جامعة نايف العربية للعلوم الأمنية
Naif Arab University for Security Sciences

Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences & Forensic Medicine
www.nauss.edu.sa
http://ajfsfm.nauss.edu.sa

الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
Arab Society for Forensic Sciences and Forensic Medicine

Case Report

# Pesticide Mixture Poisoning: A Case Report

## التسمم بمزيج من المبيدات: تقرير حالة

**Atul Bajaj [1,*], Cijo John [1], Sukhminder Kaur [1], Rohitashva M. Tripathi [1]**

[1,*] *Central Forensic Science Laboratory, Ministry of Home Affairs, Govt. of India, Chandigarh, India*

*Received 14 Aug. 2017; Accepted 15 Apr. 2018; Available Online 03 Jun. 2018*

## Abstract

Acute pesticide poisoning is reported as a major health problem worldwide and developing countries are exposed to this problem to a greater extent. The ingestion of pesticides is the most common suicide method in the world with approximately one-third of all suicide deaths occurring due to self-poisoning with pesticides. Cases of unintentional poisoning are less in number. A significant number of cases are encountered in forensic science laboratories for toxicological examination in which pesticide poisoning is reported.

We report a suicide case involving two persons due to unknown poisoning. Toxicological examination confirmed the presence of a mixture of five different pesticides in postmortem samples. Such cases of forensic interest are rarely reported.

**Keywords:** *Forensic Sciences, Toxicology, Suicide, Pesticides, Organophosphorus, Pyrethroids.*

## المستخلص

تفيد التقارير بأن التسمم الحاد بالمبيدات يمثل مشكلة صحية رئيسية في جميع أنحاء العالم، وتظهر هذه المشكلة بشكل خاص في الدول النامية. ويعد تناول المبيدات الطريقة الأكثر شيوعاً في حالات الانتحار على مستوى العالم حيث تقدر حالات الوفيات الناتجة عن الانتحار بالتسميم الذاتي بالمبيدات ثلث حالات الانتحار بشكل عام، بينما كانت حالات التسمم غير المتعمد أقل عدداً بهذه الوسيلة. ويوجد عدد كبير من الحالات الواردة إلى مختبرات السموم الجنائية والتي يُبلغ عنها كحالات تسمم بالمبيدات.

ونعرض في هذه الدراسة تقريراً عن حالة انتحار لشخصين بسبب تسمم غير معروف، وتبيَّن من خلال فحص السموم وجود خمسة مبيدات مختلفة، وتعد هذه الحالة من الحالات النادرة والمهمة التي يتم الإبلاغ عنها في مجال الأدلة الجنائية.

**الكلمات المفتاحية:** علوم الأدلة الجنائية، علم السموم، الانتحار، المبيدات، الفسفور العضوي، البيريثرويد.

Production and hosting by NAUSS

* Corresponding Author: Atul Bajaj
Email: atulbajaj37@gmail.com

doi:10.26735/16586794.2018.003

## 1. Introduction

In an effort to improve food supplies, there has been an extensive increase in the production and use of pesticides globally. Pesticides help in controlling pests and insects in a cost-effective way, and have become a common household item. They are chemicals that are intentionally added to the natural environment with the purpose of removing some form of life [1].

Poisoning and related mortality due to pesticides is a major public health problem worldwide [2-3], and developing countries are exposed to this problem to a greater extent [4]. The ingestion of pesticides is the most common suicide method in the world with approximately one-third of all suicide deaths occurring as a result of self-poisoning with pesticides [5].

In this paper, we report a case involving the death of a young couple after consumption of unknown poison. Toxicological analysis revealed the presence of five different pesticides in postmortem samples. The literature survey shows other cases where a mixture of pesticides was used with the intention of self-harm. The present case is the first report of suicide by two persons using pesticide mixture.

## 2. Case Report

A 19-year-old male and a 16-year-old female were brought to the casualty services of the hospital in an unconscious state by their family members. Both the victims were declared dead on arrival. Local police were informed by the hospital and an investigation was initiated to establish the cause of death. Upon investigation, it was revealed that both the deceased were found in an unconscious state in a farmhouse located in a village next to the city. However, the family members failed to provide any details such as psychic conditions or circumstances of poisoning in the case of both victims. The crime scene was searched by the investigating team, but no physical clues such as any empty container, bottles or wrappers were found.

The dead bodies of both deceased were sent for postmortem examination and were subjected to medico-legal autopsy. Autopsy findings for both the deceased resulted in similar observations. The time of death was approximated to 28-30 hours before autopsy for both the deceased. The external examination showed no signs of ante mortem injuries, needle marks, or any decomposition or putrefaction. No physical clues were found on the bodies of the victims. A bluish-green colored powdered substance in a small quantity was recovered from the clothing of the female and preserved for toxicological examination. No physical clues were found on the clothing of the male victim. However, froth was noticed from the mouth and nostrils in both the cases. Brain, lungs, liver, spleen, gastric mucosa and both kidneys were found in a congested condition during an internal examination. The observation of stomach contents revealed the presence of the green colored semisolid material. During the autopsy, samples of the stomach along with its contents, portions of the liver, spleen, small intestine and kidneys were preserved. The blood samples of both the deceased were also preserved for toxicological analysis. The collected samples were analyzed in our laboratory to identify the unknown poison by systematic toxicological examination.

## 3. Materials and Methods

### 3.1 Sample Preparation

The toxicological analysis was carried out systematically for unknown poisoning. The post-mortem samples of both the deceased were examined for identification of different groups of poisons like gaseous, volatile, and metallic poisons in addition to pesticides and drugs. Visceral samples, including stomach and its contents, liver, spleen, kidney, small intestine, and blood were analyzed individually for different groups of poisons.

One hundred grams of tissue samples were taken and cut into small pieces and divided into two portions. One portion was processed to check for the presence of any drug. The tissue samples were acidified with 50 mL acetic acid, and solid ammonium sulfate was added to it to obtain a saturated solution. This was kept in a water bath with thermostatic control at a temperature of 60ºC for protein coagulation. After complete coagulation, the tissue samples were allowed to cool at room temperature and filtered. The aqueous layer was collected, and two-step liquid-liquid extractions were carried out. In the first step, aqueous filtrate was extracted with 50 mL diethyl ether (3 times), and organic phases were collected. The remaining aqueous portion was made alkaline (pH 9.0) by adding ammonia. The alkaline aqueous phase was extracted with 50 mL chloro-

form (3 times), and organic phases were collected. Both the organic layers were filtered through anhydrous sodium sulfate and evaporated to dryness. The dried organic phases were reconstituted in methanol for analysis of drugs.

The second portion of tissues was analyzed to confirm the presence of Organophosphorus (OP), Organochlorines (OC), Carbamates and Pyrethroids groups of pesticides. The tissue samples were soaked in a sufficient quantity of n-hexane and kept overnight. The samples were filtered through anhydrous sodium sulfate. The organic layer was collected and evaporated to dryness. The dried filtrate was reconstituted in acetone for further analysis of pesticides.

One mL blood samples were added with two mL each of saturated solution of sodium tungstate and 1 N sulphuric acid and heated on the water bath at the controlled temperature of 60 °C to facilitate the deproteination of blood. The deproteinated samples were filtered to collect the aqueous layer and divided into two portions and taken up for extractions of pesticides and drugs as described above.

The bluish green colored solid material recovered during the autopsy was finely ground. HPLC grade methanol was added to it and kept for ultra-sonication for 10 minutes followed by filtration through a 0.45 $\mu$m nylon membrane filter. The filtrate was examined for the presence of any drug or pesticide with GC-MS screening procedure.

### 3.2 TLC Analysis

Thin Layer Chromatography (TLC) was used for the screening of different groups of drugs and pesticides. The reference standard drugs and pesticides along with the respective organic extracts were spotted on individual 20 x 20 cm Silica gel F254 TLC plates at a distance of 2 cm from base, and elution was carried out up to 12 cm in suitable mobile phases. After elution, the plates were made to dry at room temperature. The developed TLC plates were visualized in ultraviolet light at 254 nm followed by spraying various locating reagents. The details and observations of the same are depicted in Table-1.

### 3.3 GC-MS Analysis

Organic extracts were analyzed for confirmation of poisons using a simple in-house developed Gas Chromatography –Mass Spectroscopy (GC–MS) method which was routinely adopted in different cases for analysis of both drugs and pesticides. A GC–MS system equipped with a Finnigan Trace GC Ultra interfaced with a Thermo DSQ Quadrupole MS, and Thermo Auto sampler AS 3000 (Thermo Fisher, USA) was used. A BP-5 capillary column (30 m x 0.33 mm i.d x 0.5 µm film thickness) was used to facilitate chromatographic separation. High purity helium was used as a carrier gas at a constant flow rate of 1.2 mL/min. The programmable injection port was operated at con-

**Table 1-** *Thin Layer Chromatographic (TLC) screening protocol.*

| Mobile Phase | Target Poison/Drug | Locating Reagent | Standard used for Screening | Observations |
|---|---|---|---|---|
| Hexane: Acetone (9:1) | Organo phosphorous pesticides | Palladium Chloride solution | Malathion, methyl parathion | Yellow spot was observed with both standards and samples |
| Hexane: chloroform (1:1) | Organo chloro pesticides | Zinc chloride-diphenylamine solution- UV/ sunlight | Endosulfan | Parrot green color was observed with standard only |
| Hexane: Acetone (4:1) | Carbamates | Fast Blue-B (O-Dianisidine) | Propoxur, carbaryl | Blue spot with standards only |
| Chloroform: acetone (8:2) | Barbiturates | Mercuric chloride followed by Diphenyl carbazone | Phenobarbitone, barbitone | Purple spot in white background with standards only |
| Methanol : ammonia (100:1.5) | Benzodiazepines/ narcotics | Dragendroff's reagent | Diazepam, lorazepam, Alprazolam, morphine. | Orange spot with standards only |

stant temperature splitless mode and kept at 250° C. The MS transfer line temperature was 310° C, and source temperature was kept at 200° C. Initial oven temperature was kept at 80° C with a hold time of 1 min and was increased in linear ramp rate of 10° C to a final temperature of 300° C with a final hold time of 5 min. The sample injection volume was one $\mu$L. Electron impact ionization (EI) positive mode was selected for operation of the mass spectrometer, and full scan spectra in the mass range of 40-500 amu were recorded. Xcalibur 1.4 software was used for data acquisition and processing, and results were screened using NIST, Wiley and PMW mass spectral libraries to confirm the presence of any pesticides and drugs.

## 4. Results

Toxicological results of the autopsy samples analyzed were negative for the presence of any opiates, benzodiazepines, barbiturates or other drugs. Results were negative for the presence of any volatile poisons including ethyl alcohol and gaseous poisons. During the screening of pesticides, TLC analysis indicated the presence of pyrethroids and organophosphate groups. Further analysis by GC-MS confirmed the presence of Alpha-Cypermethrin (Alphamethrin) (22.08), Fenvalerate (22.96), Ethion (17.68), Triazophos (17.94) and Quinalphos (15.86) in both victims. The values given in brackets denote the retention time in minutes for individual compounds. Figure-1 depicts the Total Ion Chromatogram (TIC) with mass fragmentation of Quinalphos, and Figure-2 depicts the mass fragmentation pattern of remaining compounds identified in one victim.

The GC-MS analysis of the bluish green solid powder by adopting the above described method confirmed the presence of Promethazine; however, it was not detected in any biological samples. Through autopsy, stomach contents revealed the presence of a green colored semisolid material, which can be attributed to use of commercial pesticide formulation by the two deceased.

## 5. Discussion

Recent years have witnessed an enormous increase in the agricultural use of pesticides, mainly of OP, OC and pyrethroid groups, to protect crops from insects. The Asia-Pacific region witnesses agrochemical poisoning as a leading cause of mortality due to exposure to OP, OC, and aluminum phosphide compounds being an integral part of agriculture and their availability at low cost [6]. Easy

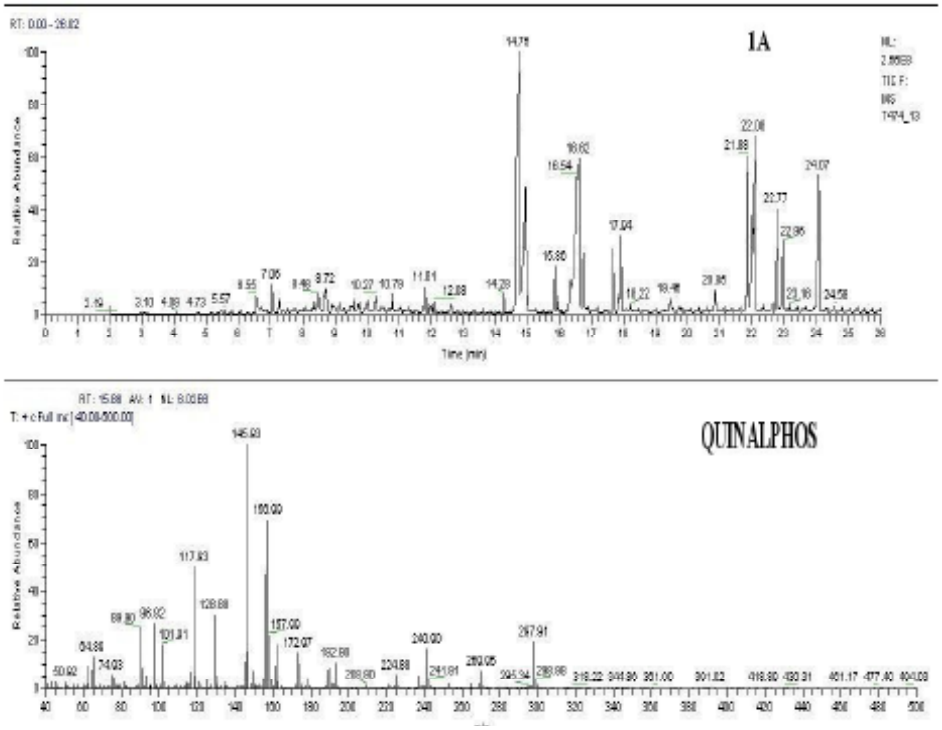


**Figure 1-** *Total Ion Chromatogram (TIC) with mass fragmentation of Quinalphos.*

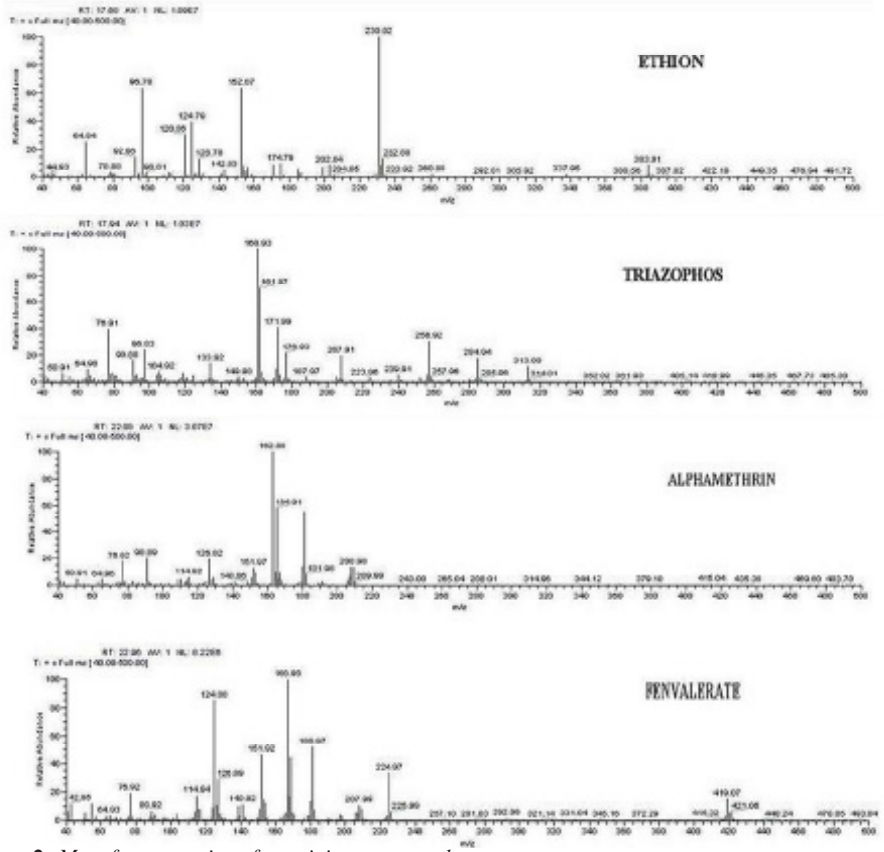


**Figure 2-** *Mass fragmentation of remaining compounds.*

availability of these pesticides in India has led to a jump in cases of human poisoning. The incidents of poisoning are common in individuals of low economic status and in suicidal deaths in India; organophosphorus pesticides are most commonly used [7].

Ethion, Quinalphos, and Triazophos, which were detected in the toxicological specimens, are OP group of pesticides. These pesticides are mainly esters, amides and thiol derivatives of phosphoric acid. Chemically Ethion {IUPAC name; O,O,O',O'-Tetraethyl S,S'-methylene bis(phosphorodithioate) molecular formula C9H22O4P2S4; Molecular weight: 384.5} is a dithiophosphoric acid derivative and is used to control a range of pests including spiders, mites, aphids, scale insects, leafhoppers and soil pests in a wide range of crops. Quinalphos {IUPAC name: O,O-Diethyl O-quinoxaline-2-Cyl phosphorothioate; Molecular formula: C12 H15N2O3PS; Molecular weight: 298.3} is an ester of phosphorothioic acid and Triazophos {IUPAC name: O,O-Diethyl 0-( l-phenyl-LH-1,2,4-triazole-3-yr) phosphorothioate; Molecular Formula: C12H16N3O3PS; Molecular weight: 313.3} is a Phosphorothioate type of OP pesticide. Triazophos is used to control insects and mites in a wide range of crops.

OP compounds inhibit acetylcholinesterase (AChE) and butyrylcholinesterase enzymes resulting in overstimulation at cholinergic synapses [8]. The toxic mechanism is based on the irreversible inhibition of AChE due to phosphorylation of the active site of the enzyme. This leads to accumulation of acetylcholine and subsequent over-activation of cholinergic receptors at the neuromuscular junctions and in the autonomic and central nervous systems. The rate and degree of AChE inhibition differs according to the structure of the OP compounds and the nature of their metabolite [9].

The other two pesticides, Alpha-Cypermethrin and Fenvalerate confirmed by GC-MS analysis, are synthetic pyrethroids. The presence of alpha-cyano group classifies the pyrethroids according to their chemical structures into two distinct types. The group which does not contain the alpha-cyano moiety in their structure are type I pyrethroids,

and the one which has alpha-cyano functional group in their structure are classified as type II pyrethroids.

Alpha-Cypermethrin (Molecular Formula: C22H19Cl2NO3; Molecular Mass 416.3), a type II pyrethroid, is a racemic mixture {IUPAC names: (S)-α-cyano-3-phenoxybenzyl-(1R, 3R)-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane-carboxylate and (R)- α -cyano-3-phenoxybenzyl-(1S,3S)-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane-carboxylate}. It is also commonly known as alphamethrin. Fenvalerate {IUPAC name: (RS)-α-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate} is another type II pyrethroid. It is a racemic mixture of four stereoisomers ([2S, αS], [2S, αR], [2R, αS] and [2R, αR]) found in approximately equal proportions owing to the presence of two chiral centers in the structure [10].

Pyrethroids impair ion transport through the membrane of nerve axons, causing muscular paralysis in the insect; death seems to follow a nervous system impairment that occurs within a few minutes after pesticide absorption. Pyrethroid insecticides act on the nerves of both insects and higher animals, inducing a transient increase in sodium permeability of the nerve membrane during excitation. This action results in relatively short trains of repetitive nerve impulses in sensory nerve fibers. Type I pyrethroids cause a moderate protraction of the sodium channel permeability in the nerve membrane; type II causes a long-lasting protraction of sodium permeability of the nerve membrane during excitation [11]. The central nervous system (CNS) effects are suggested to be related to the decrease in chloride channels gated with gamma – aminobutyric acid (GABA), modulation of nicotinic cholinergic transmission, enhancement of adrenaline release and action on calcium channels [12].

There is no dearth of literature dealing with the toxicity of pesticides. During the literature survey, we found that there are some case reports in which the pesticide mixtures are encountered. The pesticide mixtures in these cases are commercially available pesticide formulations. They are mainly pyrethroid-based formulations [13], OP based formulations [14-15] and OP- pyrethroid mixtures [16-17]. However, all these cases deal with the clinical complications with no involvement of postmortem samples.

## 6. Conclusion

In the present case report, the toxicological examination of the samples revealed the presence of a mixture of five different pesticides. The case report is of particular forensic interest as a case of double suicide in which such a mixture was used to cause death.

**Acknowledgement**

The authors are thankful to the Director of the Central Forensic Science Laboratory, Chandigarh, India for constant encouragement and support.

**Funding**

None.

**Conflict of interest**

None.

## References

1. Abu Al-Ragheb SY, Salhab AS. Pesticide Mortality: A Jordanian Experience. Am J Forensic Med Pathol. 1989; 10(3): 221-5. https://doi.org/10.1097/00000433-198909000-00010
2. Kır MZ, Öztürk G, Gürler M, Karaarslan B, Erden G, Karapirli M, Akyol Ö. Pesticide poisoning cases in Ankara and nearby cities in Turkey: an 11-year retrospective analysis. J Forensic Leg Med. 2013;20(4): 274-7. https://doi.org/10.1016/j.jflm.2012.10.003, PMid:23622474
3. Bertolote JM, Fleischmann A, Eddleston M, Gunnell D. Deaths from pesticide poisoning: a global response. Br J Psychiatry. 2006; 189(3):201–3. https://doi.org/10.1192/bjp.bp.105.020834, PMid:16946353 PMCid:PMC2493385
4. Sheu JJ, Wang JD, Wu YK. Determinants of lethality from suicidal pesticide poisoning in metropolitan Hsin Chu. Vet Hum Toxicol 1998; 40(6):332–6. PMid:9830692
5. World Health Organization. The impact of pesticides on health: Preventing intentional and unintentional deaths from pesticide poisoning. Geneva: WHO, 2006.
6. Kumar A, Verma A, Kumar A. Accidental human poisoning with a neonicotinoid insecticide, imidacloprid: A rare case report from rural India with a brief review

of literature. Egypt J Forensic Sci. 2013; 3(4): 123–6. https://doi.org/10.1016/j.ejfs.2013.05.002

7. Hundekari IA, Surykar AN, Dongre NN, Rathi DB. Acute Poisoning with Organophosphorus Pesticide: Patients Admitted to A Hospital in Bijapur, Karnataka. J Krishna Inst Medical Sci Uni. 2012; 1: 38-47.
8. Eddleston M, Eyer P, Worek F, Mohamed F, Senarathna L, von Meyer L, Juszczak E, Hittarage A, Azhar S, Dissanayake W, Sheriff MR. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. The Lancet. 2005;366(9495):1452-9. https://doi.org/10.1016/S0140-6736(05)67598-8
9. Paudyal BP. Organophosphorus Poisoning. J Nepal Med Assoc. 2008; 47(172):251-8. PMid:19079407
10. Shah PV and McGregor D. Fenvelerate: Joint FAO/WHO Meeting on Pesticide Residues; 2013; 307–61.
11. World Health Organization. Safety of Pyrethroids for public health use. Geneva: 2005.
12. Gunay N, Kekec Z, Cete Y, Eken C, Demiryurek AT. Oral deltamethrin ingestion due in a suicide attempt. Bratisl Lek Listy. 2010;111(5):303-5.
13. Yang PY, Lin JL, Hall AH, Tsao TC, Chern MS. Acute ingestion poisoning with insecticide formulations containing the pyrethroid permethrin, xylene, and surfactant: a review of 48 cases. J Toxicol Clin Toxicol. 2002;40(2):107-13. https://doi.org/10.1081/CLT-120004397, PMid:12126181
14. Mishra A, Pandya HV, Dave N, Mehta M. Multi-organ dysfunction syndrome with dual organophosphate pesticides poisoning. Toxicol int. 2013;20(3):275-7. https://doi.org/10.4103/0971-6580.121682, PMid:24403738 PMCid:PMC3877496
15. Thunga G, Sam KG, Khera K, Xavier V, Verma M. Profile of acute mixed organophosphorus poisoning. Am J Emerg Med. 2009;27(5):628-e1. https://doi.org/10.1016/j.ajem.2008.08.030, PMid:19497478
16. Nakanishi K, Naruka Y, Shimizu I, Yorozuya T, Watanabe T, Arai T. A case of Acute Fenvelrate- Dimethoate Poisoning. J Jan Soc Intensive Care Med. 1996; 2: 103-6. https://doi.org/10.3918/jsicm.3.103
17. Badrane N, Askour M, Berechid K, Abidi K, Dendane T, Zeggwagh AA. Severe oral and intravenous insecticide mixture poisoning with diabetic ketoacidosis: a case report. BMC Res Notes. 2014; 7(1): 485. https://doi.org/10.1186/1756-0500-7-485, PMid:25078103 PMCid:PMC4122673