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-36bᵀ 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...
larvae of malaria vectors (Bashir et al., 2012). Fenthion is available as dusts, emulsifiable concentrate, granules, liquid concentrates, spray concentrates, ultra-low volume (ULV) and wettable powder formulations (Meister, 1992). The frequent and extensive field use of temphos has caused the development of resistance in Chironomus yoshimatsui in the Kanda River, Tokyo (Ohno and Okamoto, 1980).

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 (McEwen 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 p冷dimethalin, atrazine, and azoxystrobine (Ishag et al., 2017, 2016; Elsalahi et al., 2015; Abdurrubman 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 × 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 K2HPO4, 0.5 g MgSO4. 7H2O, 0.5 g NaCl, 0.001 g FeSO4.7H2O, 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 fenthion 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 residues were 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-ODS (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. The solvent delay was set to 2 min.

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:

\[ R = A e^{-\alpha t} + B e^{-\beta t} \]  

(1)

Where, \( R \) is amount of fenthion and temphos at t days, \( A \) and \( B \) are the concentrations of fenthion and temphos at t=0, \( \alpha \) and \( \beta \) are the
Table 1. Main concentrations (±SD) of fenthion and temphos (mg/L) following incubation with *Bacillus safensis* in mineral salt medium (MSM).

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Fenthion (mg/L)</th>
<th>Temphos (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>400±0.000</td>
<td>400±0.000</td>
</tr>
<tr>
<td>3</td>
<td>372±0.0039</td>
<td>307±0.0060</td>
</tr>
<tr>
<td>7</td>
<td>50±0.011</td>
<td>261±0.003</td>
</tr>
<tr>
<td>14</td>
<td>34±0.001</td>
<td>152±0.002</td>
</tr>
<tr>
<td>30</td>
<td>275±0.009</td>
<td>89±0.004</td>
</tr>
<tr>
<td>LSD</td>
<td>21.5</td>
<td>12.036</td>
</tr>
</tbody>
</table>

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-36bT 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 disappearance rate constants for first and second phase model, respectively. The half-life of exponential decay was calculated according to equation:

\[ T_{1/2} = \frac{(2.303 \log 2)}{\text{rate constant}} \]  

(2)

**Figure 1.** Amount remaining (%) of fenthion after incubation with *Bacillus safensis* in mineral salt media (MSM).
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-36b<sup>T</sup> isolated from
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 aeruginosa 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. aeruginosa) 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 Pseudomonas pickettii isolated from pesticides polluted soil in the Sudan are capable and efficient in degrading pendimethalin and
Table 2. Statistical parameters of fenthion and temphos bacterial dissipation in mineral salt medium (MSM).

<table>
<thead>
<tr>
<th>Statistical parameter</th>
<th>Fenthion</th>
<th>Temphos</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_0$</td>
<td>372</td>
<td>333</td>
</tr>
<tr>
<td>$B_0$</td>
<td>350</td>
<td>261.8</td>
</tr>
<tr>
<td>$\alpha$ (days$^{-1}$)</td>
<td>0.0236</td>
<td>0.0874</td>
</tr>
<tr>
<td>$\beta$ (days$^{-1}$)</td>
<td>0.0187</td>
<td>0.0604</td>
</tr>
<tr>
<td>$t_{1/2a}$ (days)</td>
<td>0.2925</td>
<td>0.0604</td>
</tr>
<tr>
<td>$t_{1/2b}$ (days)</td>
<td>3.694</td>
<td>1.1457</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>0.559</td>
<td>0.8333</td>
</tr>
</tbody>
</table>

$A_0$ and $B_0$ are the concentration of fenthion and temphos at $t = 0$ and $\alpha$, $\beta$ are the disappearance rate constants for the first and second phase model, respectively.

Table 3. Mean lifetimes (days) and decay constants of fenthion and temphos following incubation with the B. safensis.

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Mean lifetime</th>
<th>Decay constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Fenthion</td>
<td>41.34</td>
<td>52.42</td>
</tr>
<tr>
<td>Temphos</td>
<td>11.34</td>
<td>16.40</td>
</tr>
</tbody>
</table>

atrazine under the condition of mineral salt media.

Temphos, when incubated in water with isolates from a sewage treatment lagoon or a farm pond, was found to slowly degrade after a 7 day lag period, forming products arising from the oxidation of the sulfide group and hydrolysis of the phosphate group (Daorai and Menzer, 1977). In soil, fenthion degradation ranges from 4 to 6 weeks and it occurs through photodegradation as well as anaerobic or non-photolytic organisms. However, soil particles strongly adsorb fenthion that makes it less susceptible to percolate with water through the soil (ATSDR, 2005). Recently, Ishag et al. (2016, 2017) reported at the first time degradation of chlorpyrifos, dimethoate, malathion, pendimethalin, and endosulfan by newly isolated bacterial strains form pesticides 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-36b$^T$ than 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 and efficiency of indigenous soil microorganisms (bacteria, actinomycetes, and fungi) in degrading endosulfan and lindane under the condition of selective mineral salt medium or soil. In addition, various types of synthetic and natural fertilizers were found to enhance the degradation rate (Elsaid et al., 2009).

Biodegradation of fenthion and temphos by B. safensis followed a biphasic model of initial phase of fast rate of disappearance followed by a second phase of slow disappearance. This phenomenon of biphasic disappearance rate in soil is common in many pesticides (Khaled et al., 2008; Rigas et al., 2007; Ahmed et al., 2007; Pigatello et al., 1996; Smith, 1993; Wauchope et al., 1992). The relative importance of the phase depends on the availability of the pollutants, hydrophobicity, and affinity for organic matter (Rigas et al., 2007; Pignatello and Xing, 1995).

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 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 fenthion and temphos under the conditions of mineral salt media. Based on this finding and those of previous studies (Ishag et al., 2016, 2017; Abdurrubberman 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 to be included in 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**


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


Jama AE, Nugud AD, Abdalmagid MA, Bashir AIM, Brair I, Elnaeih H (2011). Susceptibility of *Culex quinquefasciatus* Say (Diptera Culicidae) in Khartoum locality (Sudan) to Malathion, Temephos,

**Figure 6.** Degradation pathway of fenthion by *B. safensis* in MSM.


