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# Toxicity of Herbicide, Paraquat Dichloride and Insecticide, Lambda-cyhalothrin on Phosphate Solubilizing Bacteria, *Pantoa dispersa* in Aquatic Ecosystems

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** The study was aimed to determine the toxicity of paraquat dichloride and lambda-cyhalothrin on phosphate solubilizing bacteria*, Pantoa dispersa* in aquatic ecosystems.
**Study Design**: This study employs randomized block 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 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 (3.13%, 6.25, 12.50, 25.00%, 50.00% and 75.00%) and the control (0%) of the herbicide (paraquat dichloride) and insecticide, Lambda-cyhalothrin were prepared using fresh, brackish and marine water samples and

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10ml of the test organism, *Pantoa dispersa* was introduced and the survival count was determined at 0, 4hr, 8hr, 12hr and 24hr. The Mean Lethal Concentration ($LC_{50}$) 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 result in the mortality of beneficial soil bacteria like *Pantoa dispersa* which is phosphate solubilizing bacteria in aquatic ecosystems.

*Keywords: Toxicity; herbicide; insecticide; Phosphate solubilizing bacteria; Pantoa dispersa; Median Lethal Concentrations ($LC_{50}$); Pikovskaya's agar.*

## 1. INTRODUCTION

Recently, 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 is used to manage pests and improve crop productivity [1]. Pesticides therefore, include; insecticides (bug killers), herbicides (weed killers), 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 [8][9] and there has been little or no research on the effect of pesticide on the growth of phosphate solubilizing bacteria in aquatic ecosystem therefore, this study is designed to evaluate the toxicity of pesticide on phosphate solubilizing bacteria in aquatic ecosystems.

## 2. 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

Pikovskaya'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, $FeS0_4.7H_20$, 0.001g, and Distilled water, 1 liter) 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 [12,13]. 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 [14].

## 2.5 Toxicity Test Procedure

The method of Douglas et al*.* [15] 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 (3.13%, 6.25, 12.50, 25.00%, 50.00% and 75.00%) and the control (0%) were prepared from the stock toxicants. The prepared set-ups were sterilized at 121$^oC$ 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 milliliter (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 [16].

## 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. 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 $3.6\times10^3 \pm 0.00$ 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 $1.3\times10^4$ to $3.63\times10^4$ 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 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 $Ca_3(PO4)_2$.

Fig. 2 shows the percentage log survival of *Pantoa dispersa* to various concentration of herbicide in brackish water in relation to the time of exposure. The result of the toxicity shows that there was complete (100%) mortality of the population of the test organism in the brackish water when exposed to herbicide (for all the concentration) after 8hr of exposure time. High toxicity of pesticides observed in this study is in line with the study of Obire and Nrior [18] in which low concentration of 10ppm of chlorine bleach resulted in 95% mortality of *Pseudomonas floruorescens* after four hours of exposure. The Fig. 3 shows the percentage log survival of the organism, *Pantoa dispersa* to different concentration of insecticide in brackish water. After 12hours of exposure, there was complete mortality (100%) in the population of the organism compared to the control setup which was without the toxicant which there was 100% percentage log survival of the organism in relation to the time of incubation. Fig. 4 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% resulted in 100% mortality of the test organism at 24 hours of exposure.

Fig. 5 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 Fig. 6. The concentration of 75%, 50% and 25% of the toxicant, herbicide resulted in 100% 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. Fig. 7 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.

**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** | $1.2\times10^{9}$ ±0.12 CFU/g | $4.8\times10^{5}$ ±0.12 CFU/g |
| **Fresh water** | $3.8\times10^{5}$ ±0.02 CFU/ml | $3.6\times10^{3}$ ±0.00 CFU/ml |
| **Brackish water** | $6.2\times10^{6}$ ±0.7 CFU/ml | $3.1\times10^{4}$ ±0.07 CFU/ml |
| **Marine water** | $1.4\times10^{6}$ ±0.24 CFU/ml | $8.8\times10^{3}$ ±0.02 CFU/ml |

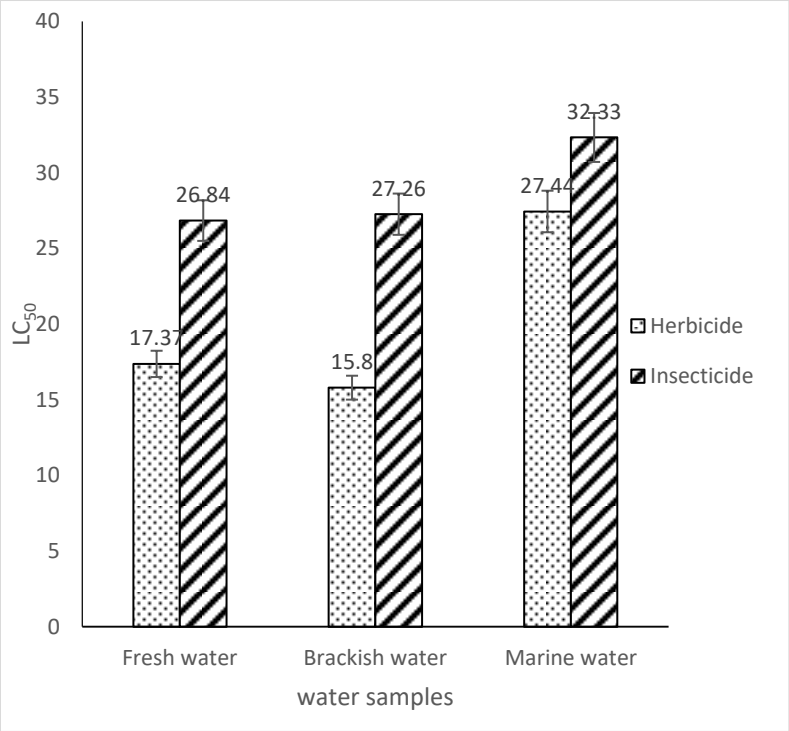


**Fig. 1. Median lethal concentration of the toxicants (herbicide and insecticide) on *Pantoa dispersa* in three water samples**

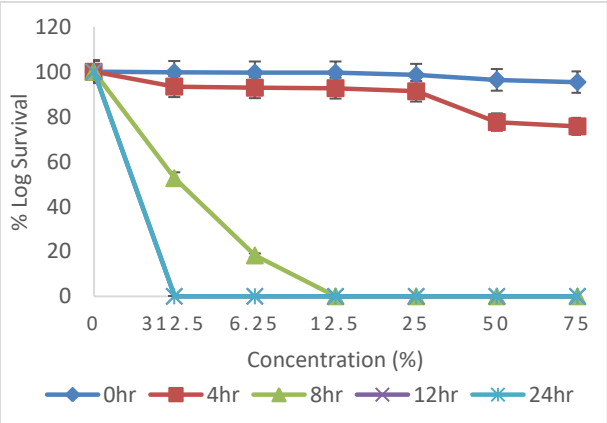


**Fig. 2. Percentage Log survival of *Pantoa dispersa* with insecticide in brackish water**

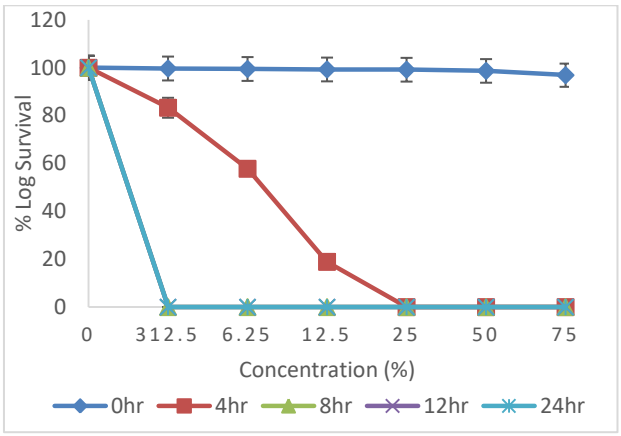


**Fig. 3. Percentage (%) Log survival of *Pantoa dispersa* with herbicide in brackish water**

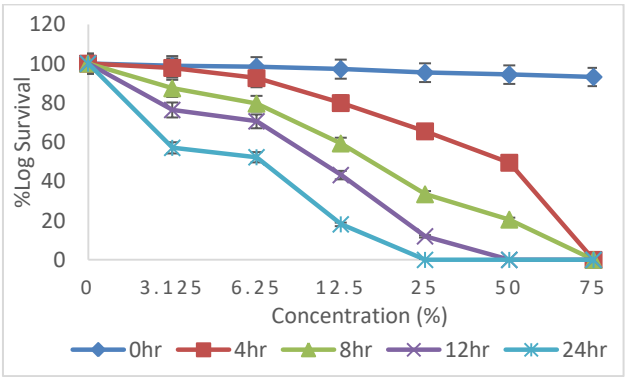


**Fig. 4. Percentage (%) Log survival of *Pantoa dispersa* with insecticide in Fresh water**

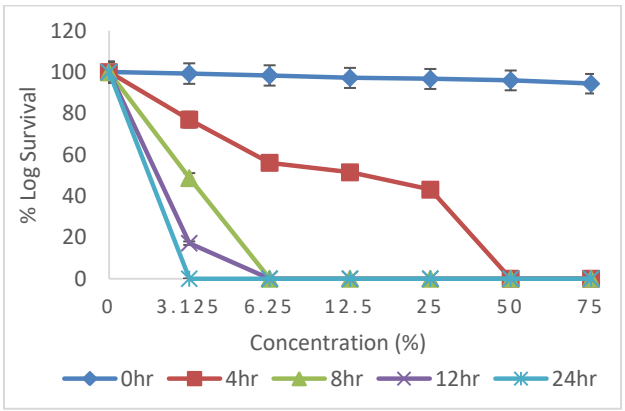


**Fig. 5. Percentage (%) Log survival of *Pantoa dispersa* with Herbicide in Fresh water**

From the study, the increase in the mortality of the test organisms in the aquatic ecosystems increased with increase in the exposure time. This is similar to the study of Wemedo et al. [19] in the study to evaluate the ecotoxicological effect of effluents on *Bacillus, Enterobacter*, *Amorphotheca, Cladosporium* and *Penicillium* species in Brackish water in which the there was reduction in the percentage survival of the test organisms as the time of exposure increased. Fig. 1 shows the $LC_{50}$ of the toxicant, herbicide and insecticides on the test organism, *Pantoa dispersa* (the lower the. $LC_{50,}$ the more toxic the toxicant). 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

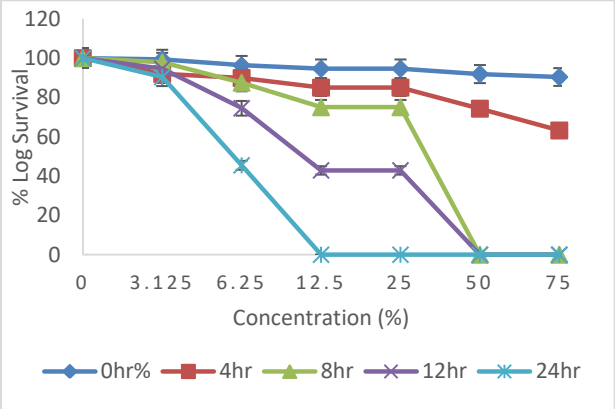


**Fig. 6. Percentage (%) Log survival of *Pontoa dispersa* with insecticide in marine water**

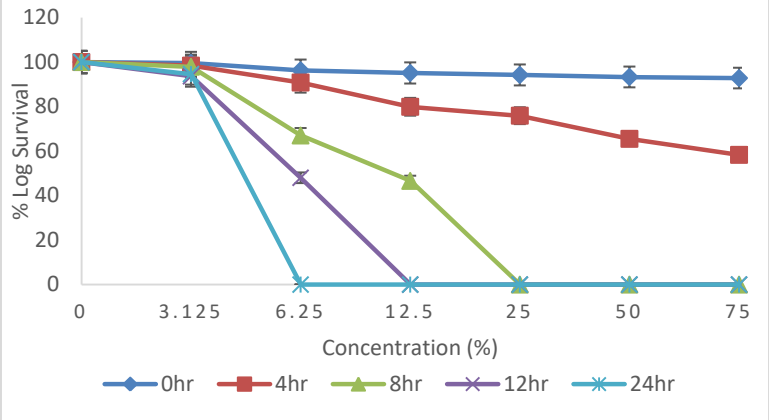


**Fig. 7. Percentage (%) Log survival of *Pontoa dispersa* with Herbicide in marine water**

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 which was less than 27.26% in brackish water compared to 32.35% recorded in marine water. The mortality of the test organism in response to the pesticides can be attributed to the enzyme's inactivation and susceptibility of the microorganism to hostile xenobiotic which might have hampered their ability to recovered gradually within the period of study [20]. Previous studies have shown that pesticides applications result in the reduction of certain microbial populations while leaving others unscathed, and also reduce competition for resources shared by the affected and unaffected populations [21].

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. [22] 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 is achieved by oxidative stress in the organism thus increasing the peroxide ion [23].

## 4. 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. From the results of the study, the herbicide, paraquat dichloride is observed to produce more toxicity effect to the test organisms, *Pantoa dispersa* in aquatic environment than the insecticide, lambda-cyhalothrin. It could be recommended that the

use of synthetic pesticides should be monitored by the government agency to prevent the extinction of important microorganisms like phosphate solubilizing bacteria in the aquatic ecosystem.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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