Original Research Article

Toxicity of herbicide, Paraquat dichloride and Insectiide, Lambda-cyhalothrin on Phosphate Solubilizing Bacteria, *Pantoa dispersa* in aquatic ecosystems

Abstract

Aim: The study was to determine the toxicity of paraquat dichloride and lambda-cyhalothrin on phosphate solubilizing bacteria*, Pantoa dispersa* in aquatic ecosystems.

Study Design: This study employs experimental design, statistical analysis of the data and interpretation.

Place and Duration of the Study: Soil sample was collected from the root nodules of leguminous plants in a sterile polythene bag from the Elele, in Etche, L.G.A, Rivers State. The fresh water sample was collected from Bane town in Khana L.G.A, brackish water sample was collected from Choba River in Obio/Akpor L.G.A of while the marine water was collected from Bonny River of Bonny L.G.A., all of Rivers State, Nigeria. The samples were collected aseptically and transported in an ice-pack immediately to the Rivers State University, Microbiology laboratory for analysis. The study lasted for three months.

Methodology: The bacterium, *Pantoa dispersa* was isolated and identified based on conventional and molecular characterization from water and soil samples. Different concentrations (3.125%, 6.25, 12.5, 25%, 50% and 75%) and the control (0%) of the herbicide (paraquat dichloride) and insecticide, Lambda-cyhalothrin were prepared using fresh, brackish and marine water samples and 10ml of the test organism, *Pantaoa dispersa* was introduced and the survival count was determined at 0, 4hr, 8hr, 12hr and 24hr. The LC_{50} of the insecticide and herbicide on *Pantoa dispersa* in the three aquatic ecosystem was determined.

Results: The LC_{50} of the herbicide (Paraquat dichloride) was recorded as 15.8% in brackish water, 17.37% in fresh water and 27.44% in marine water. While the LC_{50} of the insecticide, Lambda-cyhalothrin to *Pantoa dispersa* was 26.84% in fresh water, 27.26% in brackish water and 32.33% in marine water.

Conclusion: From the study, the herbicide, Paraquat dichloride was more toxic in the three aquatic ecosystems compared to the insecticide, Lambda-cyhalothrin. The use of these agrochemicals should be monitored as they reduce the population of beneficial soil bacteria like *Pantoa dispersa* which is a phosphate solubilizing bacteria in aquatic ecosystems.

Keywords: Toxicity, Herbicide, Paraquat dichloride, Insecticide, Lambda-cyhalothrin, Phosphate solubilizing bacteria, *Pantoa dispersa*

1.0 Introduction

Of recent, there has been increase in the amount of toxic pollutant contamination in the environment. One of these toxic pollutants which pose threat to the quality of the environment is pesticides. Pesticides usage has been on the rise over the last several years which used to manage pests and improve crop productivity [1]. Pesticides therefore, include; insecticides (bug killers), herbicides (weed killers), and fungicides (fungus killers), rodenticides (rat killers) and antimicrobials [2]. Herbicide and insecticide are classes of pesticides based on their chemical

composition and functions [3]. Their action is influenced by their effect on various mechanisms involved in photosynthesis, respiration, growth, cell and nuclear division, or in the course of protein or lipid synthesis [4].

Phosphorus is an important macronutrient which is directly involved in nucleic acids, cells division and growth of new tissues, which also control or regulate protein synthesis and energy transfer [5].

Lack of phosphate can result in significant reductions (up to 15%) of crop yield [5]. The importance of Phosphate in the soil and aquatic environment results in the application of Phosphorus for agricultural purpose to meet plant needs. The Phosphate solubilizing bacteria helps in making phosphorus available for plant uptake through the production of some organic acids [5].

Pantoa sp is a diverse group of pigmented (yellow) bacteria with growth enhancement ability for plant which have been frequently isolated from both terrestrial and aquatic environment, they exhibit antimicrobial activity, phosphate solubilizing potential, and bioremediation potential [6]. According to another study by Sharon *et al*. [7], *Pantoa* sp was identified as a phosphate solubilizing bacteria and growth enhancer of plant. The herbicide, paraquat dichloride, is more persistent in the environment when absorbed and have been found in river, lake beds, surface water and vegetations [9]. Lambda-cyhalthrin is pyrethroid insecticide which is known for their low solubility in water and moderate persistence in the soil environment [8]

Herbicide, paraquat dichloride and insecticide, lambda-cyhalothrin had been one of the most used pesticides of recent in both the southern and northern parts of Nigeria [9][8] and there has been little or no research on the effect of pesticide on the growth of phosphate solubilizing bacteria, hence the need for this study.

2.0 Materials and Methods

2.1 Sample collection

Soil sample was collected from the root nodules of leguminous plants in a sterile polythene bag from the Elele, in Etche, L.G.A, Rivers State. The fresh water sample was collected from Bane town in Khana L.G.A, brackish water sample was collected from Choba River in Obio/Akpor L.G.A of while the marine water was collected from Bonny River of Bonny L.G.A., all of Rivers State, Nigeria. The samples were collected aseptically and transported in an ice-pack immediately to the Rivers State University, Microbiology laboratory for analysis. The herbicide, paraquat dichloride with the brand Dragon and the insecticide with the brand name, Laraforce were bought from a chemical store in Mile 3 Market of Port Harcourt, Rivers State and taken to the laboratory for the toxicity test.

2.2 Isolation of the test organisms

Pikoskaya's agar (PVK) medium (Glucose, 10g, $Ca_3(PO_4)_2$, $(NH4)_2SO_4$, 0.5g, $MgSO_4.7H_20$, 0.1g, KCl, 0.2g, Yeast extract, 0.5g, $MnSO_4.H_20$, 0.002g, $FeS0_4.7H_20$, Distilled water) was used for the isolation of phosphate solubilizing bacteria [10]. The samples were resuscitated in a sterile normal saline (of 90ml) as diluent and 10-fold serial dilution was carried out. An aliquot (0.1ml) of the diluted samples were inoculated on the prepared media plate and spread with the aid of a sterile hockey stick using spread plate method of culturing. The inoculated plates were incubated for 24-48 hours at 37^oC. After incubation, the total count of the bacteria was determined by counting the colonial growth on the cultured plates and the CFU/g (colony forming unit per gram) were calculated. The different isolates of the cultures were purified by streaking the bacterial isolates on the freshly prepared nutrient agar plates based on their different cultural morphological characteristics and incubated at 37^oC for 24 hours to have a pure culture of the isolates. Pure isolates of the different organisms were preserved on nutrient agar slant and glycerol medium at 4^oC under good aseptic conditions for further analysis [11].

2.3 Identification of Bacterial Isolates

The bacterial isolates obtained from the samples were characterized and identified based on their cultural microscopic and biochemical characteristics and according to the schedule depicted by the Bergey's Manual of Determinative Bacteriology. The isolates were characterized genotypically by cloning and sequencing the 16S rRNA. The genomic DNA from a pure culture of each isolate was extracted and purified for PCR amplification of the 16S rRNA sequence using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa

2.4 Standardization of Inoculums for toxicity testing

The identified isolate of Phosphate solubilizing bacterium *Pantoa dispersa* was inoculated on freshly prepared nutrient agar and incubated at 37°C for 18 to 24h. After 24hours of growth, the organism was inoculated in a sterile broth using 0.5 MacFarland standard to measure the turbidity of the organisms [12].

2.5 Toxicity Test Procedure

The method of Douglas *et al.* [16] was adopted in the toxicity test. The toxicants (Insecticide and Herbicide) were prepared using seven (7) different conical flasks for the identified bacterium, *Pantoa dispersa* for each of the habitat water (marine, brackish and fresh water). In each set, a total of seven conical flask for the different concentrations (3.125%, 6.25, 12.5, 25%, 50% and 75%) and the control (0%) were prepared from the stock toxicants. The prepared set-ups were sterilized at 121^{o}C at 15psi for 15 minutes. The control (0%) contained the water sample habitat water) and the organism without toxicant. Test procedure for the test organisms from freshwater, brackish and marine water; Ten millilitre (10ml) of the test organism was added to each conical flask containing the different concentrations of the toxicants and control respectively. The mixture was homogenized to for 2 minutes before use. An aliquot (0.1ml) from each of the concentrations 3.125%, 6.25, 12.5, 25%, 50%, 75% and control was inoculated on a nutrient agar with the aid of a sterile hockey stick using spread plate method after which the inoculated plates were incubate at 37± 2ºC for 24hours. The same process was repeated for all the set-ups after 4h, 8h, 12h and 24hours. After incubation the counts of the test organisms were taken and expressed in Log_{10}. This process was repeated for the test organisms in the three different aquatic habitat. Percentage log survival, percentage log mortality and median lethal concentration (LC_{50}) was calculated with the formula adopted from Nrior and Okele [13].

2.6 Statistical Analysis

The data obtained during this study was analyzed statistically using SPSS version 22 for analysis of variance (ANOVA) of the data in the aquatic ecosystem studied

3.0 Results and Discussion

The results of the baseline bacterial counts of the soil and three water samples is represented in Table 1. The total Heterotrophic bacterial counts (THBC) of the three aquatic sample and soil sample ranged from $3.8\times10^{5}\pm0.02$ CFU/ml to $1.2\times10^{9}\pm$ 0.012 CFU/g (7.38 Log CFU/ml) with the Fresh water samples having the least heterotrophic bacteria count while the soil sample had the highest count of heterotrophic bacteria count. The total count of phosphate solubilizing bacteria ranged from $3.6\times10^{3}\pm0.00$ CFU/ml (3.55 Log CFU/g) to $4.8\times10^{5}\pm$ 0.12 CFU/g (5.68 Log CFU/g) as the fresh water recorded the least count of phosphate solubilizing bacteria while the highest count was recorded in the soil sample. Result of the count in this study is similar to the population recorded by Kirui *et al.* [17] in the study to determine the diversity of phosphate solubilizing bacteria from semi-arid agroecosysterm of eastern Kenya in which the population of culturable PSB ranged from 1.3×10^{4} to 3.63×10^{4} CFU/g. The organism, *Pantoa dispersa* was identified from biochemical results indicating; Gram negative rod, indole negative, citrate positive, oxidase negative, catalase negative, non-motile, MR negative, Starch negative, glucose negative and macroscopially, yellow colonies were observed. Molecular results identified *Pantoa dispersa* strain *ABRLO84* through the 16S rRNA gene sequence. *Pantoa* sp is a diverse group of pigmented (yellow) bacteria with growth enhancement ability of plant which have been frequently isolated from both terrestrial and aquatic environment, they exhibit antimicrobial activity, phosphate solubilizing potential, and bioremediation potential [6]. According to another study by Sharon *et al*. [7], *Pantoa* sp was identified as a phosphate solubilizing bacteria and growth enhancer of plant. As demonstrated in the study by Sharon *et al.* [7] to evaluate the efficient phosphate solubilizing bacteria with the capacity to enhance tomato plant growth, *Pantoa dispersa* was recorded to

Table 1: The Total Heterotrophic Bacterial Count and Total count of Phosphate Solubilizing Bacteria of the samples

Samples	**Bacterial population**	
	Total Heterotrophic Bacteria (THB) Count	**Total count of Phosphate solubilizing Bacteria (TCPSB)**
Soil	1.2×10^{9} ±0.12 CFU/g	4.8×10^{5} ±0.12 CFU/g
Fresh water	3.8×10^{5} ±0.02 CFU/ml	3.6×10^{3} ±0.00 CFU/ml
Brackish water	6.2×10^{6} ±0.7 CFU/ml	3.1×10^{4} ±0.07 CFU/ml
Marine water	1.4×10^{6} ±0.24 CFU/ml	8.8×10^{3} ±0.02 CFU/ml

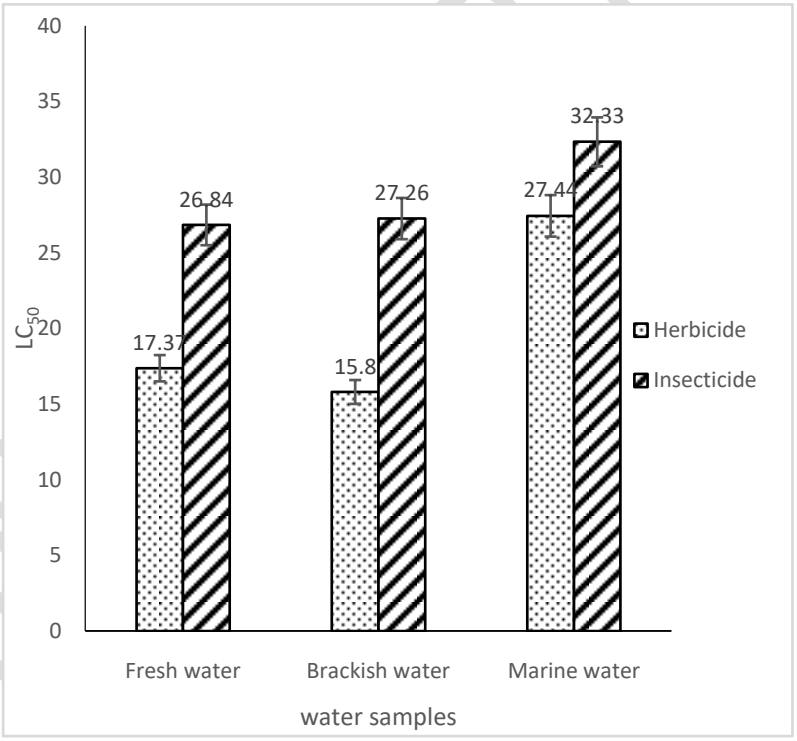

Fig 1: Median lethal concentration of the toxicants (herbicide and insecticide) on

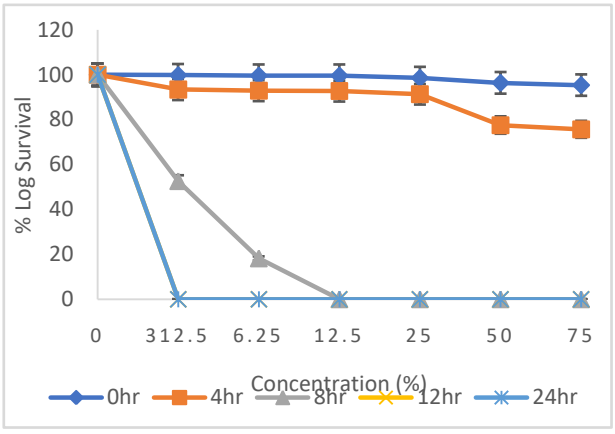

Fig 2: Percentage Log survival of *Pantoa dispersa* with insecticide in brackish water

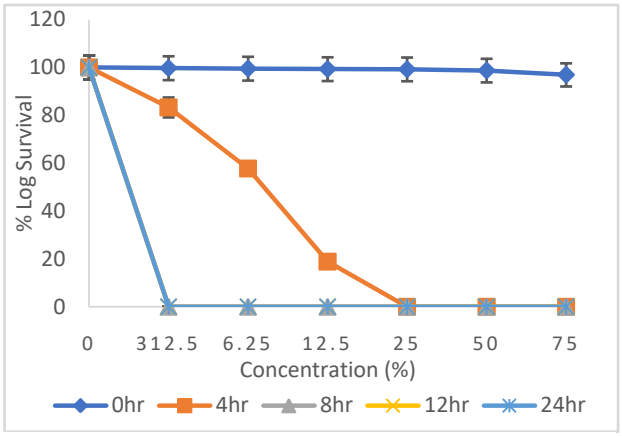

Fig 3: Percentage (%) Log survival of *Pantoa dispersa* with herbicide in brackish water

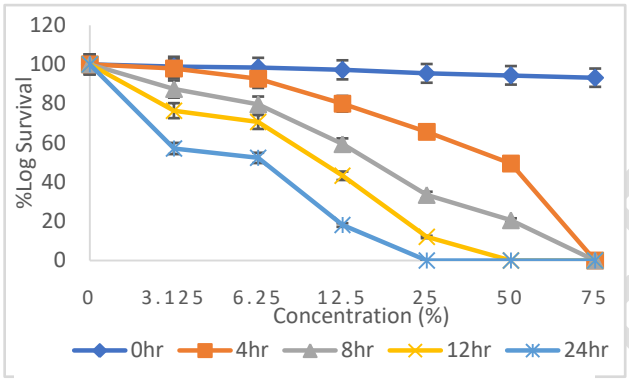

Fig 4: Percentage (%) Log survival of *Pantoa dispersa* with insecticide in Fresh water

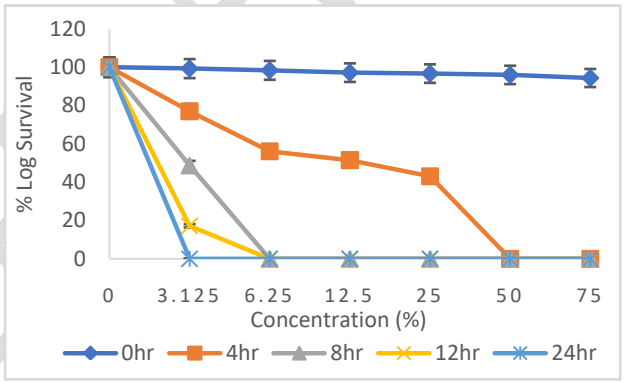

Fig 5: Percentage (%) Log survival of *Pantoa dispersa* with Herbicide in Fresh water

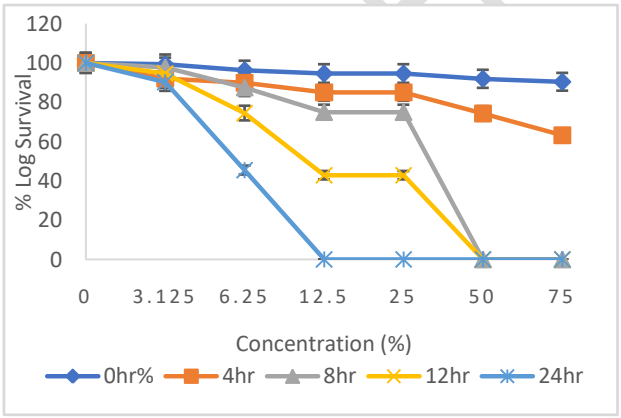

Fig 6: Percentage (%) Log survival of *Pontoa dispersa* with insecticide in marine water

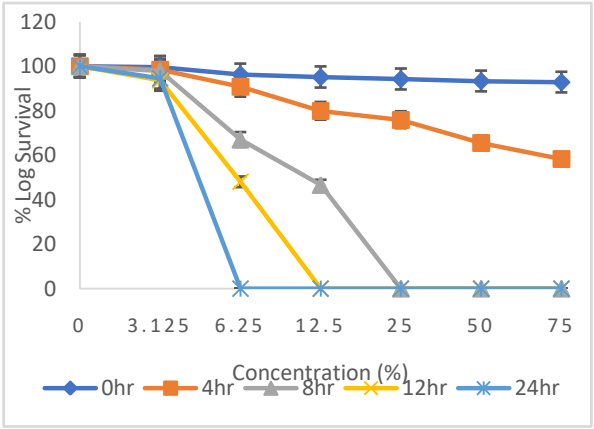

Fig 7: Percentage (%) Log survival of *Pontoa dispersa* with Herbicide in marine water

produce the highest rate of solubilization from the insoluble tricalcium complex in liquid culture hence produced a great increase in the plant growth in the presence of insoluble $Ca_3(PO4)_2$.

The result of the toxicity shows that there was complete (100%) depletion of the population of the test organism in the brackish water when exposed to herbicide (for all the concentration) after 8hr of exposure time. The figure 4 shows the percentage log survival of the organism, *Panta dispersa* to different concentration of insecticide in brackish water. After 12hours of exposure, there was complete depletion (100%) in the population of the organism compared to the control setup without the toxicant which increased in the population in relation to the time of incubation. Figure 5 shows the graphical presentation of the percentage log survival of the *Pantoa dispersa* with herbicide in fresh water. The setup of the organism containing 75% and 50% of the herbicide resulted in 100% mortality at 4 hours of exposure while the concentration of 25%, 12.5% and 6.25% resulted in 100% mortality of the test organism at 8 hour and the least concentration of 3.125% produced complete depletion of the test organism at 24 hours of exposure.

Figure 6 shows the percentage log survival of *Pantoa dispersa* with insecticide in fresh water. Complete mortality of the organism was observed in the setup containing 75% of the insecticide at 4 hours of exposure time, concentration of 50% at 12 hour, 25% at the 24 hour of exposure. The setup containing 12.5%, 6.25% and 3.125% had a percentage survival of 8.1, 52.3 and 57.1 respectively after 24 hours of exposure. The percentage log survival of the test organism, *Pantoa dispersa* to different concentration of herbicide in marine water is shown in figure 7. The concentration of 75%, 50% and 25% of the toxicant, herbicide resulted in total mortality of the test organism at the 8 hours of exposure while the 12.5% and 6.25% produced a total mortality at the 12 and 24 hours of exposure respectively. Figure 8 shows the percentage log survival of *Pantoa dispersa* to insecticide in marine water over the time of exposure. There was a total mortality of the test organism in the setups containing 75%, 50% and 25% of the concentration at the 8 hours of exposure while at 24 hour of exposure, total mortality of the test organism was observed in the setup containing 12.5% of the toxicant. Reduction of the percentage log survival of the test organism was recorded in the setup containing 6.25% and 3.125% of insecticide producing 45.5% and 90.3% respectively after 24hour of exposure. Figure 1 shows the LC_{50} of the toxicant, herbicide and insecticides on the test organism, *Pantoa dispersa*. The LC_{50} of herbicide on *Pantoa dispersa* was observed to be lower in all the water samples compared to the insecticides. The LC_{50} of herbicide was recorded as 15.8% in brackish water was lower than 17.37% in fresh water compared to LC_{50}, 27.44% recorded in marine water. The LC_{50} of insecticide on *Pantoa dispersa* was recorded as 26.64% in fresh water was less than 27.26% in brackish water compared to 32.35% recorded in marine water.From the study, the herbicide, Paraquat dichloride was more toxic in the three aquatic ecosystems than the insecticide (Lambda-cyhalothrin). Furthermore, the herbicide was more toxic to the PSB, *Pantoa dispersa* in brackish water compared to the other aquatic bodies. This is in line with the study of Thi Hue *et al*. [14] which highlighted that the herbicide, paraquat dichloride is not easily degraded chemically and microbiologically and persist longer in river water. The less toxicity of the toxicant in marine can be related to the increase in salinity of the water. The toxicity of the herbicide paraquat on the test organism isachieved by oxidative stress in the organism thus increasing the peroxide ion [15].

4.0 Conclusion

Although the application of pesticides is cost effective for plant yield in the agricultural sector especially, their uncontrollable or unmonitored use can result in the mortality of ecologically important bacteria, phosphate solubilizing bacteria, *Pantoa dispersa* in aquatic ecosystem as shown in this study. The toxicity of the herbicide, paraquat dichloride to the test organisms, *Pantoa dispersa* in aquatic environment than the insecticide, lambda-cyhalothrin as shown in this study. It could be recommended that the use of synthetic herbicide and insecticide should be monitored by the government agency as uncontrollable application of these pesticides affects the population of phosphate solubilizing bacteria like *Pantoa dispersa* in aquatic ecosystem which has been demonstrated in other studies to be a biofertilizer, increasing plant growth through their phosphate solubilizing ability both in aquatic and terrestrial habitat as these pesticides end up in the aquatic habitat through run-offs.

References

1. Wanguyun P. A. and Gerald A. (2019). Understanding Pesticide Degrading Microbe Community Using Molecular Approaches. *EM International,* 38:118-122
2. Ojo J . (2016). Pesticide Use and Health In Nigeria. *Ife Journal of Science*, 18(4):981-991
3. Alegebawy, A., Abdelkhlek, S. T., Quewahi, R. S. and Wang, M. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, 9:42-75
4. Shefali, R. K., Sankha, M. S., Kumar and R., Sonone, S. (2021). Impact of pesticides toxicity in aquatic environment. *Biointerface Research in Applied Chemistry*, 11(3):10131-10140
5. Elhaissoufi, W., Gheulam, C., Barakat, A., Zeroul, Y. and Bargaz, A. (2021). Phosphate bacterial solubilisation: A key rhizhosphere driving force enabling highr P use efficiency and crop productivity. *Journal of Advanced Research*, 8(14):2090-1232
6. Walterson, M. A. and Stavrinides, J. (2015). Pantoea: insight into a highly versatile and diverse genus within the *Enterobacteriaceae*. *FEMS Microbiology Reviews*, 39(6):968-984
7. Sharon, J. A., Glenn, G. M., Imam, S. H., and Lee, C. C. (2016). Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato growth. *Journal of Soil Science and Plant Nutrition,* 16 (2):525-536
8. Samuel, O., Victoria, N. and Joseph, N. (2020). Lambda cyhalothrin and Dichlorvos Pesticide Degradation Potential of bacteria isolated from Agricultural soil in Enugu, Enugu state, Nigeria. *International Journal of Advanced Technology and Science*, 1(2):161-172
9. Ikpesu, T. O. (2014). Assessment of occurrence and concentration of paraquat dichlorichloride in water, sediments and fish from Warri River Basin, Niger Delta, Nigeria. *Environmental Science and Pollution Research*, 22(11):8517-8525
10. Jha, K. B., Prajasj, G. M., Cletus, J., Raman, G. and Saktthivel, N. (2009). Simultaneous phosphate solubilisation potential and antifungal acticity of new fluorescent Pseudomonads strains, *Pseudomonas aeruginosa, P. plecoglossicida* and *P. mosselii. World Journal of Microbiology and Biotechnology*, 25:573-581
11. Nursofiah, S. Hartoyo, Y., Amalia, N., Febrianti, T., Febriyana, D., Saraswati, R D., Puspandari, N., Sariadji, K., Khariri1, Rukminiati1, Y., Muna, F., Susanti, I. Multihartina, P. (2021). Long-term Storage of Bacterial Isolates by Using Tryptic Soy Broth with 15% Glycerol in The Deep Freezer (-70 to -80 ^{o}C). *Earth and Environmental Science*, 913:1-7
12. Cheesbrough, M. (2005). *District Laboratory Practice in tropical countries, part 2*. Cambridge University Press, Cambridge. Pp 159-162
13. Nrior, R. R. and Okele, (2018). Toxicity of Local and industrial Refined Diesel on Nitrobacter species, a key environmental pollution biomarker. *Asian Journal of Biotechnology and Bioresource Technology*, 4(2):1-8
14. Thi Hue, T. N., Nguyen, M. P. T., Nam, H., and Tung, H. N. (2018). Paraquat in surface water of some Streams in Main Chau Province, the Northern Vietnam: Concentrations, profile and Human Risk Assessment. *Journal of Chemistry,* 11:55-65
15. Marin-Morales, M. A., Venture-Camargo, C. B. and Hoshua, M. M. (2015). Toxicity of herbicides; impact on aquatic and soil biota and human health. *Intech Open Science*, 1:399-430
16. Douglas, S. I., Nrior, R. R. and Kporman, L. B. (2018). Toxicology of spent phone bacteria on microflora in marine, brackish and fresh water ecosystem. *Journal of advance in Microbiology*, 12(2):1-10
17. Kirui, C. K., Njeru, E. M. and Runo, S. (2022). Diversity and Phosphate solubilization efficiency of Phosphate solubilizing bacteria isolated from semi-arid agroecosystem of Eastern Kenya. *Microbiology Insights*, 15:1-1
18. Ezemonye , L. I. N. , Ikpesu, E. O. and Tongo I. (2010). Distribution of endosulfan in water, sediment.

International *Journal of Environmental Studies*, 65(5): 491-504
19. Assuming-Brempong, S. and Aferi, N. (2014). Isolation of phosphate solubilizing bacteria from tropical soil. *Global Advanced Research Journal of Agricultural Science*, 3(1):8-15