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Original Research Article

EXTRACTION AND PHOTODEGRADATION OF FENITROTHION ON PLANT SURFACES

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ABSTRACT

In order to carry out risk assessments for the use of pesticides, it is necessary to understand the fate of the pesticide within the environment. The degradation on leaves is highly important because it is the first surface which the pesticide comes into contact with and from which the pesticide spreads to the wider environment. The aim of this study was to investigate the degradation of fenitrothion, an organophosphorus insecticide used widely in agriculture. Firstly a suitable extraction method was developed, followed by experiments in a solar simulator to determine the half-life of fenitrothion on lettuce (Lactuca sativa), cabbage "greyhound" (Brassica oleracea sabauda) and cabbage "goldenacre" (Brassica oleracea sabauda). The reproducibility of the method was checked. It was found the best method of extraction was sonicating the leaf in 10 mL of methanol for 10 seconds. The half-lives of fenitrothion on lettuce, cabbage "greyhound" and cabbage "goldenacre" were found to be 7.7±0.7 hrs, 2.1± 0.2 hrs and 3.9±1 hrs respectively. Repetition of the extraction method confirmed the reproducibility of the experiments with a coefficient of variation ranging from 2.5 % to 10.6 %. This research is relevant in determining the extent of pesticide residues on fruit surfaces after post- harvest application.

1. INTRODUCTION

Pesticides are extensively used in the modern world and although pesticides are beneficial for the human population, they can be highly toxic to both humans and the natural environment (Costa 2008). In the mid-1940s organochlorinated insecticides, for example dichlorodiphenyltrichloroethane (DDT), chlordane, heptachlor, aldrin, dieldrin and lindane, were commercialized (Costa 2008). Their use significantly increased over the next two

decades. However, their toxicological effects were not noticed until the 1960s when the populations of many large predator birds decreased. Eight of the "dirty dozen", which are also known as persistent organic pollutants (POP), are organochlorine insecticides (Baird and Cann 2008). It is necessary to understand how OP pesticides are toxic to both target and non-target species to be able to undertake a risk assessment. The mechanisms of toxicity of OP are based on the binding of phosphorus – oxygen bond (O=P) and alkoxy groups with the serine group in acetylcholinesterase (AChE). This results in the irreversible inhibition of AChE. AChE hydrolyzes acetylcholine, a major neurotransmitter in the central and peripheral nervous system. The inhibition of AChE causes accumulation of acetylcholine which over stimulates the receptors in nerves. The symptoms of a high dose OP poisoning in humans include increased sweating, salivation, broncho-constriction, bronchial secretion, gastrointestinal activity, diarrhoea, tremors, muscular twitching and various central nervous system effects. Death may occur as a result of respiratory failure (Costa 2008). The pesticide tolerance set by the Food and Drug Administration (USA) range from 1 to 8 μg/mL for OP pesticides (Mathew et al., 2007).

Fenitrothion was chosen to represent OP pesticides in the experiments although it is not as commonly used as other OP pesticides (Weber et al., 2009a). It was chosen because it is a model chemical that absorbs strongly in within the UV region of the solar spectrum hence susceptible to direct photodegradation. It is a phosphorothioate pesticide used to control chewing and sucking insects on rice, cereals, and vegetables, in forests as well as stored grain and cotton. It is also used in the control of mosquitoes, flies and cockroaches for public health. Fenitrothion is also non-polar molecule with a high octanol: water coefficient (log Kow) so it may adsorb to the lipid rich leaf surfaces. Plant surfaces, especially leaves, are the first reaction environment for a pesticide after application (Katagi, 2004). To understand the risk to human health and the environment from the use of pesticides, their fate and degradation within the environment are intensively investigated via models and experiments. Most of the research on the degradation of pesticides has been done in solvents, especially water. Other solvents used in research included a mixture of water and methanol and hydrogen peroxide (Pehkonen and Zhang 2002). Less data have been collected on plant and soil degradation because they are heterogeneous surfaces which can lead to complicated reactions with many variables, making their rationalization difficult. (Katagi 2004, Lavieille et al., 2008). This study was therefore designed to investigate the rate of photodegradation of fenitrothion, an organophosphorus insecticide on leaf surfaces. In order to do this, it was necessary to first establish a suitable method for extracting the fenitrothion from the leaves. After the extraction method was determined, the half-lives of fenitrothion on different leaves were determined when exposed to light.

2. MATERIALS AND METHODS

2.1. Materials

Fenitrothion (98.3 % purity) was purchased from Sigma Aldrich UK. Methanol, dichloromethane (DCM), acetone and hexane were of HPLC grade (Fisher, UK). Water used was purified on a Milli-RO plus 30® and Milli-Q® purifier (Millipore, MA, USA) (electrical

resistivity 18 MΩ cm^{-1}, TOC <5 ppb). The plant chosen for this study: lettuce, cabbage where selected because they are usually grown in protected environments (poly – tunnels). The selected plants were grown in controlled environments and used when they were about 3 – 4 weeks old.

The Suntest CPS^{+} solar simulator (Atlas MTT, Illinois US), equipped with an immersion water-cooling unit, referred to as Atlas from here on, was used to simulate solar radiation. Analyses were performed on a Surveyor liquid chromatography (LC) system (Thermo Finnigan, MA, USA) with a Photodiode Array (PDA) detector. Analytic separation was conducted on a reversed-phase column (Synergi 4u fusion, 150 x 4.6 mm, Phenomenex, CA, USA) at room temperature. The mobile phase was run with methanol/water with a 10 µl injection volume at a flow of 1 mL min^{-1} over a 20 minutes runtime. The mobile phase gradient was as follows: started at 95% water and 5% methanol then to 25 % water and 75% methanol in 10 minutes and held for 4 minutes. Then to 100% methanol in 2 minutes and held for 2 minutes. The solvent combination was then returned to the initial ratio in preparation for the next injection.

2.2. Extraction Experiments

In order to establish the best extraction method of fenitrothion, 7 cm^2 discs were cut out from lettuce (Lactuca sativa) using a punch hole, without the major vascular bundle. Ten microliters of 5 mg/mL fenitrothion in acetone was pipetted onto the discs and allowed to evaporate at room temperature. The fenitrothion was then extracted by washing the lettuce surface with 25 mL of DCM or acetone. The solutions were subsequently evaporated under a gentle stream of nitrogen (99% purity) to ~500 µL. The samples were then cleaned using a silica column and eluted with 20 mL of DCM and the resulting solution was evaporated to dryness. The solution was then reconstituted with 1 mL methanol. One in ten dilutions was conducted and then the samples were injected on to the LC. Extraction was also carried out by washing the disc with 1 mL of water and methanol and directly injecting the solution onto the LC.

To improve the recovery of fenitrothion, different methods of extraction using methanol was investigated. Methanol was used because it involved less clean up steps. One hundred microliters of 100 µg/mL fenitrothion in acetone was pipetted on to fifteen 7 cm^2 discs of maize (Zea mays) using a punch hole. Extraction was performed by either washing, dipping or sonicating the discs. The wash method was completed as previously described (Ukpebor, 2011) on six discs. The dipping method was performed on three discs by dipping them six times in 20 mL of methanol in their individual beakers. The sonicating method was conducted by placing three discs separately into 20 mL of methanol and sonicating for approximately 10 seconds. One disc per method was used as blank to account for any background concentration from the plants. The dipping and sonicating methanol solutions were then rotary evaporated to about 500 µL and transferred to a micro amber vial and finally made up to 1 mL. The wash method was repeated six times on lettuce to give a comparison to the recovery on different species. The recovery between the maize and lettuce was contrasted by using the Student's t-test.

In order to determine if the recovery was affected by the state of the leaf, 10 μL of 5 mg/mL fenitrothion in acetone was pipetted on to a leaf still attached to the plant. The spiked area was then cut into a 7 cm2 disc which was then extracted with a 1 mL methanol wash. This was repeated three times. This was done in order to compare the recovery from attached and detached leaves. A Student's t-test was performed on the attached and detached data.

2.3. Photodegradation Experiments

Light exposure was carried out under solar filter in the Atlas whose conditions were set so the radiance was 500 Wm^{-2} and the temperature at 25˚C. Twenty microliters of 5 mg/mL fenitrothion in acetone was pipetted on ten 7 cm^2 discs of cabbage "greyhound" (*Brassica oleracea sabauda*). Eight discs were placed into quartz-glass vessels with quartz lids while two discs were extracted at time zero by sonicating for 10 seconds in 10 mL of methanol. Four quartz vessels were placed into the Atlas and the other four were covered with aluminium foil and placed into a water bath as controls in a dark area of the lab. At 15 minute intervals, two of the samples were extracted. Two 1 mL samples of the methanol solution were transferred to micro amber vials for analysis. Triplicate experiments were carried out. Further experiments were carried out as above using cabbage "goldenacre" and on lettuce so that comparisons could be made between species and different varieties. The lettuce experiments were different in that twelve discs were used and samples were taken at 30 minute intervals over a two and half hour period. The Student's t-test was carried out on the data to find out if the controls were significantly different from the samples placed into the Atlas.

The data from the LC was then analysed in order to establish the pseudo first order kinetics and half-life of photodegradation. This was done according to the first order rate expression shown in Equation (1). The differentiated form of Equation (1) is shown in Equation (2). In the equations, r is the rate of reaction, C_t is the concentration at a specific time, C_o is the original concentration and k is the rate constant of that the reaction. Data were plotted using the natural log of the change of concentration verse time in order to find k. Once k was known, it was used to find the pseudo-half-life ($t_{1/2}$) by using Equation (3).

$$r = -\frac{dC}{dt} = kC_t \tag{1}$$

$$\ln \frac{C_t}{C_o} = -kt \tag{2}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{3}$$

2.4. Validation Experiments

In order to determine the reproducibility of the method, four experiments were conducted in which discs (n= 4 to 10) of cabbage "greyhound" were extracted immediately after evaporation. In order to understand how the different conditions affected the control in the

degradation tests some of the samples were placed in the fridge. Some were also covered in aluminium foil and placed in the Atlas for an hour and then extracted. This ensured that volatilisation losses and other artefacts could be quantitatively assessed. The extraction was then carried out by sonicating the disc for 10 seconds in 10 mL of methanol. To ensure that fenitrothion did not absorbed to the surface of the quartz vessels, four disc of lettuce were placed into the Atlas and four were covered with aluminium foil and placed in a water bath on the bench top as a control. The Atlas conditions were set as they were in the photodegradation work. A sample was extracted every 30 minutes over a 2 hour period. Extraction was done by removing the discs from the quartz and placing into a beaker with 10 mL of methanol and sonicating for 10 seconds. The quartz vessels were washed with approximated 600 µL of methanol and made up to 1 mL before injection onto the LC.

2.5. Statistical Analysis

Statistical analysis of the data was carried out using Excel, SigmaStat and SigmaPlot.

3. RESULTS AND DISCUSSION

3.1. Extraction Experiments

It was observed that acetone gave the highest recovery of 77 % on lettuce as seen in Figure 1. DCM and methanol removed 75 % and 64 % respectively while water had the lowest recovery at 7 %. Since methanol could be directly injected into the LC without further sample clean-up it was decide to continue working with it. The methanol method was modified to improve the recovery. The methanol recovery was increased significantly to 100.5 ± 2.8% on maize when it was sonicated for 10 seconds (Figure 2).

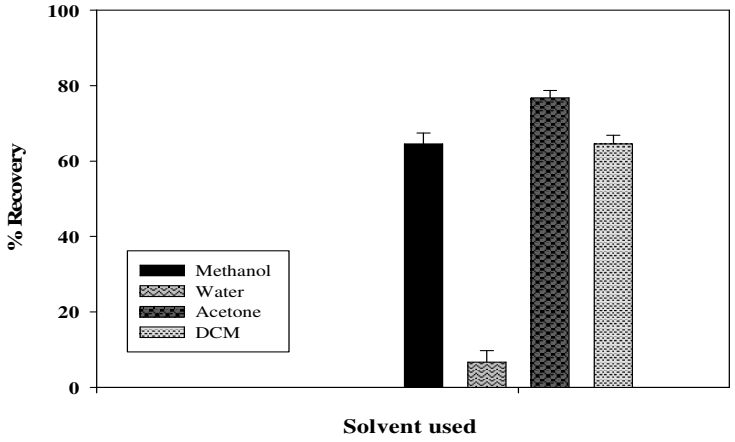


Figure 1: Recovery of fenitrothion from lettuce leaves using different extraction solvents

The dipping method produced similar results to the washing method; i.e. 59.9 ± 4.2 % and 64.5 ± 4.3 % respectively as shown in Figure 2. The t-test between the washing of lettuce and cabbage revealed that there was not a significant difference with a p-value of 0.68 (n=5). There was also no significant difference between the extraction from attached and detached leaves (p-value of 0.36, n = 3).

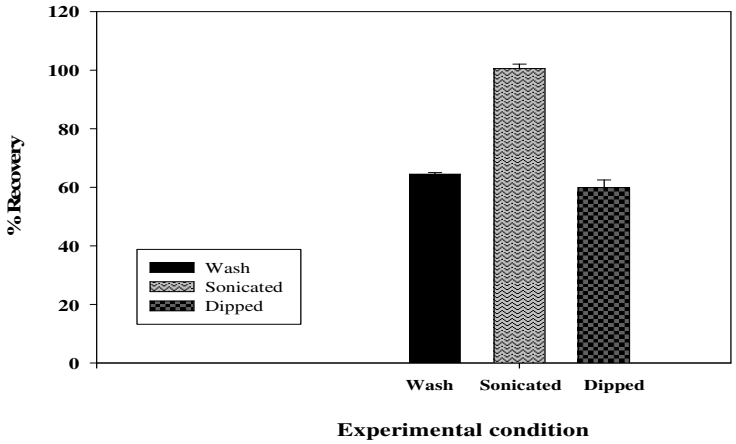
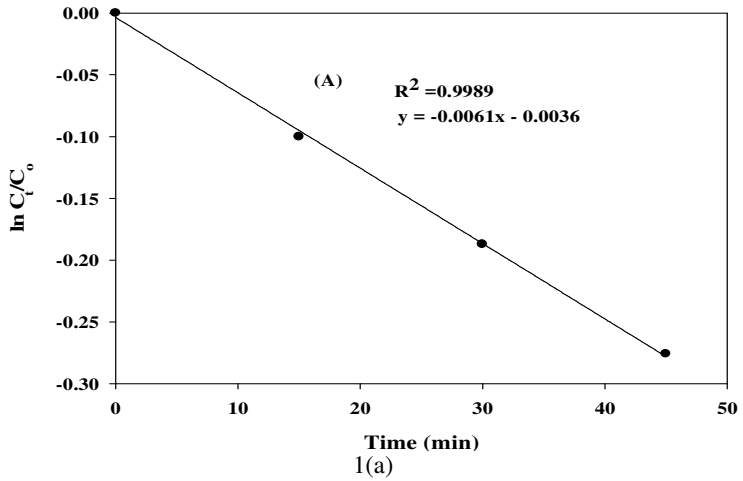


Figure 2: The percentage recovery of fenitrothion on lettuce leaf discs using methanol under different extraction methods

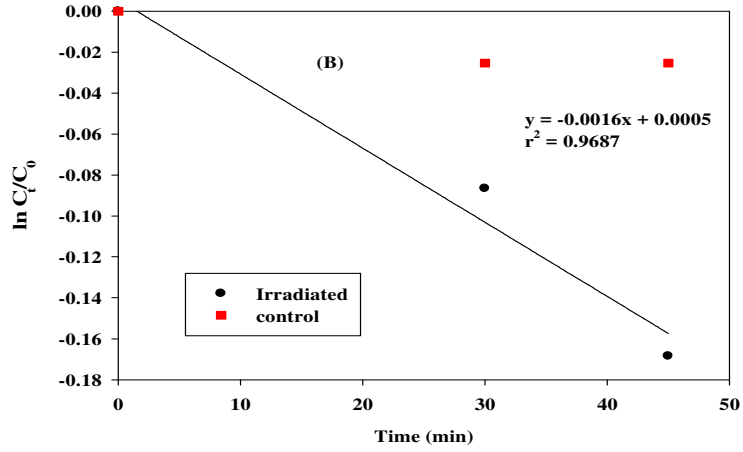
Fenitrothion is slightly soluble in water, which gave the lowest recovery at 7 %. It was surprising that the methanol recovery was lower than the acetone, because fenitrothion is soluble in both of these solvents at the same concentration 500 g/L at 20ºC. Methanol was the preferred solvent, because DCM and acetone are aggressive solvents and strips the leaf of the cuticular waxes and organic material which could interfere with the analysis. In order to remove these waxes and organic material a silica column would be used before injection onto the LC. This process has an added disadvantage of adding another step in the analysis with the attendant risk of reduced recovery. Methanol was therefore used as it made the experiment simple with reproducible and high recoveries. It was also observed that sonicating the leaf discs significantly increased the recoveries with values of 100.5 ± 5.2 % on maize. The reproducibility was confirmed with coefficient of variation (CV) arranging from 2.5 % to 10.6 % in five experiments in which the extraction was done directly after fenitrothion evaporated. It is suggested that if this method is to be repeated on different species that an initial experiment is run to find out the recoveries.

3.2. Extraction Experiments

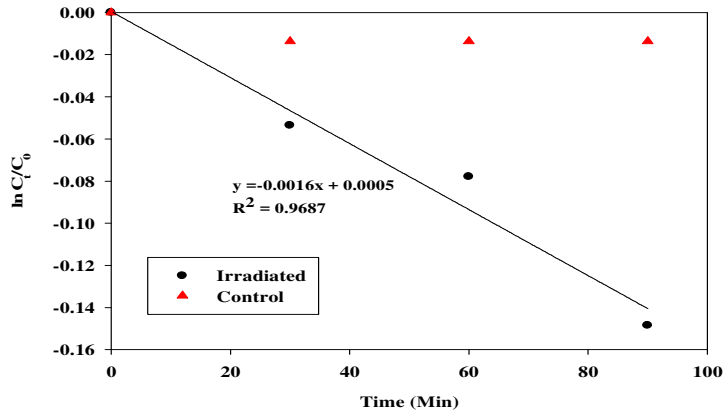
Figure 3 show the degradation of fenitrothion on different leaves. The controls did vary (not shown in the graphs). The reason for the variation was observed to be the pipetting (i.e. the quantity of pesticide solution applied each time).



1(a)



1(b)



1(c)

Figure 3: First order degradation of fenitrothion on a) cabbage "greyhound", b) cabbage "goldenacre" and c) lettuce, illumated in the Altas Solar Simulator

The half-life for lettuce was the longest at 7.7 ± 0.7 hours. Against expectations, cabbage "greyhound" and "goldenacre" did not have similar half-lives; they were 2.1 ± 0.2 hours and 3.9 ± 1 hours respectively (Figure 4). The error bars in Figure 4 represent the standard deviation (n = 3).

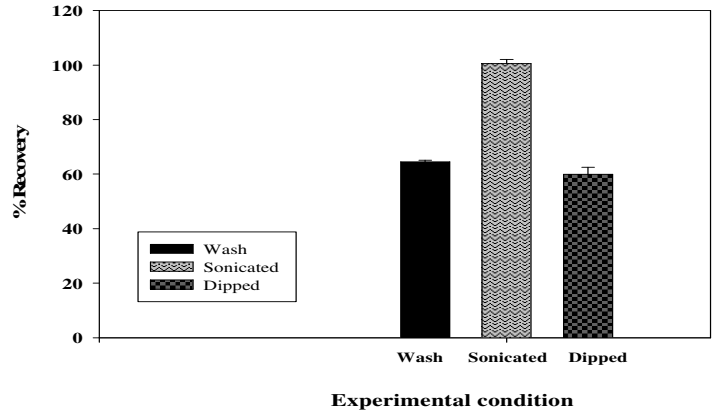


Figure 4: The half-life of fenitrothion on different leaves when exposed to artificial sunlight in the Altas

3.3. Validation of Experiments

Extractions were repeated on cabbage "greyhound" to find out if the concentration obtained fluctuated. The results from these experiments are shown in Table 1. The coefficient of variation or relative standard deviation percentage (% RSD) from these experiments ranged from 2.5 % to 10.6 %, highlighting the reproducibility of the extraction method. The average percentage recovery was 69.1 ± 5.2%.

$$\%\text{recovery} = \frac{\text{concentration of chemical obtained}}{\text{concentration of chemical applied}} \times 100 \quad (1)$$

$$CV = \frac{\sigma}{\mu} \quad (2)$$

Where CV = coefficient of variation (relative standard deviation – RSD), σ is the standard deviation and μ is the mean.

Table 1: Average recovery of fenitrothion using methanol and sonicating for 10 seconds

Experiment number	Number of samples	Average recovery concentrations (μg/mL)	S.D.	%RSD
1	08	24.35	1.20	4.92
2	10	6.40	0.45	7.01
3	05	7.44	0.37	4.91
4	04	7.04	0.17	2.47
5	12	10.76	1.14	10.61

To ensure that fenitrothion was not lost from the leaf surface to the quartz-glassed vessels, four experiments were carried out with lettuce and run similarly to the Atlas experiments. The difference is that the quartz-glassed vessels were washed with methanol and analysed. It was found that an average of 0.41 ± 0.08 µg/mL was lost to the quartz vessel surface. This is equivalent to 4.1% of the fenitrothion applied to the surface. The t-test showed that there was no significant difference between the control and of the Atlas quartz washes with a p-value of 0.12 (n = 17).

Control experiments did not reveal any significant loss of the applied pesticide, thus the pesticide loss on the plant surface was attributable to photodegradation only. Washing of the quartz vessel revealed an insignificant loss as a result of adsorption to the glass walls. The half-life of fenitrothion on leaves was found to be in the range of 2.1 ± 0.2 to7.7 ± 0.7 hours. This value is significantly lower than the half-life of fenitrothion (8.7 hours) as previously reported in similar experimental condition but in aqueous solution (MilliQ water) (Weber et al., 2009a). The rate of photodegradation (k = 1.0E-03 to 6.0E-03 hr^{-1}) was found to be faster on leaves than in water, a trend that ter Halle et al. (2006) also reported when studying the photodegradation of sulcotrione on maize.

It was observed during the experiments that the area that the drop of fenitrothion covered was different each time. This could affect the rate of diffusion into the leaf and thus affect the concentration of fenitrothion that remains on the surface to be photodegraded. The validation experiments showed that diffusion and evaporation is minimal during the experiments.

The experiment was designed to mimic the real environment as possible. The Atlas is suitable for environmental-relevant photochemical degradation when the irradiance is set to less than 500 Wm^2 (Weber et al., 2009b). The major pathways in solution include the oxidative replacement of sulphur to oxygen to form fenitrooxon, hydrolysis to give 3-methyl-4-nitrophenol, denitration to give denitrofenitrothion and oxidation of the methyl group to give a carboxylate, carbooxyfenitrothion and carboxyfentrooxon (Figure 5) (Zayed and Mahdy 2008, Weber et al. 2009c). It is known that in some cases the products of degradation are more toxic than their parent compound (Costa 2008). For all phosphorothioates pesticides, including fenitrothion their oxon analogues are more of a concern because they are in their activated forms which have a higher aceylcholinesterse inhibition activity (Durand et al. 1994).

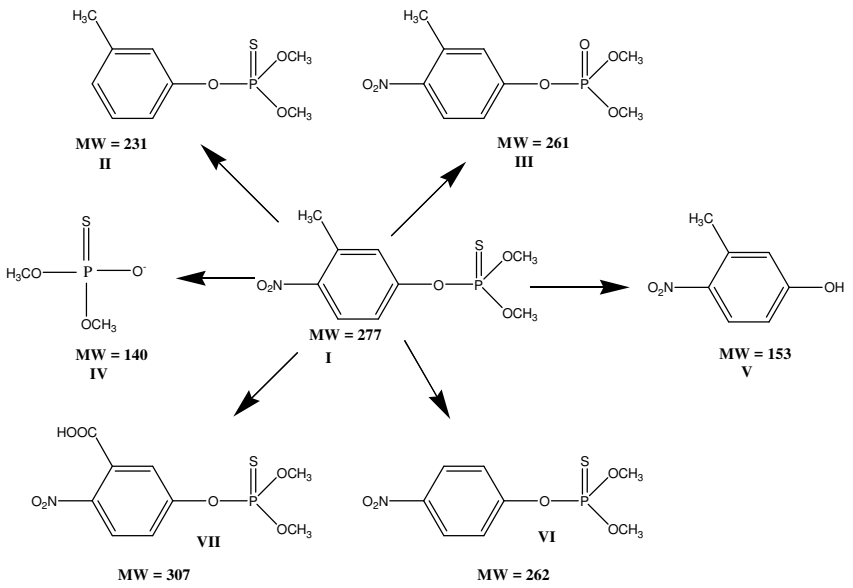


Figure 5: The molecular structure of the major photolytic products of fenitiothion (Zayed and Mahdy 2008) (I) Fenitrothion (II) denitrofenitrothion (III)Fenitrooxon (IV) phosphate ion (V) 3 – methy- 4 nitrophenol (VI) (VII) carbooxyfenitrothion.

4. CONCLUSION

Pesticides are highly important to modern farming as they improve the yield of crops. Their use is highly regulated for they can be highly toxic to non-target species. In order to carry out a risk assessment for the use of pesticide it is necessary to understand their fate within the environment. The degradation of fenitrothion was investigated on lettuce and cabbage leaves. Extraction of fenitrothion was found to be best when the leaf was sonicated for ten seconds in methanol. The validation of the experiments also showed that not much fenitrothion was been lost to the quartz vessels; 0.41 ± 0.08 µg/mL or 4.1% of fenitrothion was recovered from the quartz vessels. The half-lives of fenitrothion on lettuce, cabbage "greyhound" and cabbage "goldenacre" were found to be 7.7 ± 0.7, 2.1 ± 0.2 and 3.9 ± 1 hours respectively.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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