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Biodegradation of Dichlorovos (Organophosphate Pesticide) in Soil by Bacterial Isolates

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Abstract
Excessive and continuous dispersion of pesticides which are toxic heterogeneous compound in the environment results in environmental pollution with ecological effects that require remediation. This study investigated the potential of microbial isolates to biodegrade or cleans up agricultural soil artificially contaminated with Dichlorvos (2, 2-dichlorovinyldimethylphosphate) pesticide. A bacterial consortium which degraded Dichlorvos pesticide was isolated from agricultural soil using pour plate method. This consortium was composed of four pure strains which were characterized based on their morphological and biochemical characteristics. The strains were presumptively identifies as *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp. and *Acinetobacte*r sp. The consortium and the four bacteria were evaluated in order to discover their ability to biodegrade Dichlorvos pesticide in medium supplied with different nutrients (NH_4NO_3, KH_2PO_4 and NPK (20:10:10) fertilizer). The results showed that the bacterial consortium and the four bacteria isolates were able to grow in nutrient medium containing Dichlorvos as the only carbon source. Moreover, the bacterial consortium was able to remove greater amount of DDP in soil amended with inorganic fertilizer (NPK) than those amended with NH_4NO_3 and KH_2PO_4, respectively. These results indicate that the isolated strains can be used for waste biodegradation or bioremediation of organophosphate pesticide- contaminated soil or water.
Keywords: bacteria; biodegradation; dichlorvos; nutrient; organophosphate pesticides

1. Introduction

Pesticides are organic compounds manufactured and used for pest control (Ortiz – Hernandez and Sanchez-Salinas, 2010). Pesticide therefore can be defined as any chemical substance or mixture of substances intended for preventing, destroying, repelling, or mitigating the effect of any pest of plants and animals. They include herbicides, insecticides, rodenticides, fungicides, molluscides, nematicides, avicides, repellents and attractants used in agriculture, public health, horticulture, food storage or a chemical substance used for a similar purpose (NAFDAC, 1996). Pesticides can be classified into five chemical groups which are organochlorine, organophosphate, carbamate, synthetic pyrethroids, avermectin and formamidine (Naqvi et al., 2011; Nsikak and Aruwajoye, 2011; Natala and Ochoje, 2009). The use of Pesticide in Nigeria has been on the increase ever since its introduction in early fifties for cocoa production. It has been estimated that about 125,000 - 130,000 metric tons of pesticides are applied every year in Nigeria (Asogwa and Dongo, 2009). The extensive use of pesticides leads to an accumulation of a huge amount of residues in the environment, thereby causing a substantial environmental health hazard due to uptake and accumulation of these toxic compounds in the food chain and drinking water (Mohammed, 2009). Organophosphate pesticides are a group of highly toxic heterogeneous compounds that share a phosphoric acid derivative chemical structure widely used for plant protection and pest control. There are currently 140 organophosphate compounds being used as pesticides and as plant growth regulators around the world (Kang et al., 2006). These compounds are components of more than 100 types of commercially available pesticides (such as Paraoxon, Parathion, Malathion, Diazinon and Dichlorvos). Most synthetic organophosphate compounds are widely used as insecticides in agriculture. These compounds are powerful inhibitors of acetylcholinesterase, a vital enzyme involved in neurotransmission, in the form of acetylcholine substitutes (Bakry et al., 2006) and various clinical effects, e.g. neck muscle weakness and diarrhea can occur due to organophosphate poisoning in humans (Serdar and Gibson, 1985; Grimsley et al., 1998). The toxicity of pesticides from exposure to contaminated food is mostly unknown but there is growing evidence of cancer, neurological damage, endocrine disruption and birth defects consequential from exposure (Miller and Sharpe, 1998; IARC, 2001; ATSDR, 2005).

The conventional methods employed for the remediation of organophosphate contaminated sites mainly chemical treatment, recycling, pyrolysis, incineration and landfills are less efficient and costly and can also lead to the formation of toxic intermediates (Dua et al., 2002; Richins et al., 1997). Many bacteria (microorganisms) that are able to degrade organophosphate pesticide have been isolated from soil around the world (Zhongli et al., 2001; Chang et al., 2005; Horne et al., 2002). Enzymatic detoxification of organophosphate pesticides by some bacterial species has been reported (Chen-Goodspeed et al., 2001; Kim et al., 2005). One of the most important enzymes that is able to hydrolyze a considerable number of synthetic organophosphate compounds is phosphotriesterase (PTE) (Mulbry et al., 1986; Mulbry, 2000). Isolation of indigenous bacteria capable of

metabolizing organophosphate compounds has received considerable attention (Richins et al., 1997; Mulchaldini et al., 1999). In view of compelling evidence of health effects on humans based on studies especially in developed countries and weak implementation of government policy on regulation / ban or surveillance program for pesticides levels in the environment and foods in Nigeria, there is need for evaluation of organophosphate pesticides detoxification. The objective of this study is to isolate and characterize an organophosphate pesticide degrading bacterial consortium obtained from a contaminated agricultural soil and to test its potential use in bioremediation of soil contaminated with Dichlorvos pesticide as well as to observe the effect of different nutrients on the degradation of Dichlorvos pesticide in soil.

2. Materials and Methods

2.1 Materials

Analytical grade dichlorvos (2, 2 - dichlorovinyldimethylphosphate, 97% pure), nutrient agar and other chemicals used as media components (products of Sigma-Aldrich, USA) as well as NPK (20:10:10) fertilizer were purchased from local distributors in Lagos, Nigeria.

2.2 Sample Collection and Artificial Contamination

Soil samples were collected from Ladoke Akintola University of Technology agricultural farm and were characterized for physical and chemical properties. The characterized properties are presented in Table 1. One kilogram (1 kg) of the characterized soil was dispensed into four different thin walled aluminum cores. Different quantity of the organophosphate pesticide (O, 10, 20 and 30 ml) was each added to the soil in the different aluminum core, respectively. The aluminum cores and its contents were placed under a green vegetable area within the university premises. Green tissue effect help in the supply of oxygen needed for microbial growth and the trees present help in the temperature regulation during sunny day. The aluminum cores were left in the site for 28 days (4weeks) with regular interval of stirring and addition of well water to ensure proper agitation, aeration and even temperature distribution required for microorganism development.

Table 1: Physicochemical Characteristics of Uncontaminated Soil

Parameters	Value
Moisture content (%)	9.20 ± 0.02
Total nitrogen (%)	0.20 ± 0.01
Total organic carbon (%)	0.45 ± 0.03
Available phosphorus (%)	0.10 ± 0.02
Potassium (%)	0.16 ± 0.03
pH	6.1 ± 0.02

2.3 Isolation and Characterization of Bacteria

One gram each of the pesticide contaminated soil (soil contaminated with 20 ml and 30 ml organophosphate pesticide) was taken in twelve sterilized test tubes and 9 ml each of de-ionized water was added for serial dilution. These soil samples were then introduced into several sterilized Petri-dishes that contain prepared nutrient agar medium. The Petri-dishes and its contents were allowed to incubate at room temperature for 72 hrs to allow for microbial growth. The consortium bacteria isolated from soil contaminated with 20 and 30 ml of Dichlorvos pesticide was selected for colony purification and for further evaluation of the capacity of isolated bacteria to hydrolyze organophosphate pesticide. Several different colonies were chosen from the isolated consortium. Those colonies considered as visually different were streaked separately on nutrient agar plates containing 30 ml of organophosphate pesticide and incubated at 37^{o}C. This procedure was repeated several times to ensure the purity of the isolated colonies. In order to characterize the isolated bacteria, the sample was subjected to a wide range of biochemical tests including growth on different NaCl concentrations, indol, catalase and oxidase. The shape and morphology of bacterial cells were determined by light microscopy (Granados and Villaverde, 1996). Identification was made following Bergey's Manual of Determinitive Bacteriology (Holt et al, 1994).

2.4 Biodegradation Experimental Design

The degradation experiments with the pure colonies and consortia isolated were carried out. Ten grams of sterilized soil sample was introduced into five different Petri-dishes and labeled A, B, C, D and E. Two ml of deionized water was added to each of the Petri-dish so as to moisten the soil and followed by the addition of 2 ml of Dichlorvos pesticide. No nutrient was added to Petri-dish A. Three different types of nutrient was added to Petri-dish B, C, and D, respectively. To Petri-dish B was added 1.6 g of ammonium nitrate (NH_4NO_3), 2.7 g of potassium dihydrogen phosphate (KH_2PO_4) to Petri-dish C and 4.7 g NPK fertilizer to Petri-dish D, respectively. The contents in each of the Petri-dish A, B, C and D were inoculated with the isolated bacterial consortia and then incubated for 28 days at room temperature. Petri-dish E that contained no nutrient and was not inoculated with the isolated bacterial consortia served as control. The same procedure was repeated for Petri-dishes to be inoculated with the pure colonies. All the experiments were done in duplicate. Samples were withdrawn at 7

days interval for residual pesticide determination.

2.5 Extraction and Determination of Residual Pesticide

Ten millilitre of hexane was added to each Petri-dish after their respective days and the mixtures were shaken thoroughly to allow hexane mix properly and extract all the residual pesticides in the soil sample. The mixtures were left for 48 hrs in a separating funnel after which it separated into two layers: liquid layer made up of hexane, water and the extracted residual pesticide on top and, solid layer (residue) that is made up of sand particles at the bottom of the funnel. The liquid layer (supernatant) was decanted and centrifuged at 300 rpm for about 60 seconds, after which it was transferred into a separating funnel. After separation, the residual pesticide layer was poured into dispensable Petri-dishes and placed in the air for 5 minutes to allow for the evaporation of hexane solution and the residual pesticide measured using syringes.

3. Results and Discussion

Four bacterial strains capable of utilizing organophosphate pesticide as the sole carbon source for growth were isolated from the agricultural soil sample in the presence of Dichlorvos pesticide. They were presumptively identified according to their physical and biochemical characteristics as presented in Table 2. The S1, S2, S3 and S4 strains were identified as *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp., and *Acinetobacter* sp, respectively.

Table 2: The Physical and Biochemical Characteristics of Bacterial Isolates

Strain	Shape	GR	MT	CT	OX	IN	CI	GL
S1	Circular	-	+	+	-	+	-	+
S2	Irregular	-	-	-	-	+	-	-
S3	Circular	-	+	-	+	+	-	+
S4	Circular	-	+	-	-	-	+	+

GR = Gram staining; MT = Motility; CT = Catalase; OX = Oxidase,; IN =Indol; CI = Citrate; GL = Glucose; + = positive reaction; - = negative reaction.

3.1 Pesticide Degradation by Isolated Bacteria

No degradation was observed in case of sterilized soil that was not inoculated with bacteria and the level of pesticide was maintained throughout the experiment, while degradation was observed in sterilized soil that was inoculated with bacteria. This showed that soil bacteria play vital role in pesticide degradation (Chaudhry and Ali, 1988; Naqvi et al., 2011). Fig. 1 shows the degradation of Dichlorvos pesticide by the isolated bacterial consortium under different nutrient application. It is seen in Fig. 1 that the consortium was able to a very large extent remove (or degrade) the organophosphate pesticide from the contaminated soil under no nutrient and nutrient application respectively. The percentage removal of Dichlorvos pesticide from the soil was relatively higher for soil amended with NPK than for soil amended with NH_4NO_3 (75%) and KH_2PO_4 (82.5%) respectively. The % removal of Dichlorvos pesticide for soil not amended with any nutrient was 70% and when compared with those amended with nutrients indicated that biostimulation of soil with nutrients of nitrogen and phosphorus source enhances the removal or degradation rate of pesticide contaminated soil. Naqvi et al. (2011) reported that the addition of nutrients increased the rate of degradation of carbaryl pesticide and nitrogen amended soil had the most significant effect on the degradation of carbaryl pesticide which was about more than 85%. Previous findings have showed that nutrient addition stimulate the degradation of different types of pollutants. It has been observed that fertilizers increased the rate of degradation of carbaryl pesticide in soil (Roling et al., 2002; Roling et al., 2004). The effect of phosphorus addition on degradation of benzylamine and caprolactum by means of selected microbes has been studied and found that phosphorus addition positively increase the rate of degradation of both compounds (Steffensen and Alexander, 1995).

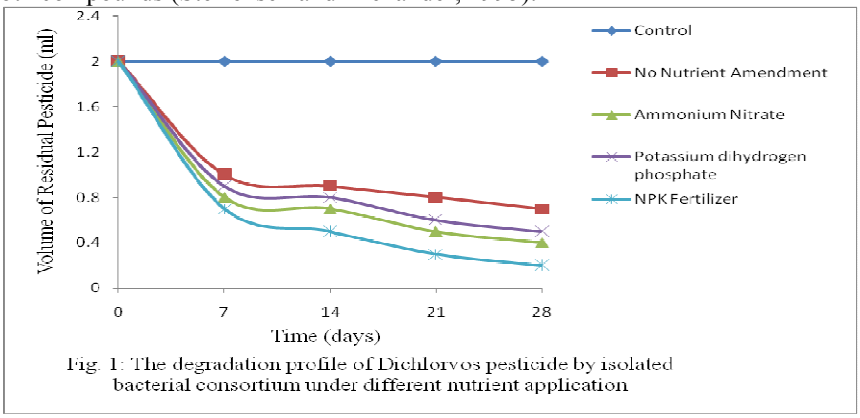

Fig. 1: The degradation profile of Dichlorvos pesticide by isolated bacterial consortium under different nutrient application

Dichlorvos pesticide degradation with the pure strain separated from the isolated consortium was also analyzed.

Fig 2 shows the results obtained from measuring the volume of residual pesticide for each type of bacteria in Dichlorvos pesticide contaminated soil incubated for one week period. It can be seen that the S1 strain (*Proteus vulgaris*) showed the highest removal rate (70%) in comparison to the other strains. The strain that showed the lowest removal rate (45%) was S4 (*Vibrio* sp.). Ortiz-Hernandez and Sanchez-Salinas (2010) have reported that *Serratia* sp., *Vibrio metschinkouii* and *Proteus vulgaris* could biodegrade tetrachlorvinphos organophosphate pesticide in soil, while Sabit et al. (2011) reported that *Acinetobacter* sp can biodegrade Malathion pesticide.

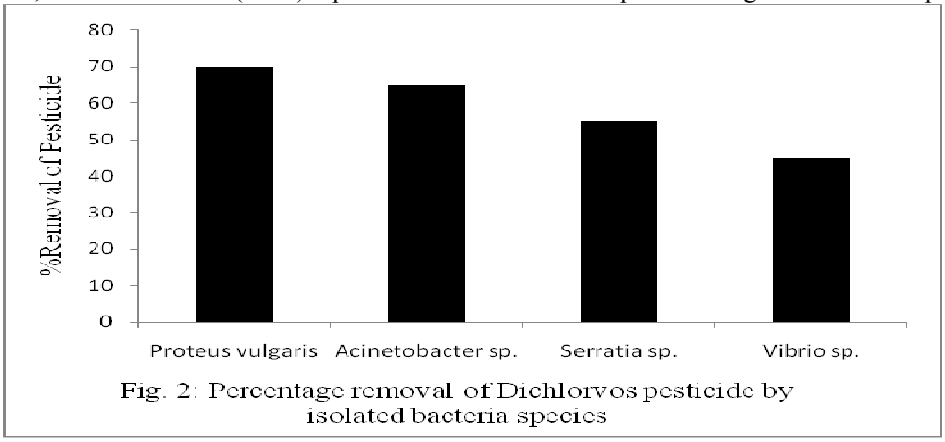

Fig. 2: Percentage removal of Dichlorvos pesticide by isolated bacteria species

4. Conclusion

The present study reports isolation of a bacterial consortium that is capable of utilizing Dichlorvos pesticide as a soil source of carbon. Utilization of xenobiotic compounds by soil microbes is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. The results of the present study suggest that the bacteria which were isolated are able to grow in an environmental in the presence of added pesticide and may thus be used for bioremediation of pesticide contaminated soil and water.

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