Full Length Research Paper

Biodegradation of fenthion and temphos in liquid media by *Bacillus safensis* isolated from pesticides polluted soil in the Sudan

Azhari Omer Abdelbagi¹, Adam Ishag Abdallah Wady¹, Abd Elaziz Sulieman Ahmed Ishag¹*, Ahmed Mohammed Ali Hammad¹, Mohamed Abdalla Omer Abdalla² and Jang-Hyun Hur³

¹Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Sudan.
²Department of Botany, Faculty of Agriculture, University of Khartoum, Sudan.
³Department of Biological Environment, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea.

Received 5 September, 2017; Accepted 26 January, 2018

The objective of this study was to evaluate the capability of the bacteria *Bacillus safensis* strain FO-36b T isolated from pesticide-polluted soil in degrading fenthion and temphos in mineral salt media (MSM). Fenthion and temphos were incubated with the isolated bacteria. Samples were drawn at 0, 3, 7, 14, and 30 days to analyze residual fenthion and temphos content with gas chromatography (GC) and high-performance liquid chromatography (HPLC), respectively. The loss of the initial pesticide concentration (400 mg/L) over time was determined and used to compute the half-lives using a biphasic model. Gas chromatography-mass spectrometry (GC-MS) was used to identify the major metabolites as well as to re-confirm the identity of starting material (fenthion). The results showed that the bacterium was still viable at the end of each incubation period. The biodegradation of fenthion and temphos followed a biphasic model. The half-lives of fenthion in the first and in the second phase were 0.29 and 3.69 days, respectively, whereas the corresponding values for temphos were 0.11 and 1.15 days. Only one metabolite “iso-fenthion” (O, S-dimethyl O-[3-methyl-4-(methylthio)phenyl] phosphorothioate) was detected in fenthion culture, while no metabolites were detected in temphos culture. Based on the half-lives, this bacterium was able to degrade temphos at a faster rate than fenthion.

Key words: Biodegradation, fenthion, temphos, bacteria, pesticides-contaminated soil, Sudan.

INTRODUCTION

Fenthion and temphos are organophosphorus insecticides used as larvicides in fresh and polluted waters, under urban malaria schemes (UMS). The use of the same larvicide for a long-time may, however, cause resistance in mosquito larvae (Mittal et al., 1999). Fenthion and temphos are used in Sudan to control...
Fenthion is moderately toxic to mammals if ingested, inhaled, or absorbed through skin (Smith 1993) and highly toxic to birds. Based on its high toxicity to birds, fenthion is used in various parts of the world for weaver bird control as well as for the control of pigeons around public buildings. It has contact action and it is readily absorbed through skin. It is applied as a paste to roosting areas when utilized for such purposes (McCewen and Stephenson, 1979). Fenthion is classified by the U.S. Environmental Protection Agency (EPA) as a Restricted Use Pesticide (RUP) due to the special handling warranted by its toxicity (VanDrieshe, 1985).

Temphos is considered as a basic larvicide for immature stages of mosquito (Jamal et al., 2011). Its aerial application over aquatic sites may contaminate surface and drinking waters. The human population may be exposed to temphos via ingestion of some fish/seafood, drinking water, and dermal contact with consumer products containing this compound.

In water, temphos might be adsorbed to organic matter and slowly released to achieve steady state. Remediation of some elements pollutant using sorption process by various source materials of natural organic matter in aqueous solution was reported (Butnariu et al., 2015). Temphos adsorption to sediment steadily increased to a maximum after two days of exposure, but temphos degradation products were shown to adsorb less strongly to soils. Absorption would be expected to be less than 3% of applied dose. In mammals, elimination of mainly unchanged temphos is in the feces and urine. It might also be released to the environment through various waste streams (CASRN, 2015). US EPA concluded that there was no evidence of carcinogenicity of temphos. Temphos formulations were classified as slightly toxic end-use products (EPA toxicity class III) (US EPA, 2001).

Biodegradation is a common mechanism for fenthion and temphos degradation in the environment (HSDB, 2003). The potential use of Sudanese soil microorganisms in cleaning pesticides polluted soils in Sudan and dump sites was first argued by Abdelbagi et al. (2000, 2003).

Strains of microorganisms isolated from pesticides polluted soils in Sudan were reported to have great capability for the degradation of some pesticides such as malathion, chlorpyrifos, dimethoate, benomyl, thiram, oxyfluorfen, lindane, endosulfan pemendimethalin, atrazine, and azoxystrobin (Ishag et al., 2017, 2016; Elsalaﬁ et al., 2015; Abdurruhman et al., 2015; Shaer et al., 2013; Elhussein et al., 2011; Mohamed et al., 2011; Elsaid et al., 2009; Elsaid and Abdelbagi, 2010; Osman, 2006). Their degradation capability can be enhanced by many activators such as farm manure and synthetic fertilizers (Elsaid et al., 2009). This study was initiated to evaluate the potential capability of the indigenous bacteria Bacillus safensis isolated from pesticides polluted soils in degrading fenthion and temphos under the condition of mineral salt media. To study the biodegradation of fenthion and temphos, the specific objectives were: (1) to characterize biodegradation rates on mineral salt media and (2) to identify bio-degradation products especially of toxicological concern.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Analytical standards of the organophosphorus insecticides temphos (94.9% pure) and fenthion (95.5% pure) were obtained from the Agricultural Research Corporation, Sudan. Solvents (99.8% pure; acetone, n-hexane, ethanol, dichloromethane and other solvents) were obtained from Fischer, company, UK.

**Isolation and identification of microorganisms from pesticides polluted soils**

Surface soil samples were randomly collected from pesticides polluted storage soil in Hasahisa, (Gezira scheme) using a soil auger (10 cm length x 5 cm diameter). Five augers were taken and mixed thoroughly to make the composite sample (1 kg). The collected samples were placed in labeled paper bags and immediately transported to the pesticides laboratory, Crop Protection Department, Faculty of Agriculture, University of Khartoum, and then sent to the Microbiology Laboratory, Faculty of Veterinary Medicine, University of Khartoum for isolation and identification of the types of bacteria present. Isolation and identification were done according to the methods described by Cowan and Steele (1993). The identified isolate have been reconfirmed by molecular biotechnology (Ishag et al., 2016, 2017)

The identified bacterial strain was subcultured on meat peptone agar for 24 h prior to their use in biodegradation study using mineral salt media (almost organic carbon free media).

**Preparation of media**

**Meat peptone agar (MPA)**

This media was prepared by adding 5 g meat, 7.5 g of peptone, 5 g NaCl, and 15 g agar to 1 L distilled water according to the methods of Tepper et al. (1993) and kept in a refrigerator at 5°C for further use.

**Mineral salt medium (MSM)**

MSM was prepared following the method described by Tepper et al. (1993); 1 g KH2PO4, 0.5 g MgSO4, 7H2O, 0.5 g NaCl, 0.001 g FeS04·7H2O, 0.01 g MnSO4·4H2O, and 0.05 g CaCO3 were added to a conical flask (1500 mL) and then, the volume was completed to 1 L by adding distilled water. The media were autoclaved for 20 min at 121°C and then allowed to cool at room temperature and kept in a refrigerator at 5°C for further use.
Preparation of the microbial inoculums

Two hundred milliliters of MPA were taken and placed in a 250 mL conical flask and inoculated with bacteria using sterilized loops. Inoculated flask was then closed with sterilized cotton and kept in an incubator (thermostatic cabinet, Austria) at 25°C for 24 h prior to use in biodegradation experiment.

Microbial degradation of fenthion and temphos in mineral salt media

The aim of this experiment was to evaluate the capability of the isolated bacteria *B. safensis* in degrading temphos and fenthion in mineral salt media. A total of 30 clean test tubes were sterilized in an oven for 3 h at 180°C. Ten milliliters of mineral salt media (MSM) were taken from the stock flask into each test tube. One milliliter of inoculum was added to each test tube. The cultured test tubes were incubated at 25°C with 400 mg/L temphos and fenthion for 0, 3, 7, 14, and 30 days. The experimental units were arranged in a Completely Randomized Design (CRD) with two replicates. Control sets without bacterial inoculums were incubated under the same conditions. The recovery sets were immediately extracted and kept in the refrigerator for analysis by Gas Chromatograph (GC) for fenthot and High-Performance Liquid Chromatography (HPLC) for temphos.

Effect of temphos and fenthion on cultured bacteria

One milliliter of culture was taken by sterilized pipette from each test tube at the end of each period of 3, 7, 14 and 30 days and placed in a Petri dish containing sterilized meat peptone agar (MPA). The inoculated plates were then incubated at 37°C for 72 h.

Extraction of fenthion and temphos from the culture

Treated cultures were centrifuged at 800 rpm for 10 min to separate the microorganisms from the media. The supernatant was removed by careful decanting and placed in 100 ml separatory funnel and 10 ml of dichloromethane, and 10 ml saturated sodium chloride solution were added. The contents were vigorously shaken for 5 min and allowed to stand for 3 min until separation of layers. The dichloromethane layer was collected in a clean test tube and the aqueous layer was re-extracted twice with 10 ml dichloromethane. Dichloromethane fractions were recombined in a clean test tube and dried up by passing through anhydrous sodium sulfate on a filter paper. The solvent was stripped off by rotary evaporator at 70°C till dryness and the residue was reconstituted in 10 ml n-hexane and stored in the refrigerator at 5°C for Gas Chromatograph (GC) and High-Performance Liquid Chromatography (HPLC) analysis. The identity of starting materials and breakdown products were confirmed by GC-MS.

Gas chromatographic analysis

A Shimadzu GC Qp2010 system (Japan) Gas chromatograph (GC) equipped with flame ionization detector (FID) and DB-5 splitless injection fused silica capillary column of 30 m length and 0.25 mm ID was used for fenthion analysis extracts. The stationary phase (0.25 mm thickness) was 5% phenyl, methylpolysiloxane. Detector and injection temperatures were 330 and 300°C, respectively. Nitrogen was used as carrier gas at a flow rate of 4.23 ml min⁻¹. The oven temperature was programmed as follows: initial temperature was 50°C for 1 min, increased at 5°C min⁻¹ until 75°C, held for 2 min, increased again at 10°C min⁻¹ until 160°C, held for 6 min, increased by 5°C min⁻¹ until 180°C and then held for 3 min, and finally increased by 3°C min⁻¹ until the final temperature which was 240°C, with holding time of 10 min. Flow rates of the makeup gas (helium), hydrogen, and air were 30, 40, and 400 mL min⁻¹, respectively. Analysis of sample was done by duplicate injections of 1 µL each. Three concentrations (62.5, 125 and 250 mg/L) of the analytical standard of fenthion (95.5% pure) was injected under the same condition and response was used for the construction of the standard curve. Data was processed by GC solution software version 2.3. The limit of detection (LOD) of fenthion was 1.8 mg/L. The recovery of fenthion from the media was greater than 98%.

Gas chromatography with mass spectrometry (GC-MS) instrumentation

Three representative samples were reanalyzed using Shimadzu GC-MS Qp2010 system (Japan) with an AOC-5000 autosampler. The gas chromatograph was fitted with RSH-MS capillary column of 30 m × 0.25 mm ID, 0.25 µm film thicknesses from Restek (UK). Helium (purity ≥ 99.999%) was used as a carrier gas at a flow rate of 1.22 ml min⁻¹. The splitless injection temperature was 200°C. The oven temperature was programmed from an initial temperature of 100°C, held for 3 min, then increased to 180°C at 16°C min⁻¹, held for 6 min, and finally, increased by 16°C min⁻¹ to 240°C at which it was held for 3 min. The mass spectrometer was operated with electron impact (EI) source in the scan mode. The electron energy was 70 eV, and the interface temperature was maintained at 200°C. The solvent delay was set to 2 min.

High-performance liquid chromatography analysis

A Shimadzu (Kyoto, Japan) CLASS-VP, Version 5.22 High-Performance Liquid Chromatography (HPLC) equipped with a UV/Visible detector was used for analysis of extracts of temphos. Separation was performed on a Luna C18 column. The instrument system consisted of LC-10 ADvp binary pump, DGU-14 An online degasser, SPD-M10-Avp Luna absorbance detector, Sil-10 ADvp auto-injector, CTO-10 ASvp column oven fitted with Shim- Pack VP-OBS (150 × 4.6 mm, 10 µm) column and a similar pre-column (4 × 4 mm. ID). Samples were auto-injected. The detector was connected to the computer for data processing. The working condition of the HPLC was a binary gradient, with the mobile phase being acetonitrile: water (60:40), the flow rate was 1 ml min⁻¹, injection volume was 10 µL and the wavelength of the UV/Vis detector was fixed at 210 nm. Analyses of samples were done by duplicate injections of 10 µL each. Five concentrations (10, 20, 40, 80 and 100 mg/L) of the analytical standard of temphos (94.9 pure) were injected under the same condition and response was used for the construction of the standard curve. The limit of detection (LOD) of temphos was 1.58 mg/L. The recovery of temphos from the media was greater than 98%.

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) and means were separated by the LSD. The probability of 0.05 or less was considered significant (SAS 2004). A biphasic model was assumed in order to calculate the loss of fenthion and temphos from the media inoculated with the bacteria. Calculations were done according to the following equation:

$$ R = A_0e^{-ct} + B_0e^{-bt} $$

(1)

Where, R = amount of fenthion and temphos at t days, A₀ and B₀ are the concentrations of fenthion and temphos at t=0, c and β are the
Table 1. Main concentrations (±SD) of fenthion and temphos (mg/L) following incubation with *Bacillus safensis* in mineral salt medium (MSM).

| Time (days) | Fenthion (mg/L) | Temphos (mg/L) |
|------------|-----------------|----------------|
| 0          | 400^a±0.000     | 400^a±0.000    |
| 3          | 372^b±0.0039    | 307^a±0.0060   |
| 7          | 50^c±0.011      | 261^c±0.003    |
| 14         | 34^d±0.001      | 152^d±0.002    |
| 30         | 275^e±0.009     | 89^e±0.004     |
| LSD        | 21.5            | 12.036         |

Means followed with the same letter(s) in the same column are not significantly different at p=0.05 according to LSD.

## RESULTS

Biodegradation of fenthion and temphos in mineral salt media (MSM)

The indigenous bacteria *B. safensis* strain FO-36b^T^ showed capability in degrading fenthion and temphos in mineral salt media (MSM). Data in Table 1 indicates that the concentrations of fenthion and temphos declined with the increase in the incubation periods. The concentration of fenthion (400 mg/L) was found to be 400, 372.8, 350.8, 334.6, and 275.5 mg/L after 0, 3, 7, 14, and 30 days of incubation, respectively, while the concentration of temphos (400 mg/L) was found to be 400, 307.7, 261.9, 152.4, and 89.3 mg/L following the same order. Generally, the rate of fenthion disappearance was high up to day 14 and slow thereafter while that for temphos was from day 7 onward (Table 1 and Figures 1 and 2). There were significant differences between the levels of fenthion and temphos at various time intervals. Less than 68% of the initial concentration was recorded at 30 days after the incubation of fenthion with the bacteria, whereas 22% of the initial amount was found after 30 days of incubation of temphos with the bacteria. Despite the significant drop in the starting material, only one metabolite was detected "iso-fenthion" (O, S-dimethyl O-[3-methyl-4-(methylthio) phenyl] phosphorothioate) in fenthion (Figures 3, 4 and 5). The recovery of the
fenthion and temphos from the media was greater than 98%. There was no change in the cultured bacteria after each incubation period. Generally, the results in Table 3 show that the degradation constant decreased with increase in the incubation period, while the mean lifetime is directly proportional to the incubation period.

**Biodegradation kinetics**

The data in Table 2 indicates that there was a faster rate of disappearance in the first phase than in the second. This is clearly reflected in the half-life values obtained. The half-life of fenthion and temphos in the first phase were estimated at 0.29 d and 0.11 days, respectively, while the corresponding values for the second phase were 3.69 and 1.15 days.

**DISCUSSION**

The results of biodegradation of fenthion and temphos by the bacteria *B. safensis* strain FO-36bT isolated from...
Figure 4. Mass spectrum of fenthion.

Figure 5. Mass spectrum of iso-fenthion O, S-dimethyl O-[3-methyl-4-(methylthio) phenyl] phosphorothioate.

pesticides polluted soil in Sudan was studied under mineral salt medium (MSM). Results indicate that the isolated organism is capable and efficient in degrading fenthion and temphos. The bacteria reduced the half-life of fenthion to 0.29 days in the first phase ($t_{1/2}$) and 3.69 days in the second phase ($t_{1/2}$) while for temphos it was reduced to 0.11 days in the first and 1.15 days in the second phase.

This reduction can be considered very significant compared to the reported fenthion half-lives 14 to 40 days. The degradation of temphos was followed by first-order kinetics, with a half-life of 17.2 days in the soil (CASRN, 2015). *Bacillus cereus*, *Bacillus mycoides*, and *Pseudomonas aerginosa* were reported as degrades of organic compounds such as petroleum products (Okerentugba and Ezeronye, 2003; Dhanarani et al., 2016) while *B. safensis* strain CFA-06 was reported to degrade aromatic compounds and petroleum aromatics (Francie et al., 2015). *B. safensis* Gram-positive and it has environmental relevance in biocatalysis and bioremediation studies (Kothari et al., 2013). Lateef et al. (2015) reported that *B. safensis* has promising biotechnological applications due to its ability to produce various industrial enzymes and industrially applicable secondary metabolites. Abiotic factors such as pH and temperature were found to have effects on biodegradability of chlorpyrifos by test microorganism (EPA, 1997). The current result agrees with those of Shaer et al. (2013) who showed that bacterial strains (*B. cereus*, *B. mycoides*, and *P. aerginosa*) isolated from pesticide-polluted soil are capable of degrading pendimethalin under the condition of mineral salt media. Further, this study agrees with Abdurruhman et al. (2015) who mentioned that bacteria *Psedomonas pickettii* isolated from pesticides polluted soil in the Sudan are capable and efficient in degrading pendimethalin and
The detected fenthion is in line with Khaled, 2007; Pignatello (2000, 2003) that indigenous soil microorganisms could be of great potential in reducing the level of contamination by pesticides in highly polluted soils. They obtained encouraging results with degradation of different pesticides and also found that the concentration of chlorpyrifos was sharply reduced in culture of B. safensis strain FO-36bT compared to the other tested pesticides. The current study agrees with the argument of Abdelbagi et al. (2000, 2003) that indigenous soil microorganisms could be of great potential in reducing the level of contamination by pesticides in highly polluted storage soil in the Sudan. Their suggestion is in line with Elzorgani (1982) who mentioned that irrespective of a large amount of dichlorodiphenyltrichloroethane (DDT) and other pesticides applied in Gezira scheme, Sudan, yet their soil level is not high which indicate a possible and efficient degradation factors in these soils. This argument was later confirmed by Ali (2005) and Elsaid et al. (2011, 2010, 2009), who demonstrated the capability of soil microorganisms to degrade chlorpyrifos and other organophosphorus pesticides. Their study also reported that the formation of isomalathion (phenyl phosphorothioate) was detected in fenthion (Khaled et al., 2009).

Despite the drop in the starting material of temphos, no metabolites were detected. However, one metabolite “iso-fenthion” (O, S-dimethyl O-[3-methyl-4-(methylthio) phenyl] phosphorothioate) was detected in fenthion culture (Figures 3, 4 and 5). The detected fenthion metabolite (Figure 6) could be formed by rearrangement of sulfur and oxygen atom. Kouichiro and Yasuo (2006) reported that the formation of isomalathion is due to oxidation of malathion by cytochrome P-450. The absence of detectable levels of breakdown products on pesticides biodegradation studies involving bacteria and fungi was reported by many authors (Khaled et al., 2008; Ishag et al., 2016, 2017).

The bacterium was found alive after the end of each incubation period, indicating that the degradation process was not complete. This suggests that further incubation may be necessary to complete the degradation process.
incubation period and even after the end of the whole experiment (30 days). The current results of B. safensis indicate its ability to live in such media.

The current results indicate that the strain of the bacteria B. safensis isolated from pesticides polluted soil was capable of degrading both fenithion and temphos under the conditions of minimal salt media. Based on this finding and those of previous studies (Ishag et al., 2016, 2017; Abdurrufman et al., 2015; Shaer et al., 2013; Elsaid and Abdelbagi, 2010; Elsaid et al., 2011, 2010, 2009; Osman, 2006; Ali, 2005), one can argue the significant of carrying further studies on this topic such as effects of environmental factors on soil media on the rate of degradation. Isolation and characterization of the responsible enzymes in this bacterium also deserve future work. Studies of the role of other indigenous microorganism deserve future work.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abdelbagi AO, Elmahi MA, Osman, DG (2000). Chlorinated hydrocarbon insecticide residues in the Sudanese soils of limited or no pesticide use. Arab J. Plant Prot. 18:35-39.

Abdelbagi AO, Elmahi MA, Osman D (2003). Organochlorine insecticides residues in Sudanese soils of intensive pesticide use surface soil of Qurashi pesticide store. U. of K. J. Agric. Sci. 11(1):59-68.

Abdurrufman AM, Abdelbagi AO, Alsheikh E, Ahmed AS, Elsaid GE (2015). Biodegradation of pendimethalin and atrazine by Pseudomonas picketti isolated from pesticides-polluted soils under laboratory conditions. J. Biotechnol. Sci. Res. 2(3):94-102.

Ahmed BAI (2007). Field evaluation of Temephos and Aegnine (MMF) against immature stages of Anopheles arabiensis patron (Diptera: Culicidae) the vector of malaria in Khartoum, Sudan. M.Sc. Thesis, University of Sains, Malaysia.

Ahmed IA, Gamal Hi, Khaled AO (2007). Biodegradation kinetics of bromoxynil as a pollution control technology. J. Egyp. Aquat. Res. 33(3):111-121.

Ali TM (2005). The potential of naturally occurring soil microorganisms in degrading Endosulfan α, β, and Lindane: A case study of Qurashi pesticides store (Hasaheesa)- Sudan. M.Sc. (Agric) thesis, University of Khartoum, Sudan.

Agency for Toxic Substances and Disease Registry (ATSDR) (2005). Toxicological information about insecticides used for eradicating mosquitoes (West Nile Virus Control). Department of Health and Human Services: Agency for Toxic Substances and Disease Registry.

Barrow GJ, Felthman RKA (2003). Cowan, and Steele's manual for identification of medical bacteria, Third Edition, Press Syndicate of the University of Cambridge, Cambridge P 317.

Bashir AI, Jamal AE, Abdamagid ME (2012). Emergence of culex quinquefasciatus Say larvae (Diptera Culicidae) resistance to same organophosphate insecticides in Khartoum state, Sudan. Sudanese J. Public Health 7(1):17-20.

Butnaru M, Negrea P, Lupa C, Ciopec M, Negrea A, Pentea M, Sarac I, Samfris I (2015). Remediation of rare earth element pollutants by sorption process using organic natural sorbents. Int. J. Environ. Res. Public Health 12(9):11278-11287.

CASRN (2015). Temephos. Human Health Effects: 3383-96-8. HSDB database.

Daorai A, Menzer RE (1977). Behavior of Abate in microorganisms isolated from polluted water. Arch Environ. Contam. Toxicol. 5(1):229-240.

Dhanarani S, Viswanathan E, Piruthiviraj P, Arivalagan P, Kalianatt N (2016). Comparative study on the biosorption of aluminum by free and immobilized cells of Bacillus safensis KTSMBNL 26 isolated from explosive contaminated soil. J. Taiwan Inst. Chem. Eng. 69:61-67.

Elhussein A, Osman A, Shariif A (2011). Isolation characterization, identification and potentiality of fungicide thiram (TMTD) degraders under laboratory conditions. Int. J. Appl. Environ. Sci. 6(2):193-199.

Elsaid OG, Abdelbagi AO (2010). Comparative biodegradation of Endosulfan by mutant and their native microorganisms. Res. J. Agric. Biol. Sci. 6 (6):953-961.

Elsaid OG, Abdelbagi AO, Elsheikh EAE (2011). Accelerating the rate of Endosulfan degradation by bacteria and actinomycetes. Inter. J. Appl. Environ. Sci. 6 (1):11-12.

Elsaid OG, Abdelbagi AO, Elsheikh EAE (2010). Pesticide resistant bacteria strain. Inter. J. Environ. Sci. 1(2):123-131.

Elsaid OG, Abdelbagi AO, Elsheikh EAE (2009). Effects of fertilizers (activators) in enhancing microbial degradation of Endosulfan in soils. Res. J. Environ. Toxicol. 3(2):76-85.

Elsalahi R, Elhussein A, Osman A, Shaerif A (2015). Microbial degradation of fungicide benomyl in soil as influenced by addition of NPK. Int. J. Curr. Microbiol. Appl. Sci. 4(5):756-771.

Elzorgani GA (1982). The status of DTD residues in Sudan. Progress report. Agricultural Research Corporation. Wad Medani, Sudan.

Environmental Protection Agency (1997). Review of Chlorpyrifos poisoning data, Washington, DC, USA.

Francie SA, Celio FF, Marco A, Marco A, Cicero AL, Clelton A, Antonio R, Patricia FL, Valerio J (2015). Identification of oxidoreductases from the petroleum contaminated soil. J. Taiwan Inst. Chem. Eng. 69:17-20.

Ishag ASA, Abdelbagi AO, Elsaid OG, Elsheikh EAE, Hammad AMA, Hur J-H (2017). Biodegradation of endosulfan and pendimethalin by three strains of bacteria isolated from pesticides-polluted soils in the Sudan. J. Appl. Biol. Chem. 60(3):287-297.

Ishag ASA, Abdelbagi AO, Elsaid OG, Elsheikh EAE, Hammad AMA, Hur J-H, Mark DL (2016). Biodegradation of chlorpyrifos, malathion, and dimethoate by three strains of bacteria isolated from pesticides polluted soils in Sudan. J. Agric. Food. Chem. 64(45):8491-8498.

Jamae AE, Nuqad AO, Abdalmagid MA, Bashir AM, Brair I, Elnaeim H (2011). Susceptibility of Culex quinquefasciatus Say (Diptera Culicidae) in Khartoum locality (Sudan) to Malathion, Temephos,
Lambdacyhalothrin and Permethrin insecticides. Sudanese J. Public Health 6(2):56-62.

Khaled AO, Gamal HI, Ahmed IA, Abdul Rahman A (2008). Biodegradation kinetics of dicofol by selected microorganisms. J. Pestic. Physiol. 81(3):180-185.

Kothari VV, Kothari RK, Bhatt VC, Nathani NM, Koring PG, Joshi CG, Vyas BRM (2013). Genome sequence of salt-tolerant Bacillus safensis strain VK, isolated from saline desert area of Gujarat, India. Genome Announc. 1(5):13-671.

Kouichiro T, Yasuo S (2006). Detection of human butyrylcholinesterase-nerve gas adducts by liquid chromatography–mass spectrometric analysis after in gel chymotryptic digestion. J. Chromatogr. B. 838:21-30.

Lateef A, Adelere IA, Gueguim-Kana EB (2015). The biology and potential biotechnological applications of Bacillus safensis. Biologia 70(4):411-419.

McEwen FL, Stephenson GR (1979). The use and significance of pesticides in the environment. NY: John Wiley and Sons, Inc. 66: p. 108.

Meister RT (1992). Farm Chemicals Handbook. "92th" eds. Meister Publishing Company, Willoughby, OH.

Mittal PK, Batra CP, Adak T (1999). Susceptibility status of Culex quinquefasciatus larvae to fenthion in Delhi: a note on the possible development of resistance. Indian Malar. 36:81-84.

Mohamed A, Elhussein A, Elsiddig M, Osman A (2011). Degradation of oxyfluorfen herbicide by soil microorganisms. Biotechnology 10(3):274-279.

Ohno M, Okamoto H (1980). Test on the susceptibility of the last in star larvae of chironomus yoshimatsui Martin and Sublette (Diptera, Chironomidae) Collected at the kanda River to tow organophosphorus insecticides. Annual Report. Tokyo Metropol. Public Health Res. Lab. 31:261-264.

Okerentugba PO, Ezeronye OU (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Afr. J. Biotechnol. 2(9):288-292.

Osman A (2006). Degradation of fungicide azoxystrobin by soil microorganisms. U. K. J. Agric. Sci. 14(1):124-134.

Pignatello JJ, Xing B (1995). Mechanisms of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30(1):1-1.

Rigas F, Papadopoulo K, Dritsa V (2007). Bioremediation of a soil contaminated by lindane utilizing the fungus Ganoderma austral via response surface methodology. J. Hazard. Mater. 140:325-332.

Shaer IB, Abdelbar AO, Alsheikh E, Ahmed AS, Elsaid GGE (2013). Biodegradation of pendimethalin by three strains of bacteria isolation from pesticides polluted soil. U. of K. J. Agric. Sci. 21(12):233-252.

Smith GJ (1993). Toxicology and pesticide use in relation to wildlife: organophosphorus and carbamate compounds. U.S. Department of the Interior, Fish, and Wildlife. C.K. Smoley. Boca Raton. p 510.

Tepper EZ, Shiilinkova UK, Perverzeva GE (1994). Manual of microbiology, Mosco, Kolas. 4th Edition. P 170.

United State Environmental Protection Agency (US EPA) (2001). Interim reregistration eligibility decision for fenthion. United States Environmental Protection Agency.

VanDrieshe RG (1985). Pesticide facts. Cooperative Extension Service. Department of Entomology. University of Massachusetts. Amherst, MA; Cooperative Extension Service.

Wauchope RD, Butler TM, Hornsby AG, Augustijn-Beckers PWM, Burt JP (1992). SCS/ARS/CES Pesticide properties database for environmental decision making. Rev. Environ. Contam. Toxicol. 123:157-164.