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# Forensic Entomology: When Puparia of Insect Stages is the Only Link to Cause

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**Abstract.** High performance liquid chromatography (HPLC) was used to conduct an entomotoxicological analysis on puparia cases and adult insects bred from monocrotophos poisoned carrions. The results indicate that, the poison was better detected in puparia cases compared to adults. This is valuable as puparia abundance at carrion in the late stages of decomposition is a common phenomenon compared to maggots and adults. Hence, standard operating procedures for collecting entomological evidence at death scene involving heavily decomposed remains should focus on collecting puparia, as they could be a link to cause of death, especially when foul play by poisoning is suspected.

**Keywords:** puparia, insect, entomotoxicology, forensic entomology, decomposition

## Introduction

Forensic entomology is the study of insects involved in any legal action, and can include urban and stored products entomology (Anderson, 2000). Application of entomological knowledge to criminal investigations requires an in depth information about insects. Many workers, besides studying insect fauna succession, have focused on the detection of drugs, toxins, poisons and other chemicals through analysis of maggots that had fed on intoxicated tissues. Gunatilake and Goff (1989) detected organophosphate Malathion poisoning in the larvae present on the putrefying body of a boy. Kintz *et al.* (1990) performed toxicological analysis of fly larvae collected from putrefied remains and used to calculate the post-mortem interval (PMI). Pounder (1991) has stressed the importance of entomotoxicology and the possibility of its use in solving drug-related cases where no body tissue is left for analysis. Wolff *et al* (2004) used high performance liquid chromatography (HPLC) to determine and quantify parathion in insects collected from decomposing rabbits previously injected with commercial methyl parathion.

Many studies have also focused on the effect of detected substances on the developmental rates of carrion insects. Notably, Goff *et al.* (1989) investigated the effect of cocaine and its metabolite benzoly-cocaine on the rate of development of Sarcophagid *Boettcherisca peregrina* and found that cocaine accelerated the growth rate of maggots feeding on the tissue. Heroin has also been highlighted to effect accelerated growth on maggot metabolism (Goff *et al.*, 1991). From rats euthanized in different ways, Patrican and Vaidynanathan (1995) noted that, chemicals like sodium pentobartital delayed the oviposition of calliphorids by seven days and rats decomposed twice as long to decompose.

Globally today, there is an upsurge in suicides or homicides through poisoning with drugs. Rahat *et al.* (2005) identified two widespread kinds of poisoning in India as being due to consumption of agrochemicals and overdose of medicine. They highlighted that, in emergency wards, two types of pesticide poisoning are common, ingestion of organophosphates and aluminum phosphide.

Arun *et al.* (2005) reported that, organophosphate are used extensively in horticulture and agriculture. Because of their easy availability and the rapidity of their action (even in smaller doses), they are some of the popular poisons for suicide (Davies, 1987). Insects play an invaluable part in the decomposition process of carrion. Due to their ability to retain some death agents in different stages of their life cycle, insects can reveal information in the unraveling of criminal cases especially murder. This has therefore prompted the need to examine the suitability of puparia stage in the developmental stages of carrion insects.

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## Materials and Methods

**Study site and experimental layout.** The study site was the Demonstration farm of Benson Idahosa University, Ugbor Village, Benin City, Edo State Nigeria. The area lies within the rainforest vegetation belt of Nigeria and is characterized by two distinct seasons of wet and dry. The latitude of the study area is 06°17′01.6′N and longitude 005°36′10.6′ E on an elevation of 73 m obtained from GPSmap76 Csx Global Positioning System model.

**Pig specimens used, killing exercise and experimental layout.** Three pigs, (*Sus scrofa* L.) purchased from a piggery farm in Ekae village, Sapele Road Benin City of mean weight 23.21±1 kg were used in the study (Catts and Golf 1992). Each pig was killed by lethal injection of 5 mL monocrotophos poison (venous poisoning). A total of 12 white pigs (*Sus scrofa* L.) were used in all.

Each dead pig was immediately packed in a heavy-duty polythene thrash bag, labeled and transported to the study site. The time of death and deposition were noted for each pig on a daily data collection sheet. This procedure was repeated four times during the 2006-2007 and 2007-2008 seasons.

**Entomotoxicological analysis.** The entomotoxicological analysis of the empty puparia cases and adults bred from the monocrotophos poisoned carrions were done using a modified method of Fotch *et al*., (1957). Dried empty puparia cases (0.4 g) was weighed and homogenized in 10 mL of acetone/methanol (2:1). The homogenate was rinsed into a beaker with 10 mL of acetone and subjected to mechanical shaking overnight.

The suspension was then centrifuged and the supernatant was decanted and filtrated using Buckner funnel. The filtrate was re-filtered using filter paper (Whatman No. I). Purified sample (10 mL) was then analysed by HPLC (Zhong *et al.*, 2006). The procedure above was also carried out for adults bred from the monocrotrophos poisoned carrions. In this case, 0.5 g of the dried adults was homogenized in 10 mL of acetone/methanol (2:1).

## Results and Discussion

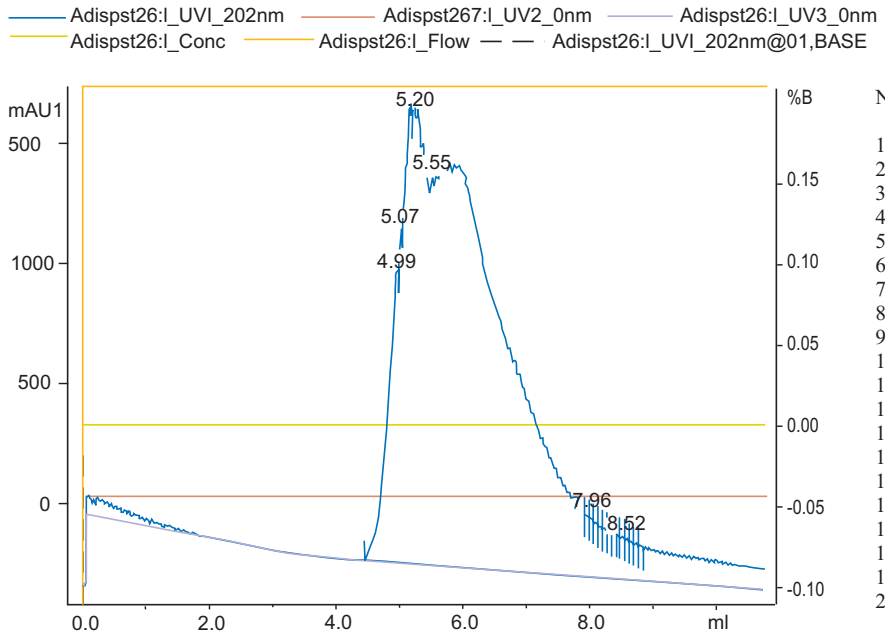
The incidence of death arising from poisons is a common world-wide phenomenon. Kintz *et al*. (1990) and Wolff *et al.* (2004) among others have at various times and in different circumstances detected and quantified drugs or poisonous substances from various insect stages thereby solving various drug or poison related cases.

High performance liquid chromatograms of the monocrotophos standard, puparia and adult insects bred from the monocrotophos poisoned carrions are presented in Figs. 1-3. Table 1 is the summary of the analysis. The results revealed similarity in the profiles obtained but showed differences in peak values, an indication that the detected substances are different in the puparia and the adults compared to the standard monocrotophos used. Monocrotophos standard gave 96 peaks, while extracts from puparia and adults recorded 104 and 99 peaks respectively.

It is not uncommon to find toxicants that caused death in tissues of carrion as well as in insects and other animals that feed on such carrions. This is probably because of the complex web synergy that occurs in any ecosystem where the principle of eat and be eaten operates. When carrion is eaten by other animals such as insects, they have a tendency to pick up and accumulate the poisonous substances that caused the death of such animal. These accumulated substances may be evident when body tissues or parts of such animals are analyzed. In this present work, entomotoxicological analysis carried out using the HPLC for the monocrotophos standard and the extracts from puparia and adult insects had similar profiles. Even though the obtained peaks differ in numbers and the detected substances are not exactly in the same proportion at the different stages of development of the insects bred from the monocrotophos poisoned carrions

**Table 1.** HPLC analysis of puparia cases, adults and standard used.

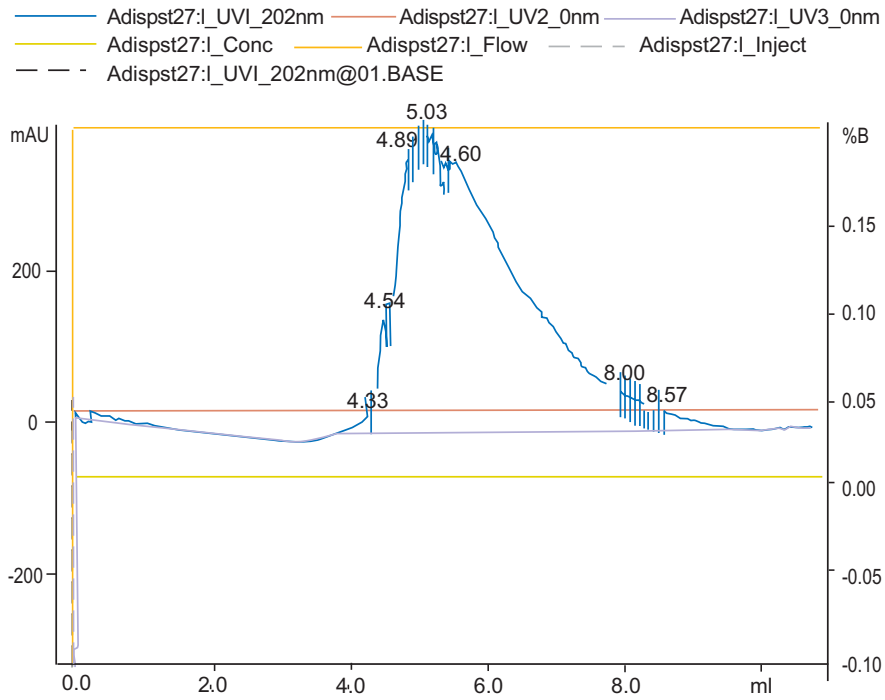
| Sources | No. of detected peaks | Total area (mAU*mL) | Area in evaluated peaks (mAU*mL) | Ratio peak area/ total area | Total peak width (mL) |
|---|---|---|---|---|---|
| Standard monocrotophos | 96 | 3784.13 | 3526.81 | 0.93 | 4.39 |
| Puparia case | 104 | 793.48 | 770.71 | 0.97 | 4.33 |
| Adult | 99 | 505.21 | 480.60 | 0.95 | 4.30 |




| No. | Ret ml | Area mAU*ml | Height mAU |
|---|---|---|---|
| 1 | 4.99 | 242.3396 | 1173.557 |
| 2 | 5.07 | 77.6373 | 1343.794 |
| 3 | 5.20 | 240.2301 | 1827.443 |
| 4 | 5.27 | 491.2921 | 1886.980 |
| 5 | 5.55 | 96.3739 | 1560.320 |
| 6 | 5.62 | 109.1768 | 1603.422 |
| 7 | 5.69 | 2101.5252 | 1627.989 |
| 8 | 7.96 | 16.9982 | 252.705 |
| 9 | 8.03 | 16.3602 | 237.625 |
| 10 | 8.10 | 15.1060 | 224.747 |
| 11 | 8.17 | 14.3203 | 212.989 |
| 12 | 8.24 | 13.6669 | 203.234 |
| 13 | 8.31 | 13.4283 | 195.397 |
| 14 | 8.38 | 12.6648 | 188.750 |
| 15 | 8.45 | 12.2075 | 182.363 |
| 16 | 8.52 | 13.6802 | 174.633 |
| 17 | 8.59 | 11.3764 | 166.336 |
| 18 | 8.66 | 10.5533 | 157.911 |
| 19 | 8.73 | 10.0425 | 150.795 |
| 20 | 8.80 | 9.8306 | 143.795 |

Total number of detected peaks = 96; Total area = 3784.1322 mAU*ml; Area in evaluated peaks = 3526.8102 mAU* ml ; Ratio peak area/total area = 0.932000; Total peak width = 4.39 ml;Calculated from : adispst26: UV1 202 nm; Baseline adispst26:1_UVI_202 nm@01,BASE

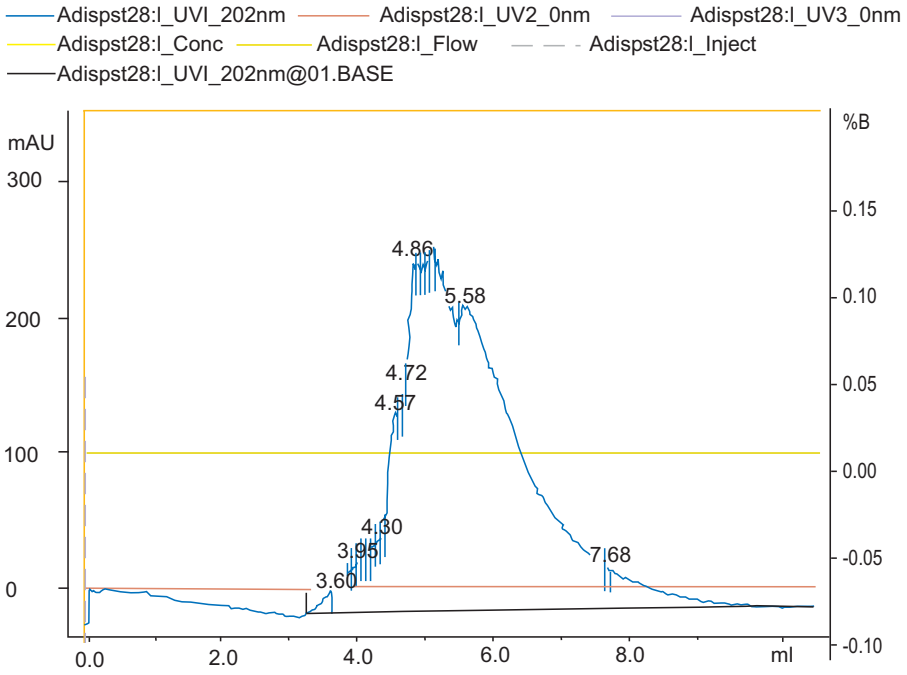
**Fig. 1.** Chromatogram of standard monocrotophos used with other relevant information.




| No. | Ret ml | Area mAU*ml | Height mAU |
|---|---|---|---|
| 1 | 4.33 | 1.7036 | 28.418 |
| 2 | 4.54 | 18.2611 | 143.134 |
| 3 | 4.60 | 10.3092 | 151.469 |
| 4 | 4.89 | 70.1130 | 347.795 |
| 5 | 4.96 | 25.6839 | 365.874 |
| 6 | 5.03 | 26.6313 | 379.199 |
| 7 | 5.10 | 27.5014 | 390.589 |
| 8 | 5.17 | 26.9967 | 390.947 |
| 9 | 5.24 | 28.8855 | 382.285 |
| 10 | 5.31 | 73.5936 | 370.557 |
| 11 | 5.60 | 43.4681 | 345.675 |
| 12 | 8.00 | 3.2637 | 48.600 |
| 13 | 8.07 | 3.0536 | 45.959 |
| 14 | 8.14 | 2.9196 | 42.562 |
| 15 | 8.21 | 2.6585 | 39.827 |
| 16 | 8.28 | 2.4690 | 37.085 |
| 17 | 8.35 | 2.3402 | 34.391 |
| 18 | 8.42 | 2.1106 | 31.898 |
| 19 | 8.49 | 1.9374 | 29.363 |
| 20 | 8.57 | 1.8114 | 26.879 |

Total number of detected peaks = 104; Total area - 793.4822 mAU*ml; Aroa in evaluated peaks = 770.7116 mAU* ml; Ratio peak area/total area = 0.971303; Total peak width = 4.33 ml; Calculated from : adispst27:1 UV1 202 nm; Baseline adispst27:1_UVI_202 nm@01,BASE

**Fig. 2.** Chromatogram of puparia cases bred from monocrotophos poisoned carrions.




| No. | Rot ml | Arca mAU*ml | Height mAU |
|---|---|---|---|
| 1 | 3.60 | 2.7130 | 15.444 |
| 2 | 3.88 | 2.1206 | 31.095 |
| 3 | 3.95 | 2.2968 | 34.897 |
| 4 | 4.02 | 2.5955 | 38.267 |
| 5 | 4.08 | 2.7561 | 41.435 |
| 6 | 4.16 | 2.9624 | 44.299 |
| 7 | 4.23 | 3.2686 | 47.775 |
| 8 | 4.30 | 3.4567 | 51.524 |
| 9 | 4.36 | 3.0262 | 53.718 |
| 10 | 4.57 | 23.7816 | 140.901 |
| 11 | 4.64 | 10.3045 | 147.407 |
| 12 | 4.72 | 8.4839 | 160.514 |
| 13 | 4.86 | 33.1885 | 245.291 |
| 14 | 4.92 | 17.4596 | 253.071 |
| 15 | 5.00 | 17.0246 | 253.219 |
| 16 | 5.07 | 17.7990 | 259.798 |
| 17 | 5.14 | 19.8304 | 258.445 |
| 18 | 5.21 | 75.8171 | 248.898 |
| 19 | 5.58 | 229.7163 | 214.363 |
| 20 | 7.68 | 2.0013 | 29.189 |

Total number of detected peaks = 99; Total area - 505.2071 mAU*ml; Area in evaluated peaks = 480.6025 mAU* ml; Ratio peak area/total area = 0.951298; Total peak width = 4.30 ml; Calculated from : adispst28:1 UV1_202_nm; Baseline adispst28:1_UVI_202 nm@01,BASE

**Fig.3.** Chromatogram of adult fly bred from monocrotophos poisoned carrions.

compared to the monocrotophos standard used, there is a clear pattern of similarity. This could be accounted for in that, the parent monocrotophos compound initially injected into the pig may have been metabolized into other more soluble components hence, the detection of components at 4.33 mL for puparia (Fig. 2) and 3.60 mL for adults (Fig. 3) compared with the 4.99 mL value of the monocrotophos standard (Fig. 1). The metabolism of the components perhaps continued through the stages of the invaded insects as solubility of subsequent components reduced as evident in the adults' chromatogram (Fig. 3). This possibly increased solubility of the components and may have accounted for the increase in the number of detected peaks from 96 in standard monocrotophos to 104 in puparia and 99 in the adults.

Earlier researchers including Anderson (1995), Goff and Lord (1994), Wilson *et al.* (1993), and Nolte *et al.* (1992) used larvae for toxicological analysis. Miller *et al.* (1994) reported the first major work on the detection of drugs like amitriptyline and nortriptyline in fly puparia and beetles. In this present work, the analyses of the fly puparia and adults flies bred from monocrotophos poisoned carrions indicate that the toxicant may have been metabolized to produce components other than the original toxicant administered. Thus the peak number detected at the puparia and adult stages were quit different from the peaks obtained for the standard monocrotophos used (Fig. 1). If the applied toxicant were not metabolized, the peaks obtained at the stages of insect development would possess same number of peaks in their profiles as for the standard monocrotophos used. Hence, differences in chromato-gram forms and number of peak are a strong indication that the killing agent (monocrotophos) has undergone some level of metabolism to other components.

The detection of toxicant in chitinised insect tissues (fly puparia) is very significant. This is because maggots are known to have specified period within the carcasses after which they develop into the pupa and adult stages that fly away. Thus, the use of maggot as the base of entomotoxicology would be unreliable. However, fly puparia can still be available in abundance as they are seen littering the areas even years after death has occurred. Coupled with the ability to withstand climatic

conditions, fly puparia may therefore be the only source of reliable entomological evidence even in the absence of all other tissues and stages of insects.

## Conclusion

On the basis of time spent on carrions or carcasses and the ability of empty puparia to withstand climatic conditions, it is suggested that, attention should be given to puparia cases as one of the critical stages where entomological data can be extracted in the phase of uncertainty of information. This may become imperative as larvae and adults stages of insect developments on carrion soon disappear as metamorphosis progresses. This act of disappearance by the two stages of insect development (larva and adult) resultantly limits their use in evidence gathering. Hence, the puparia of the insects bred from the carrion may just be the only link of the cause of death in the phase of uncertainity.

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