, *Bacillus sonorensis*

I. INTRODUCTION

The discovery and application of pesticides have saved humans from problems associated with pest infestation [1]. Cypermethrin, a synthetic pyrethroid pesticide widely employed in agricultural practices, plays a pivotal role in modern agrochemical strategies of pest control and management systems [2; 3]. While the use of pesticides such as cypermethrin has been instrumental in enhancing crop yields and mitigating pest-related damages, its toxicity and persistence in the environment raise concerns about its potential ecological impact [4; 5].

The accumulation of cypermethrin in environmental entities such as soil and water systems can lead to adverse effects on non-target organisms, disrupting ecosystems and posing a threat to biodiversity [6; 7]. The urgent need to address the environmental effects of cypermethrin usage has spurred research efforts to explore sustainable and eco-friendly approaches to its degradation.

Microorganisms through their unique adaptation and inherent metabolic versatility can degrade and remediate pollutants that have been ravaging the complex web of different ecosystems [8]. Protecting the delicate balance of nature is crucial, especially when considering the increasing rise in synthetic contaminants. Identification and characterization of

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microorganisms is one of the most important aspects in the field of microbiology. Phenotypic traits such as morphological, physiological and cultural characteristics are among the classical features used in the identification of microorganisms [9]. However, with the advent of molecular biology techniques, the genotypic methods based on Deoxyribonucleic acid (DNA) offer more precise and best tools for microbial identification in the environment [10].

Phenotypic profiling of microorganisms involves the evaluation and analysis of observable characteristics of microorganisms, such as growth patterns, morphology, and metabolic activities [11]. Therefore, characterizing bacteria based on observable traits provides benefits such as direct observation, quantification, and measurement of cultured cells without requiring expensive technology [12]. Genotypic profiling plays a crucial role in modern microbiology by allowing researchers to delve deep into the genetic composition of microorganisms. Genotypic profiling enables the examination of genetic markers within bacterial strains for their identification or functional profiling. Through techniques such as DNA sequencing and polymerase chain reaction (PCR), genetic markers unique to each microorganism can be identified and analyzed [13]. This provides invaluable information for the precise identification of the organisms and the assessment of their functional attributes. The integration of both phenotypic and genotypic approaches can enhance the precision and reliability of identifying bacteria species from the environment.

The identification of bacterial strains with potential cypermethrin-degrading capabilities

can pave the way for the formulation of microbial consortia, which could enhance the degradation efficiency of toxic chemicals in complex environmental matrices. As the global community grapples with the challenges of sustainable development, the adoption of eco-friendly strategies for managing pollutants is imperative. The screening and characterization of potential cypermethrin-degrading bacteria through phenotypic and molecular characterization represent a vital step towards developing environmentally friendly approaches for pesticide remediation.

II. MATERIALS AND METHODS

A. The study area

The study was conducted using soil samples from the research and teaching farm of Kwara State University, Malete which is situated within latitude $08^{\circ} 7^1$ North and Longitude $04^{\circ} 44^1$ E and had a history of repeated cypermethrin input for cowpeas insect infestation control [14].

B. Chemicals used

The commercial formulation of cypermethrin was procured from an agrochemical vendor in Ilorin, Kwara State, Nigeria. All the reagents and chemicals used in this study were of analytical grade and purchased from the Central Research Laboratory, Ilorin, Kwara State, Nigeria.

C. Soil sample collection and preparation

The composite surface soil sample (0-15 cm layer) used for the analyses was collected from the Cowpea farm of Teaching and Research farm of Kwara State University, Malete, Nigeria in September 2020. The soil composition comprises 29.0 % total organic carbon content,

9.6 g/kg phosphorus content, 7.1 pH value, and 18.3 % moisture content [14].

The composite sample was pretreated by removing plant debris and fauna through hand-picking. The samples were gently sieved through a mesh of about 2 mm fraction and spiked with 25 % w/v cypermethrin [32]. The sample was stored in a sterile polythene bag at 4 °C for further study.

D. Isolation of potential cypermethrin-degrading bacteria

The potential cypermethrin-degrading bacterial groups were isolated following the techniques of International Standard Organization method 7827 [15; 16]. The isolation was carried out using enrichment techniques in a mineral salt medium, “MSM” (1.8g K₄H₂SO₃; 4.0g NH₄Cl; 0.2g MgSO₄; 0.1g NaCl; 0.01g FeSO₄; 1L dH₂O; 15g/L agar) where cypermethrin serves as sole carbon source.

Five grams of composite soil was introduced into 95 ml of MSM in an Erlenmeyer flask. Cypermethrin was added as the sole source of carbon. The initial concentration of cypermethrin in the enrichment medium was 0.75 mg/L and subsequently, the medium was spiked with cypermethrin to give a final concentration of 3 mg/L. The flask was incubated at room temperature (27 ± 2 °C) on a benchtop shaker (MaxQ 4450 Model) at a speed of 100 rpm for one week.

From the enriched samples, repeated enrichment was carried out for another three weeks by transferring 5 ml of the culture broth into a sterile flask containing 95 ml of mineral salt medium (MSM) spiked with 3 mg/L cypermethrin. The culture was further incubated and kept at room temperature on a benchtop shaker at 100 rpm.

After four successful transfers, a loopful of the enriched culture broth was inoculated on MSM supplemented with agar (15g/L agar) and incubated at room temperature (27 ± 2 °C) for 48 h.

Repeated sub-culturing was carried out on nutrient agar and pure isolates were stocked on agar slants for further studies.

E. Growth-dependent screening for cypermethrin utilization

The bacterial isolates were screened for their ability to utilize cypermethrin based on their growth in cypermethrin-spiked mineral salt medium (MSM) [15].

The suspension of the isolates from the stock culture was standardized with 0.5 McFarland standard. Then, 5 ml of the standardized aliquots of the isolates were introduced into a flask containing 50 ml of MSM broth spiked with cypermethrin (3 mg/L). The MSM broth medium without the cypermethrin, inoculated with 5 ml of the respective isolates serves as the control. All the set-ups were prepared in three replicates and incubated on a benchtop shaker (100rpm) at room temperature. The growth rate was determined by measuring the optical density (OD 600nm) with a spectrophotometer (UV/Vis 721D Model) at 3-day intervals for fifteen days.

F. Identification of potential cypermethrin-degrading bacterial isolates

The potential cypermethrin-degrading bacterial isolates were characterized based on their phenotypic features (morphological and biochemical characteristics) using the standard identification criteria [17]. The features used for identification tentatively include Gram staining,

spore staining, motility test, catalase test, oxidase test, citrate utilization, starch hydrolysis, urease test, indole test, methyl red test and Voges-Proskauer test [17].

The isolates were further subjected to molecular characterization for proper identification.

G. Molecular characterization of potential cypermethrin-degrading bacterial isolates

Molecular characterization of potential cypermethrin (PCD) degrading bacterial isolates was carried out by sequencing the 16S rRNA gene of the isolated bacteria [18].

The nucleic acid contents of the pure overnight culture of potential cypermethrin degrading bacterial isolate were isolated using the phenol-chloroform technique[19]. The gene fragments specific for the highly variable region of the bacterial 16s rRNA gene were amplified by polymerase chain reaction (PCR) using the universal primer 16S forward primer (5'-GGACTACAGGGTATCTAAT - 3') and reverse primer (3'-GGACTACAGGGTATCTAAT - 5').

The purified nucleotide sequence products were sequenced using the automated Sangers dideoxy method. The sequences were edited by the bioinformatics software Chromas. The homology of the 16S rRNA gene sequences was checked with the 16S rRNA gene sequences of other organisms that had already been submitted to the Gene Bank database using the basic local alignment search tool for nucleotide (BLASTN) algorithm. The phylogenetic tree was created using the neighbour-joining method with Molecular evolutionary genetics analysis software (MEGA 11).

III. RESULTS AND DISCUSSION

A. Results

i. Characterization of potential cypermethrin degrading bacterial isolates.

The phenotypic features of the potential cypermethrin-degrading bacterial isolates (PCD) are presented in Table 1.

Only two of the isolates (PCD1 and PCD2 respectively) were Gram-positive rods with the presence of spore, while the remaining isolate (PCD3) was a Gram-negative rod (Table 1).

All the isolates showed positive results for the motility test. The catalase and oxidase tests were positive for all the isolates but none of the isolates tested positive for urease, indole, methyl red and Voges Proskauer tests (Table 1).

Two of the isolates (PCD2 and PCD3) were positive for the citrate utilization test, and only one of the isolates (PCD2) tested positive for the starch hydrolysis test (Table 1).

Based on the phenotypic features the probable isolates of potential cypermethrin degrading bacteria were 2 *Bacillus* species and 1 *Achromobacter* species.

ii. Molecular identification of potential cypermethrin degrading bacterial species.

The amplified DNA of the potential cypermethrin-degrading bacterial isolates is shown in Plate 1. The neighbour-joining

Table 1: Phenotypic features of potential cypermethrin degrading bacterial isolates.

Characteristics	Bacterial Isolates		
	PCD1	PCD2	PCD3
Gram reaction	+	+	-
Cell shape	rod	Rod	rod
Endospore	Central	subterminal	absence
Motility	+	+	+
Catalase test	+	+	+
Oxidase test	+	+	+
Citrate utilization	-	+	+
Starch hydrolysis	-	+	-
Urease test	-	-	-
Indole test	-	-	-
Methyl red test	-	-	-
Voges proskauer test	-	-	-
Probable Isolates	<i>Bacillus</i> species	<i>Bacillus</i> species	<i>Achromobacter</i> species

Keys: positive (+); negative (-); PCD (Potential cypermethrin degrading)

phylogenetic tree of partial 16s rRNA gene sequence of potential cypermethrin degrading (PCD) bacterial isolates is shown in Figure 1. The comparison of the sequenced results of the 16s rRNA gene of the isolates with previously published 16s rRNA gene of bacterial nucleotide on the National Center for

Biotechnology Information (NCBI) database revealed that the isolates belong to the following genera; *Lysinibacillus* (PCD1), *Bacillus* (PCD2) and *Achromobacter* (PCD3); (Figure 1).

The isolate PCD1 showed the highest percentage homology (99%) with the partial 16s

rRNA gene sequence of *Lysinibacillus fusiformis* strain NBRC15717 with accession number NR112569. The isolate PCD1 formed a phylogenetic cluster with *L. fusiformis* (Figure 1).

The isolate PCD2 showed the highest percentage homology (99%) with the partial 16S rRNA gene sequence of *Bacillus sonorensis* strain NBRC101234 with accession number NR113993. The isolate PCD2 formed a phylogenetic cluster with *B. sonorensis* (Figure 1).

The sequence from the isolate PCD3 exhibited the highest gene sequence similarities of 96% with *Achromobacter xylosoxidans* strain Hugh2838 with accession number NR04492, *A. rublandii* strain EY3918 with accession number NR027197 and *A. denitrificans* strain NBRC15125 with accession number KY174335 respectively.

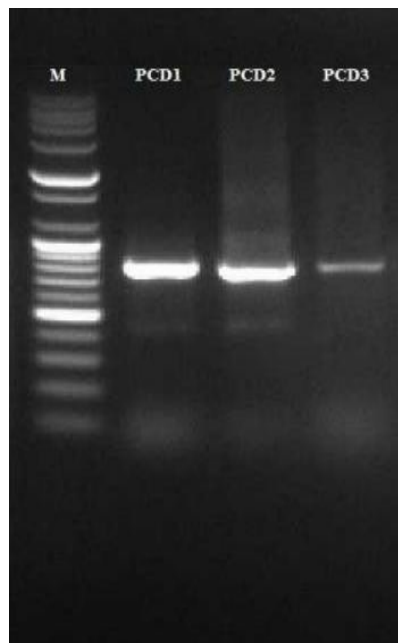


Plate 1: 16S rDNA amplified nucleic acid product of bacterial isolates PCD1, PCD2 and PCD3

Key: PCD means potential cypermethrin degrader; M represents the molecular marker

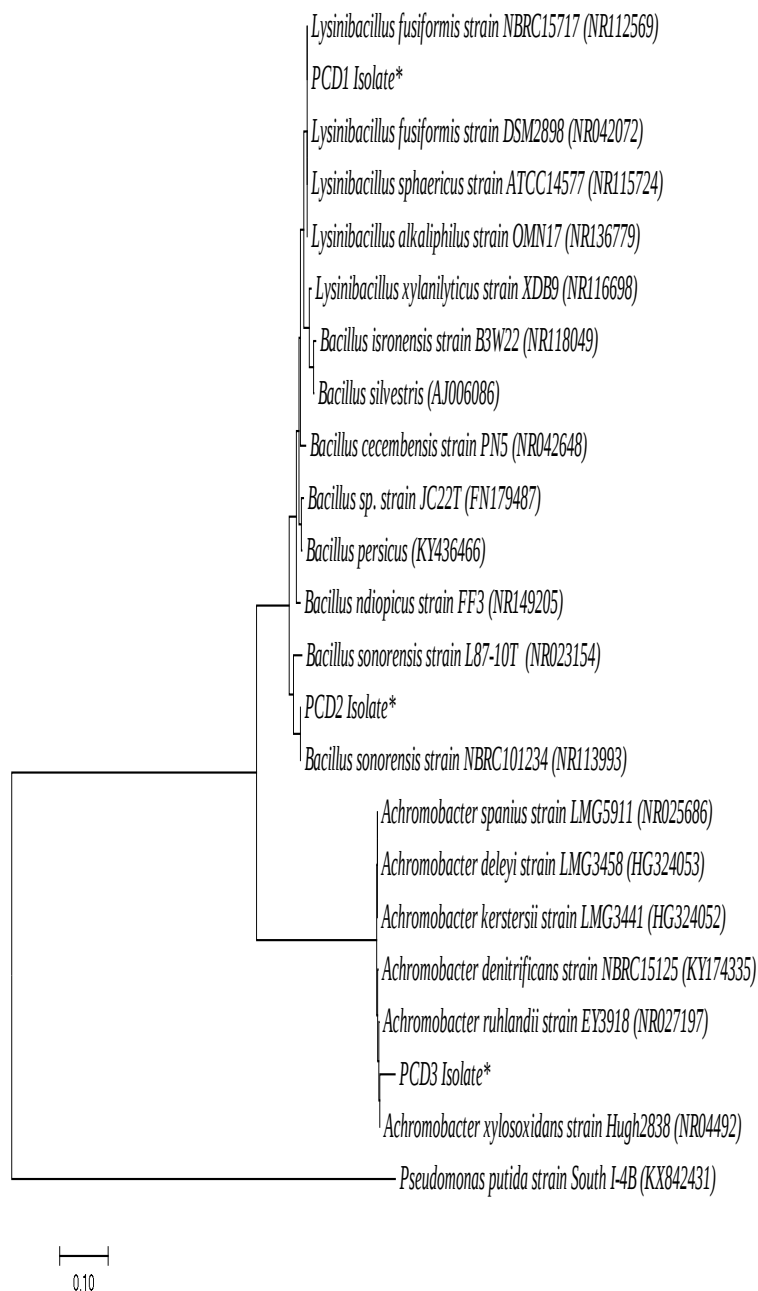


Figure 2: Phylogenetic relationship of the potential cypermethrin degrading (PCD) bacterial isolates

Key:* indicate the quarry isolates; PCD1: *Lysinibacillus fusiformis* (MF973057), PCD2: *Bacillus sonorensis* (MF973058), and PCD3: *Achromobacter* sp. (MF973059).

iii. Growth-dependent screening for cypermethrin utilization

The result for the growth-dependent utilization of cypermethrin determined by OD values at 600 nm is shown in Figure 1. The increases in

the OD values were observed for all the isolates on the third day (Figure 1). The highest OD values were recorded on the ninth day for the PCD1 (0.80) and the sixth day for the PCD2 (0.60) and PCD3 (0.65) respectively (Figure 1).

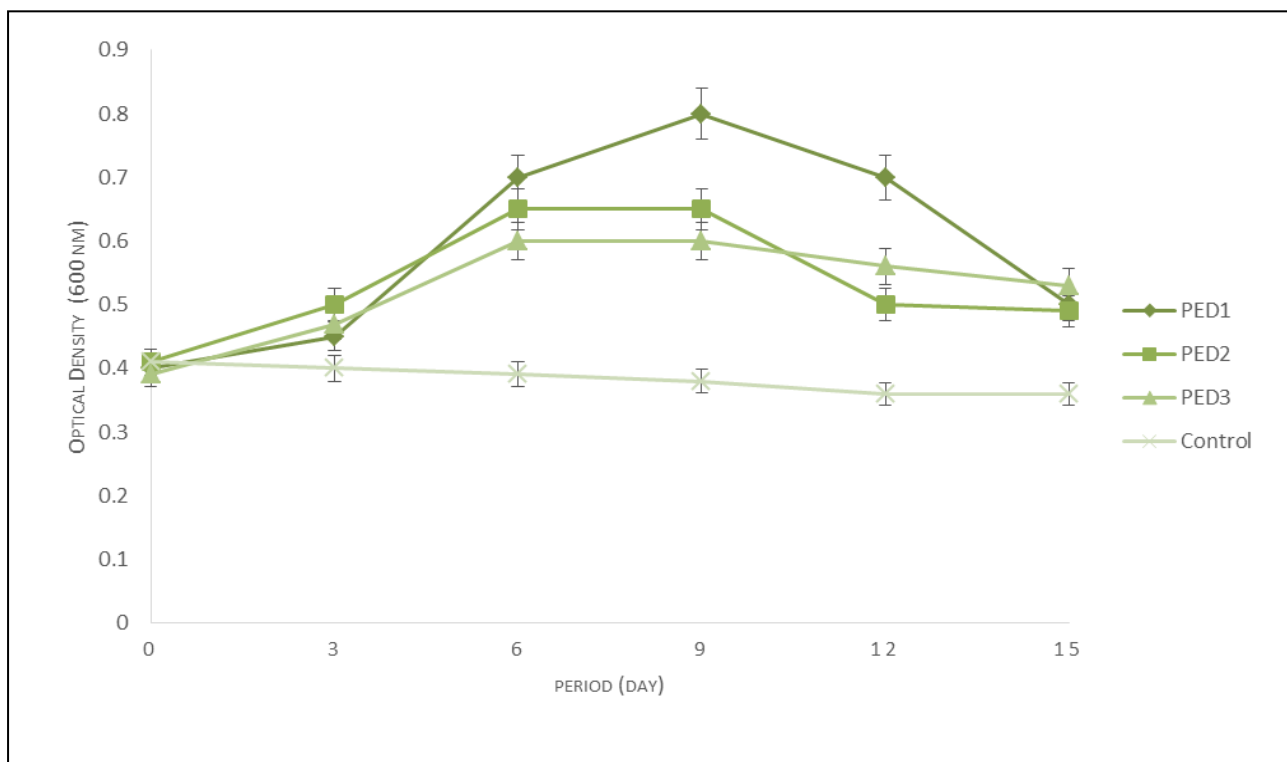


Figure 1: Growth-dependent screening for cypermethrin utilization

Key: PCD potential cypermethrin degrading bacteria

A. Discussion

The concern about the possibility of synthetic agrochemicals causing non-target effects on other organisms in the environment has been one of the critical issues among environmental stakeholders. Therefore, to reduce the environmental and public health risks associated with pesticide use, it is necessary to develop methods to remove or minimize the concentration in the environment. Among various approaches for the remediation of a

contaminated environment, the biological technique offers the most promising and effective strategy due to its cost-effectiveness and capability to turn toxic chemicals into innocuous components [20]. Evidence has shown that the success of bioremediation depends on isolating microorganisms capable of degrading the pollutant compounds [21] and contaminated sites are considered to be a good source for the isolation of pollutant-degrading microorganisms [22].

Soil bacteria possess diverse catabolic pathways for the degradation of chemical pollutants and this potential can be exploited to design an effective bioremediation strategy [23; 24]. Hence, the indigenous bacterial strains capable of metabolizing cypermethrin were isolated in this study.

Morphological, biochemical and molecular characterization indicated that the isolates belong to the genus *Lysinibacillus* (PCD1), *Bacillus* (PCD2) and *Achromobacter* (PCD3). Phylogenetic analysis constructed through the neighbour-joining method showed that the respective genus was the closest relative of the isolates. On the standard means of prokaryotic classification based on molecular technique, gene sequences with percentage similarity values of $\geq 95\%$ and $\geq 99\%$ could be utilized for classifying prokaryotes into the same genus and species, respectively [25]. Thus, sequence analysis revealed that PCD1 and PCD2 shared 99% homology with *Lysinibacillus fusiformis* strain NBRC15717 and *Bacillus sonorensis* strain NBRC101234 respectively. Thus, isolated PCD1 and PCD2 were identified as *L. fusiformis* strain PCD1 and *B. sonorensis* strain PCD2 respectively. The percentage homology value of the 16S rRNA gene sequence of PCD3 (96%) with their respective closest relative confirmed that this isolate belongs to the genus *Achromobacter* with properties different from those that were on the database. Thus, PCD3 was identified as *Achromobacter* sp. strain PCD3. The nucleotide sequences of the 16S rRNA gene of the isolates were deposited in the GenBank database under the submission code "SUB3052529" with the accession numbers MF973057 (PCD1), MF973058 (PCD2) and MF973059 (PCD3) respectively.

The report has revealed that the genus *Lysinibacillus*, which was previously classified as *Bacillus* based on phenotypic characteristics, is known for its remarkable capability to produce endospores [26]. Furthermore, it has been discovered that *Lysinibacillus* strains play a crucial role in the biodegradation of persistent substances in the environment [27]. The genus *Achromobacter*, which belongs to the class Betaproteobacteria and the family Alcaligenaceae, has been found in various environments such as water, soil, plant tissues, and clinical samples [28]. Previous research has shown that certain strains of *Achromobacter* can bioremediate xenobiotic pollutants in the environment. Many characteristics of this bacterial genus have been documented, including its capability to degrade substances such as arsenite, biphenyl, haloaromatic acids, and polycyclic hydrocarbons [29].

The increases in the population of PCD isolates with respect to the optical density (OD 600 nm) further confirmed the potential capability of the bacterial isolates to degrade cypermethrin. Reports have confirmed the correlation between the increases in optical density values and the population increases of bacterial cells [16; 30]. Although studies have shown the possibility of the strains of the bacteria to metabolize different persistent organic pollutants [31-33], further studies are required to determine the suitability of PCD strains in the bioremediation of cypermethrin.

IV. CONCLUSION

In this study, three potential cypermethrin-degrading (PCD) bacterial strains were isolated: PCD1, PCD2 and PCD3 respectively. Through standard phenotypic and molecular characterization, the bacteria were identified as

Lysinibacillus fusiformis strain PCD1, *Bacillus sonorensis* strain PCD2, and *Achromobacter* sp. strain PCD3.

The accession numbers of the isolates were MF973057, MF973058 and MF973059 for *Lysinibacillus fusiformis* strain PCD1, *Bacillus sonorensis* strain PCD2, and *Achromobacter* sp. strain respectively.

The increases in optical density values further confirmed the potential capability of the isolates to utilize cypermethrin. Further study is required to evaluate the suitability of these strains for bioremediation purposes.

REFERENCES

- [1] Madaki, M.Y., Lehberger, M., Bavorova, M., Igbasan, T. I. and Kachele, H. "Effectiveness of Pesticide Stakeholders' Information on Pesticide Handling Knowledge and Behaviour of Smallholder Farmers in Ogun State, Nigeria", *Environment, Development and Sustainability*, 2023, pp1-20.
- [2] Aborisade, W. T. and Atuanya, E. I. "Ecotoxicological Studies of Pesticide Formulations on Soil Sentinel's Microflora (*Nitrosomonas* and *Nitrobacter* spp.) and Mesofauna (*Ephyriodrilus* spp.)", *International Journal of Biological Innovations*, vol. 5, Number 1, 2023, pp 1-13.
- [3] Scheepers, L. D., Freercks, R. and Merwe, E. V. "Acute Cypermethrin and other Pyrethroid Poisoning - An Organophosphate-Like Poisoning", *Toxicology Reports*, vol. 11, 2023, pp 107-110.
- [4] Tudi, M., Daniel-Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C. and Phung, D. T. "Agriculture Development, Pesticide Application and Its Impact on the Environment", *International Journal of Environmental Research and Public Health*, vol. 18, Number 3, 2021, pp 1-23.
- [5] Devi, P. I., Manjula, M. and Bhavani, R. V. "Agrochemicals, Environment, and Human Health", *Annual Review of Environment and Resources*, vol. 47, 2022, pp 399-421.
- [6] Aborisade, W. T. and Atuanya, E. I. "Effects of an Organophosphate (Glyphosate) and a Quaternary Ammonium (Paraquat) herbicides Formulation on Soils' Culturable Bacterial and Fungal Populations", *International Research Journal of Biological Sciences*, vol. 11, Number 1, 2022, pp 1-11.
- [7] Casado, N., Berenguer, C. V., Camara, J. S. and Pereira, J. A. M. "What Are We Eating? Surveying The Presence of Toxic Molecules in The Food Supply Chain Using Chromatographic Approaches", *MDPI Journal of Molecules*, vol. 29, Number 3, 2024, pp 1-34.
- [8] Pathak, V. M., Verma, V. K., Rawat, B. S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N. and Cunill, J. M. "Current Status of Pesticide Effects on The Environment, Human Health and Its Eco-Friendly Management As Bioremediation: A Comprehensive Review", *Frontier of Microbiology*, vol. 13, 2022, pp 1-29.
- [9] Bekele, G. K., Gebrie, S. A., Mekonen, E., Fida, T. T., Woldeamayrat, A. A., Abda, E. M., Tafesse, M. and Assefa, F. "Isolation and Characterization of Diesel-Degrading Bacteria from Hydrocarbon-Contaminated Sites, Flower farms, and Soda lakes", *Hindawi International Journal of Microbiology*, 2022, pp 1-12.
- [10] Buszewski, B., Rogowska, A., Pomastowski, P. Zloch, M. and Railean-Plugaru, V. "Identification of Microorganisms by Modern Analytical Techniques", *Journal of AOAC International*, vol. 100, Number 6, 2017, pp 1607-1623.
- [11] Franco-Duarte, R., Cernakova, L., Kadam, S., Kaushik, K. S., Salehi, B.,

- Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stepien, K., Leszczewicz, M. Relison-Tintino, S., Alexandrino-de- Souza, V. C., Sharifi-Rad, J., Coutinho, H. D. M., Martins, N. and Rodrigues, C. F. "Advances in Chemical and Biological Methods to Identify Microorganisms from Past to Present. *MDPI Journal of Microorganisms*", vol. 7, Number 5, 2019, pp 1-32.
- [12] Zhang, Z., Shah, A. M., Mohamed, H., Tsiklauri, N. and Song, Y. "Isolation and Screening of Microorganisms for the Effective Pretreatment of Lignocellulosic Agricultural Wastes", *Hindawi Biomedical Research International*, 2021, pp 1-16.
- [13] Alown, F., Alsharidah, A. and Shamsah, S. "Genotypic Characterization of Soil Bacteria in the Umm Al-Namil Island, Kuwait", *Saudi Journal of Biological Sciences*, vol. 28, Number 7, 2021, pp 3847-3854.
- [14] Aborisade, W. T., AbdulSalam, Z. B. and Abdulmumini, S. A. "Effects of Seed Coating Agents on Some Agronomic Properties and Rhizobacterial Population of *Vigna unguiculata*. *Badeggi Journal of Agricultural Research and Environment*, vol. 3, Number 2, 2021, pp 28-36.
- [15] Atuanya, E. I. and Ekanem, N. O. "Degradative Potentials of Indigenous Bacteria from Rubber Processing Factory Effluent", *Journal of Advance Medicine and Pharmaceutical Sciences*, vol. 2, Number 2, 2008, pp 87-92.
- [16] Aina, O. R., Atuanya, E. I., Oshoma, C. E., Omotayo, A. E. and Olaleye, O. N. (2021). "Biodegradation Potential of Rhizospheric Microorganisms of *Rhizophora Racemosa* in Crude Oil Contaminated Mangrove Swamp In The Niger Delta" *African Journal of Health, Safety and Environment*, vol. 2, Number 2, 2021, pp 91-102.
- [17] Cheesbrough, M. "District Laboratory Practice in Tropical Countries, Low Price Edition Part 2. Cambridge University Press London" 2000, Pp. 434.
- [18] Harisha, S. "Biotechnology Procedures and Experiments Handbook. Infinity Science Press LLC, Hingham, Massachusetts", 2007, Pp. 710.
- [19] Wright, M. H., Adelskov, J. and Greene, A. C. "Bacterial DNA Extraction Using Individual Enzymes and Phenol/Chloroform Separation", *Journal of Microbiology and Biology Education*, vol. 18, Number 2, 2017, pp 1-3.
- [20] Akbar, S., Sultan, S. and Kertesz, M. "Bacterial Community Analysis of Cypermethrin Enrichment Cultures and Bioremediation of Cypermethrin Contaminated Soils", *Journal of Basic Microbiology* vol. 55, 2015, pp 819-829.
- [21] Moneke, A. N., Okpala, G. N. and Anyanwu, C. U. "Biodegradation of Glyphosate Herbicide In Vitro Using Bacterial Isolates from Four Rice Fields" *African Journal of Biotechnology*, vol. 9, Number 26, 2010, pp 4067-4074.
- [22] Bhatt, P., Sharma, A., Gangola, S., Khati, P., Kumar, G. and Srivastava, A. "Novel Pathway of Cypermethrin Biodegradation in A *Bacillus* sp. strain SG2 Isolated from Cypermethrin Contaminated Agriculture Field" *Journal of Biotechnology*, vol. 6, 2016, pp 45-56.
- [23] Gessesse, K., Tekle, T., Abda, E. M., Kamaraj, M. and Fassil, A. (2021). "Factors Influencing the Bacterial Bioremediation of Hydrocarbon Contaminants in the Soil: Mechanisms and Impacts. *Hindawi Journal of Chemistry*, 2021, pp 1-17.
- [24] Kumari, S. and Das, S. "Bacterial Enzymatic Degradation of Recalcitrant Organic Pollutants: Catabolic Pathways and Genetic Regulations", *Environmental Sciences Pollution Research International*, vol. 30, Number 33, 2023, pp 79676-79705.

- [25] Clarridge, J. E. "Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases" *Clinical Microbiology Reviews*, vol. 17, Number 4, 2004, pp 840–862.
- [26] Jeon, J. M., Park, S. J., Choi, T. R., Park, J. H., Yang, Y. H. and Yoon, H. J. "Biodegradation of Polyethylene and Polypropylene by *Lysinibacillus* species JJY0216 Isolated from Soil Grove", *Polymer Degradation and Stability*, vol. 191, 2021. pp 1-8.
- [27] Jamal, Q. M. S. and Ahmad V. *Lysinibacilli: A Biological Factories Intended for Bio-Insecticidal, Bio-Control, and Bioremediation Activities*. *Journal of Fungi*, vol. 8, 2022. pp 1-19.
- [28] Hong, Y. H., Ye, C. C., Zhou, Q. Z., Wu, X. Y., Yuan, J. P., Peng, J., Deng, H. and Wang, J. H. "Genome Sequencing Reveals the Potential of *Achromobacter* sp. HZ01 for Bioremediation" *Frontiers in Microbiology*, Vol. 8, 2017. pp 1-14.
- [29] Marzec-Grzadziel, A. and Galazka, A. "Sequencing of the Whole Genome of a Bacterium of the Genus *Achromobacter* Reveals Its Potential for Xenobiotics Biodegradation" *MDPI Journal of Agriculture*, vol. 13, 2023. pp 1-15.
- [30] Onuoha T. and Adeola M. O. "Isolation and Screening of Potential Pesticide Degrading Bacteria from Selected Consumable Vegetable Commonly Sold in Benin City Markets" *Academia Journal of Medicinal Plants*, vol. 7, Number 3, 2019, pp 082-085.
- [31] Tonelli-Fernandes, A. F., Braza, V. S., Bauermeister, A., Paschoal, J. A. R., Lopes, N. P. and Stehling, E. G. "Degradation of Atrazine by *Pseudomonas* sp. and *Achromobacter* sp. Isolated from Brazilian Agricultural Soil" *International Biodeterioration and Biodegradation*, vol. 130, 2018. pp 1-6.
- [32] Abdulsalam, Z. B., Eniola, K. I. T. and Awe, S. "Isolation, Characterization and Screening of Potential Lambda Cyhalothrin-Degrading Bacteria from Cultivated Soil in Moro, Kwara State, Nigeria" *Journal of Biochemistry, Microbiology and Biotechnology*, vol. 11, Number 1, 2023, pp 22-25.
- [33] Muhammad, J. B., Jagaba, A. H., Yusuf, F., Usman, S., Yakubu, N. S., Birniwa, A. H., Yakasai, H. M. and Shehu, D. "*Achromobacter xylosoxidans* Bacteria Isolated from Contaminated Agricultural Environment for a Sustainable 2,4-dichlorophenoxyacetic acid Herbicide Degradation: An Experimental Study" *Chemical and Environmental Engineering*, vol. 9, 2024, pp 1-7.
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