



Response of *Pseudomonas* species from Contaminated Soils to Selected Organic (Synthetic) Pesticides

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Authors' contributions

This work was carried out in collaboration with all authors. Author UJNN designed the study and wrote the protocol of the study. Author UAO managed the analyses and performed statistical analysis of data. Author GEU managed literature searches and materials required for the study. All authors proofread and approved the final manuscript.

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ABSTRACT

Growth response profile of three species of *Pseudomonas* isolated from pesticide contaminated soils within Uyo, Nigeria was studied using standard microbiological and analytical techniques. The ability of the isolates to tolerate varying concentrations of commercially available pesticides namely; Decis, DD force and Cyperforce was assessed over a 96 hour period. Selective enrichment cultures with graded concentrations of the pesticides were used to assay their growth response profile and the absorbance determined using CO75 digital colorimeter. The results showed that the *Pseudomonas* species differed biochemically. Their growth response at pesticide concentrations 0.0001, 0.001, 0.01, 0.1, 1 and 10% v/v differed significantly ($P < 0.05$) at 24 hours interval for four days. At 10% concentration of Decis pesticide over 96 hour, the absorbance were 0.20, 0.23 and 0.30 for *Pseudomonas* from Agriculture research farm, hospital dumpsite and municipal waste dump site respectively. This ability therefore offers a veritable tool for use in the bioremediation and ultimate restoration of pesticide contaminated soils but however requires further evaluation.

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1. INTRODUCTION

One of the major environmental problems facing the world today is the contamination of soil, water, air and food by toxic chemicals [1]. Eighty billion pounds of hazardous pesticides are produced annually and used in agricultural farms and only 10% of these are disposed of safely [2]. Expansion as well as the intensification of agricultural and industrial activities in recent decades has led to the pollution of crops, soil, sediments and ground water with pesticides, although many treatment processes have been developed to reduce the environmental impacts of this contaminations [3,4]. However, due to the magnitude of this problem and the lack of a reasonable solution, a rapid, cost-effective, ecologically responsible method of clean-up is greatly needed.

Pesticides are applied widely to control pests such as insects, weeds plant diseases, nematodes and rodents. The increased use of pesticides since 1945 has greatly aided the increase in crop production, protected livestock from diseases such as trypanosomiasis, protected man from diseases such as malaria and filariasis, decreased losses of stored grain and has generally improved man's welfare.

Despite the enormous benefits derived from pesticides, these chemicals are not problem. Major concerns associated with pesticide use are related to their persistence, toxicity, bioaccumulation and potentials to undergo a variety of transformations that provides a complex pattern of metabolites [5] referred to as transformation products (TPs). The interest in their transformation products (TPs) is because they can be present at higher levels especially in soils than the parent pesticide itself.

Generally, pesticide TPs could show lower toxicity to biota than the parent compounds [6]. However, toxicological evaluation of pesticide TPs is also an emerging issue and the few scientific works that have been reported show insufficient results to establish a generality. In some instances TPs are more toxic, so they represent a greater risk to the environment than the parent molecules [6,7].

However, the combined effects of multisites use of several pesticides in mixtures may lead to

augmentation of toxicities i.e. synergistic effect [8].

In recent years, the scientific community has shown greater concern about the possible adverse effects that the presence of these pesticides in food and water may have for human health and for the equilibrium of ecosystems [9-11]. Such concern which has been highlighted by [12] and [13] is supported by results from major monitoring studies already carried out in the past and confirmed by more recent investigations. Among the possible chronic effects of pesticides are carcinogenesis [14], neurotoxicity [15], effects on reproduction [16] and cell development effects, particularly in the early stages of life [17]. In order therefore to avoid potential human exposure to pesticides residues via food and water, knowledge of and/or understanding of the fate of the chemicals as well as effective techniques to remove and/or degrade pesticides residues is required.

The fate of pesticides in environmental matrices is controlled by chemical, biological and physical dynamics of the matrix [5]. These processes can be grouped into those that affect persistence (including chemical and microbial degradation) and those that affect mobility (involving sorption, plant uptake, volatilization, wind erosion, run-offs and leaching). Some conventional methods (photolysis, hydrolysis, ozonation, ultrasonic irradiation and ionizing radiation) have been used for the degradation of pesticides residues [18-23]. However, the removal efficiency of these techniques is somewhat limited [24] and undesired toxic compounds are sometimes formed as well. Therefore, developing an alternative approach becomes inevitable.

In their pesticide bioremediation studies, [23] found out that acetochlor (an organochlorine pesticide), a model compound of herbicide was degraded when injected into soil and water samples pre-treated with cyanobacterial mat with degradation being much faster in the water system than in soil. In a similar study by [24], it was also reported that the pesticide diuron could also be effectively removed from soil by cyanobacterial mats. This suggest that biological methods could be advantageous to decontaminate areas that have been polluted by pesticides. These methods consider the thousands of microorganisms in the environment

that in order to survive, seek for alternatives to eliminate the pesticides that are sprayed. Many native microorganisms develop complex and effective metabolic pathways that permit the biodegradation of toxic substances that are released into the environment. Bioremediation has become an attractive option as it is more efficient, ecofriendly and a cheaper approach. Following widespread use of pesticides in agriculture and general fumigations, the natural microbiota is continuously exposed to these chemicals. Therefore, microorganisms that inhabit polluted environments are armed with resistance by catabolic processes to remove the toxic compounds. Earlier reports based on cultivable bacteria suggested that xenobiotic contaminated soil is predominated by Gram-negative bacteria [25].

Bacterial strains that are able to degrade most organic pollutants have been isolated, mainly from soil. These are usually Gram-negative bacilli, most of them belonging to the genus *Pseudomonas* [26]. This study therefore aims at investigating the interaction between selected pesticides and *Pseudomonas* species with the broad objective of providing information and ground for successful interventions into environmental processes that ultimately leads to optimized strategies for tapping of the bacteria's potentials for efficient and effective bioremediation of pesticide contaminated environment.

2. MATERIALS AND METHODS

2.1 Description of Study Sites

Three sites namely University of Uyo Agriculture research farm at Use-Offot (N 05° 02' 20.8", E 007° 58' 39.5"). Municipal Waste Dump sites at Udo Street (N 05° 02' 33.3", E 007° 56' 11.8") and St. Luke's Hospital waste dump site at Anua Offot (N 05° 01' 48.21", E 007° 57' 31.38") were selected for this study. All the sites are located within Uyo metropolis, capital city of Akwa Ibom state, Nigeria and have received varying amounts of pesticides applied either as herbicides or pesticides. Soils consisted of grayish sandy clay loam, brown sandy clay loam with brown and grayish-brown mottles. Site soils were moderately acidic and well drained on low lands. The soil properties were pH 7.1±1.0, conductivity (%) 0.89±0.9, Carbon (%) 0.43±0.5, Nitrogen (%) 0.45±1.0, Phosphorus (%) 0.026±0.1 Potassium 0.89±0.1, Calcium (%) 1.14±0.2, Magnesium (%) 1.12±0.6, Sulphate

(%) 0.41±0.8. Values are presented as mean ± standard deviation.

2.2 Collection of Soil Samples

Composite soil samples from 0 cm – 15 cm depth were collected aseptically using soil auger from each of the sites. The samples were bulked and approximately 200 g each was placed in sterile polythenes and labelled accordingly. The samples were labeled A1, A2 and A3 for soils from Agricultural Farm Dump site and Anua Hospital dumpsite and Municipal waste dump site respectively. They were transported immediately in ice cold packs to the Department of Microbiology laboratory, University of Uyo for analyses.

2.3 Pesticide Source

Commercial pesticide grades namely Decis, Cyper force and DD force (99% pure, Sun Agro chemicals PVT Ltd) were obtained from an Agrochemical dealer in Uyo and used for the study.

2.4 Isolation of Test Organism

From each of the composite soil samples precisely 10 g of soil was weighed and suspended in 90 ml of sterile deionized water contained in an Erlenmeyer flask and thoroughly shaken to dislodge the protists. An aliquot of 1ml was transferred and serially diluted in test tubes. From an appropriate dilution, 0.1 ml was pipetted and spread on *Pseudomonas* agar plate containing 0.3 g l⁻¹ Cetrimethyl Diammonium Bromide (selective agent in *Pseudomonas* agar to inhibit growth of other microorganisms) and incubated at 37°C for 48 hours.

After the incubation, discrete colonies appearing was re-inoculated into fresh nutrient broth supplemented with Novobiocin (0.45 mg), Penicillin G (44.9 mg) and Cycloheximide (75 mg) and further incubated at 30°C for 48 hrs in order to suppress the growth of other microbes. By transferring subcultures to nutrient broth containing antibiotics *Pseudomonas* were selected. The isolates were further characterized according to methods described by [27].

2.5 Pesticide Tolerance Analysis by *Pseudomonas* Species

Varying concentrations (10%, 1%, 0.1%, 0.01%, 0.001% and 0.0001% v/v). There was no control

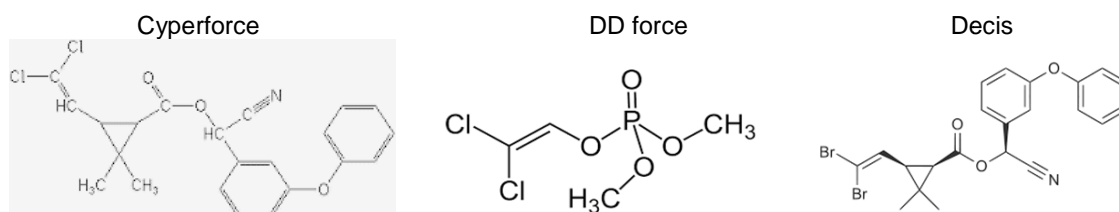


Fig. A. Chemical structure of the tested pesticides

since the study was a comparative response of *Pseudomonas* species isolated from the three sites to the three pesticides) of commercially available pesticides namely Decis, Cyperforce and DD force were incorporated into presterilized tubes of nutrient broth. Each of these tube(s) were thereafter inoculated with 1 ml of 24 hr broth culture of *Pseudomonas* sp. The tubes were incubated at 37°C for 24 hours and observed for its optical density at 550nm wave length. The absorbance of each tube was determined at 24 h interval for four days using a CO75 digital colorimeter.

3. RESULTS

The different *Pseudomonas* species isolated from each of the contaminated soil samples on cetrimide agar amended with antibiotics are presented in Table 1. Results revealed the isolation of *Pseudomonas* species PA1, PA2 and PA3 from Agricultural Research Farm, St. Luke's Hospital Dumpsite soil and Municipal Waste Dumpsite Soil respectively.

Results from the pesticide tolerance analysis revealed that *Pseudomonas* from dump site (PA3) soil was more resistant (Fig. 3) to the toxic effect of the three pesticides used in the study

with observed optical density (OD) values even at 10% concentration after 48 hours incubation whereas *Pseudomonas* from agriculture research farm soil (PA1) was more susceptible (no observed OD values) to all the tested pesticides (Fig. 1) after 48 hours incubation at the same concentration (10% v/v).

Generally, an initial decrease in OD was observed with all pesticide supplemented cultures and later increased with time and decreasing concentrations of each pesticide (Figs. 1, 2 and 3). This may be suggestive of the initial inhibitory effect of these pesticides on the test organisms. This inhibitory effect was more pronounced with DD force pesticide on PA1 even at 0.01% concentration (Fig. 1). This observed differences was statistically significant at ($p < 0.05$) and suggests that the varying concentrations of the different pesticides affected the test isolates differently with time.

Although PA3 isolate showed more resistance (Fig. 3) to the inhibitory effect of the pesticides even at higher concentrations, all three isolates however exhibited similar adaptability and growth response profile at varying pesticide concentrations, indicating their ability to utilize and hence degrade the pesticides.

Table 1. Summary of biochemical characteristics of test organisms

| Biochemical tests | Pseudomonas species and site origin | | |
|-------------------|-------------------------------------|---------------------------------------|------------------------------------|
| | PA1 (Agricultural research farm) | PA2 (St. Luke's Hospital dumpsite) | PA3 (Municipal waste dump site) |
| Methyl red | + | + | + |
| Voges Proskauer | - | - | - |
| Catalase | + | + | + |
| Oxidase | + | + | + |
| Urease | + | + | + |
| Citrate | - | - | - |
| Indole | - | - | - |
| Mannitol | + | - | - |
| Maltose | - | + | - |

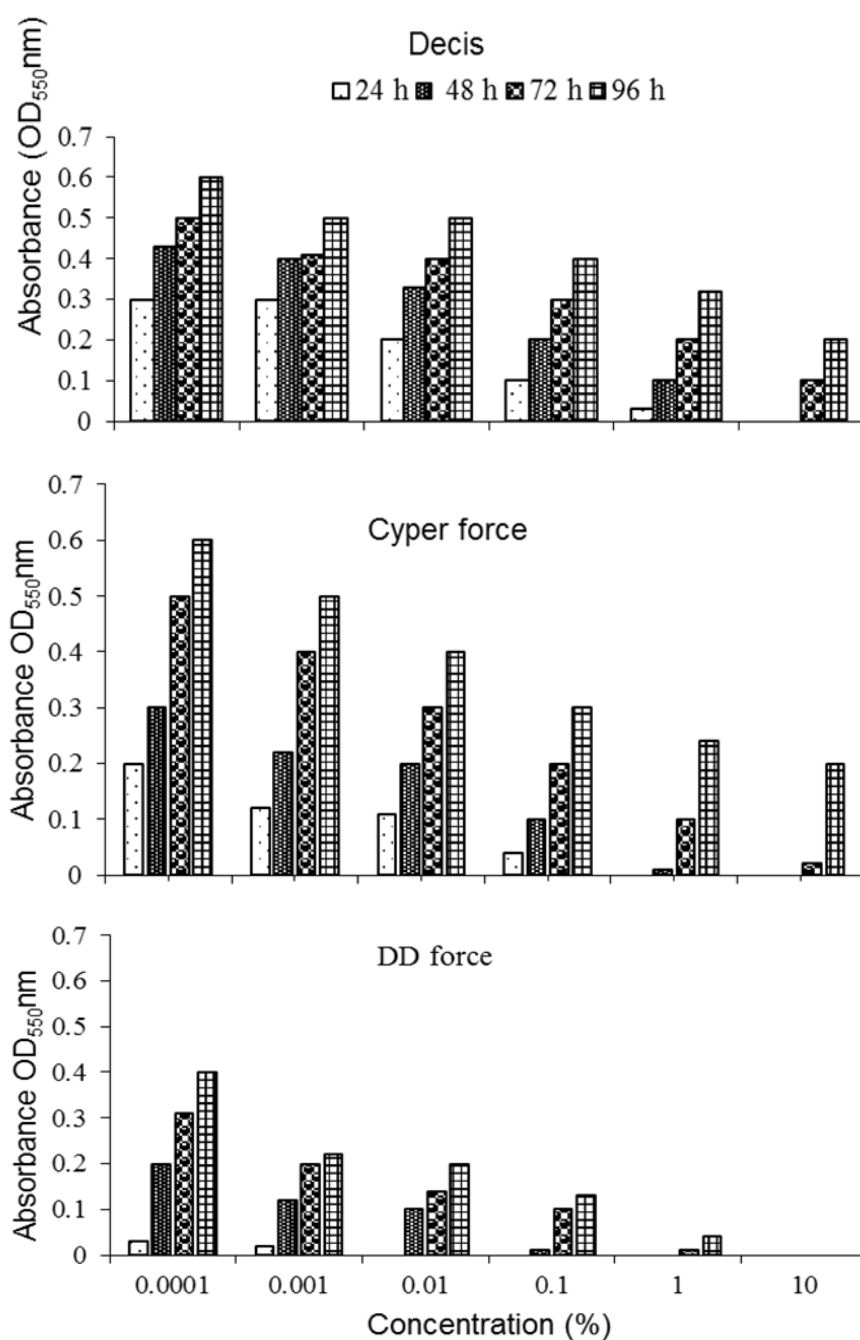


Fig. 1. Comparison of growth response profile of PA1 species to (a)-Decis, (b)-cyper force and (c)-dd force pesticides at varying concentrations over 96 hours period of time and OD_{550nm} taken as a measure of growth

4. DISCUSSION

Organic pesticides commonly used in controlling pests are inhibitory/inimical to autochthonous microflora as evinced in this study. Howbeit, this effect may be temporary or short lived [28] and

the dissipation of pesticides can persist up to seven days [29]. Furthermore, the indiscriminate and injudicious use of pesticides in soils can permit its adsorption to soil and absorption by crops which results in bioaccumulation and eventual biomagnification in successive food

chains culminating into detrimental effects/impacts on the environment and ultimately health safety [30,31].

As reported by [32], the utilization of synthetic organic pesticides has been

demonstrated predominantly by gram negative bacteria. Similarly, [33] and [34] have reported the biodegradation of pesticides by *Pseudomonas* species thus corroborating the results of this study. Also a number of studies have highlighted the ability of cyanobacteria to

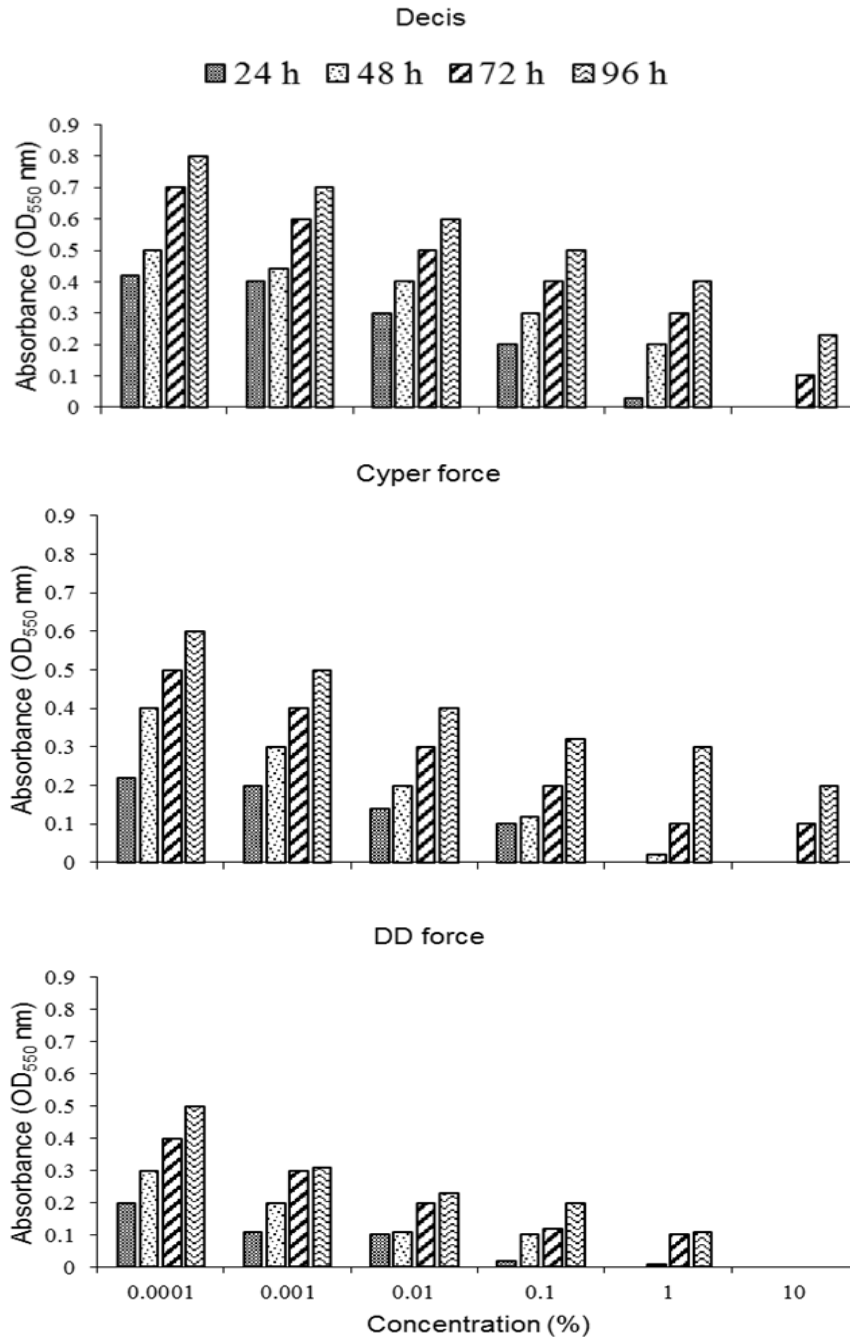


Fig. 2. Comparison of growth response profile of PA2 species to (a)-Decis, (b)-cyper force and (c)-dd force pesticides at varying concentrations over 96hours period of time and OD₅₅₀nm taken as a measure of growth

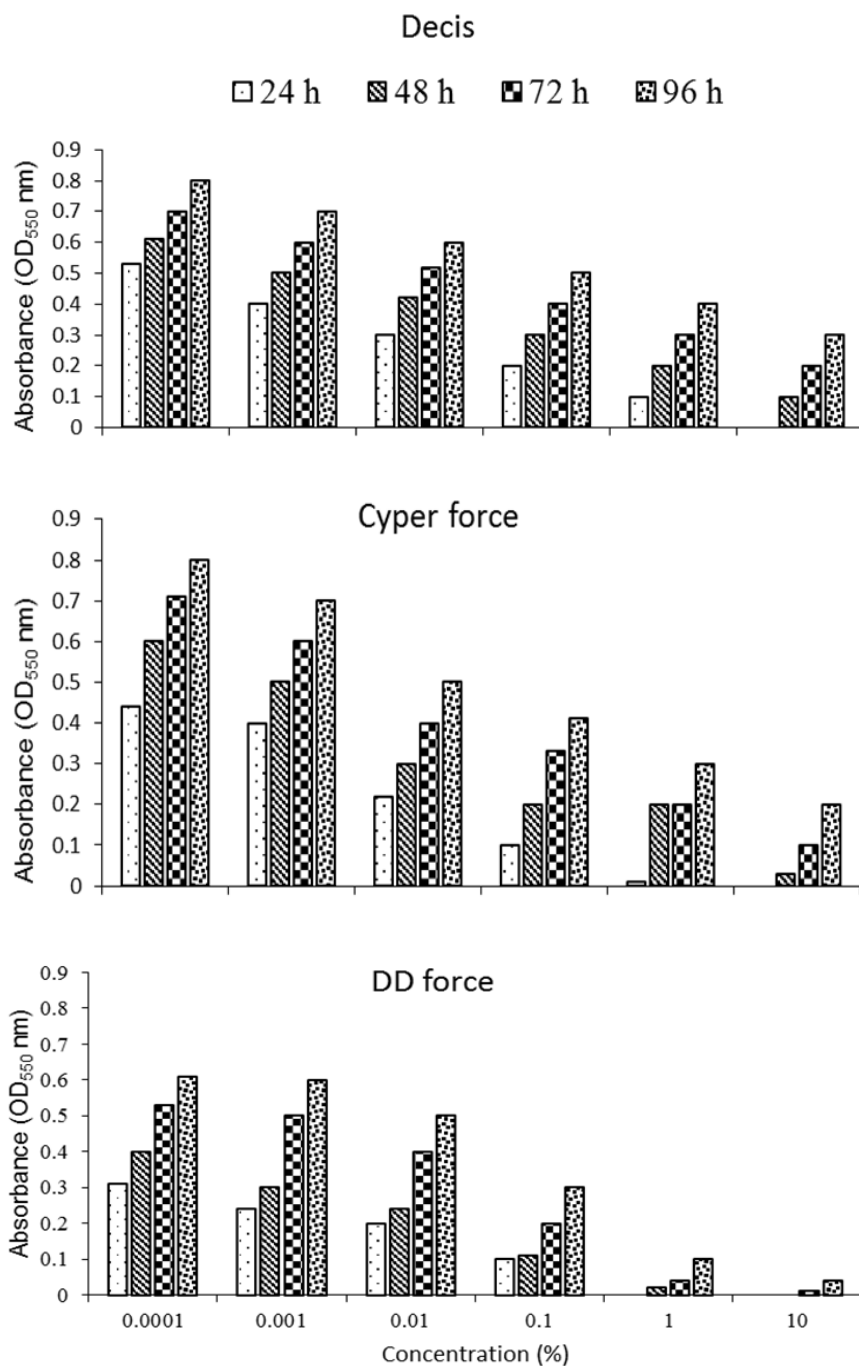


Fig. 3. Comparison of growth response profile of PA3 species to (a)-Decis (b)-cyper force and (c)-dd force pesticides at varying concentrations over 96 hours period of time and OD_{550nm} taken as a measure of growth

degrade organic pollutants including pesticides [23,35]. Recently, [36] investigated acute toxicity of Diuron, Diquat and Tertbutryn to cyanobacterial mats collected from Wadi Gaza, Palestine and reported that the algal mats

adapted and grew fast under laboratory conditions. However, there exists no report so far on the isolation of indigenous natural strains of *Pseudomonas* from these contaminated soils capable of degrading “Decis, Cyperforce and DD

force pesticides”, hence, its use for *in situ* bioremediation. This advantage therefore offers an incredible tool for eco-restoration of our degraded environment through bioremediation [37].

According to [38], the most efficient bacterial genus for the degradation of toxic compounds is *Pseudomonas*. However, microorganisms generally possess the ability to interact physically or chemically with substances leading to structural changes (biotransformation) or complete degradation (mineralization) rendering it non-toxic [39]. This ability relates to their contact time with the compound as shown in recent work of [40], environmental conditions in which they develop and their physiological versatility which arises from the production of wide array of enzymes and are considered key to the biology of many pesticides [41].

This technique of using autochthonous microbes to degrade pollutants is cost effective and ecofriendly as opposed to the setbacks of bioaugmentation which involves competition with the naturally occurring microbial communities. Nonetheless, for bioremediation purpose, it may be necessary to determine factors that will enhance (biostimulate) these strains and eventually increase their efficiency. Again, end products of degradation can be analyzed and evaluated to ensure its non-toxicity using mass spectroscopy and high performance liquid chromatography (HPLC).

5. CONCLUSION

The isolation and degradability of *Pseudomonas* strains from soils treated with different pesticides at varying concentrations offers a valuable tool for re-evaluation towards the clean-up and treatment of pesticide-contaminated soils with the ultimate aim of eco-restoration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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