INTRODUCTION

The toxicological impact of insecticidal compounds is increasing day by day as a consequence of their rapid and widespread application. It is used as ectoparasiticide in animal, production of agricultural crops and also public health programme. The consequence of indiscriminate use include apart from health hazards to non-target organisms, pollution of ecosystem and accidental poisoning. In field condition the pesticides poisoning is common due to accidental, malicious poisoning and improper use of pesticides by farmer, but the detection is very rare or undetected due to lack of analytical laboratory for identification and quantification of residue of pesticides and other toxin. Present study was undertaken to ascertain whether the cause of death of large number of goats was due to pesticide poisoning and if so, the identification and quantification of the pesticides by HPLC method.

MATERIALS AND METHODS

The viscera of six (6) animals (random sampling) like stomach contents and liver, lungs also blood smear were received from project co-coordinator, Animal Disease Research Institute, Phulnakhara, Cuttack, Orissa for diagnosis of causes of death of goats in the farm. It was suspected that, surrounding the farm field the insecticide
Fig. 1: LC Calibration report of Malathion.
Fig. 2: Chromatogram of unknown sample.

may be sprayed and after the return from grazing the animals showed frothy salivation, with nervous symptoms, colic, constriction of pupil, tetany etc. and out of 150 goats, there was death of 90 goats. The blood smears were examined. The liver and lungs were taken for histopathological examination.

The liquid chromatography consisted of Millipore Waters (Milford, USA) model 1525 pumps, 2487UV detector. The column was a Waters reversed phase C18 (Nova pack) stainless steel column (150mm × 3.9mm I.D,
and also this method (HPLC) is now widely used according to Watanabe et al. (2014).

Tissues (lungs and liver) were extracted for 4 min with acetonitrile and anhydrous sodium sulfate (0.4%) using a homogenizer. The extract was filtered through anhydrous sodium sulfate and the tissues were re-extracted twice with acetonitrile. The extract was clarified by centrifugation and filtered through anhydrous sodium sulfate. The combined acetonitrile extracts were concentrated to 20 ml and partitioned with hexane. The hexane phases were discarded and the acetonitrile phase was evaporated to dryness using a rotary vacuum evaporator. The volumes were finally adjusted to 5 ml with acetonitrile for HPLC estimation.

Reagents: HPLC grade acetonitrile, water and all other reagents / chemicals were procured from E. Merck (India). The standard compound of pesticides like malathion, etc were obtained from (Supelco-Sigma-Accustandard) and residue of organophosphorus compound in sample was compared to external standard.

Residue level determination: The tissue residue level of the unknown compound was estimated by the method of Manna et al. (2006),
after purified and clean up by solid phase extraction (SPE). A stock solution of 1 mg per litre of malathion (analytical grade >99%) was prepared as an external standard. Acetonitrile and water were used as mobile phase in gradient manner and the retention time (RT) of malathion was 7.46 min. The data were recorded and calibration curve of malathion has been done (Fig. 1). The method has been repeated 4 times for validation of the assay. With 10 ml Cap. Hamilton Syringe, 2 ml of standard and samples were injected into HPLC.

**Histopathological examination:** Small pieces of lungs, liver, were fixed in 10% neutral buffered formalin. Sections of 3-5m thicknesses were cut and stained with haematoxylin and eosin (H&E) for observation under light microscope.

**RESULTS AND DISCUSSION**

The antimortem symptoms were frothy salivation, nasal discharge, nervous symptoms, colic, constriction of pupil etc. The postmortem symptoms were bloat and inflammation in stomach and intestine, haemorrhages (Fig. 3, 4) in lungs and intestine. The histopathological changes in liver and lungs showed congestion, hemorrhages. The examination of blood smear showed no pathogenic organisms. The analysis confirmed the presence of malathion and the method of extraction –purification gave very good results without fortification of the sample (Fig. 2) The detected residue level (average within six samples) of malathion was 5277 ppb, and the death of the animals were due to organophosphorous compound poisoning (malathion. The oral LD50 for malathion in rat has been reported to 5400 mg/kg in male and 5700 mg/kg in female according to Zeid *et al.* (1993).

The detection of pesticides and its metabolites in animal tissue more accurately and specifically using Mass Spectrometry (MS) may be taken into consideration in future.

**REFERENCES**


