Simultaneous Detection of Insecticides and Growth Regulators by Single Chromogenic Spray Reagents

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Abstract

Insecticides are widely used in India for agriculture purpose[1-2]. Due to easy availability of insecticides with new trade names and mixture of different class they are knowingly or unknowingly used for homicidal and suicidal poisoning purposes. Thin layer chromatography is found to be the best option for the identification of these insecticides. Presently number of chromogenic reagents is used to identify different class of insecticides from the biological materials which is found to be tedious, laborious, time consuming and increases the analytical cost. Hence a single unique chromogenic spray reagent for detection of different commonly used insecticides like organophosphorous, carbamate, organochloro, sysnthetic pyrethroids and plant growtrh regulators by thin layer chromatoraphy technique. The present paper report if 10% of aqueous sodium hydroxide and 0.1% Bromophenol Blue in 1% silver nitrate solution in absolute alcohol at room temperature sprayed on these insecticide gives different colour spots like blue, purple, yellow, black green for different insecticides. Limit of detection of these reagents is about 10µg.

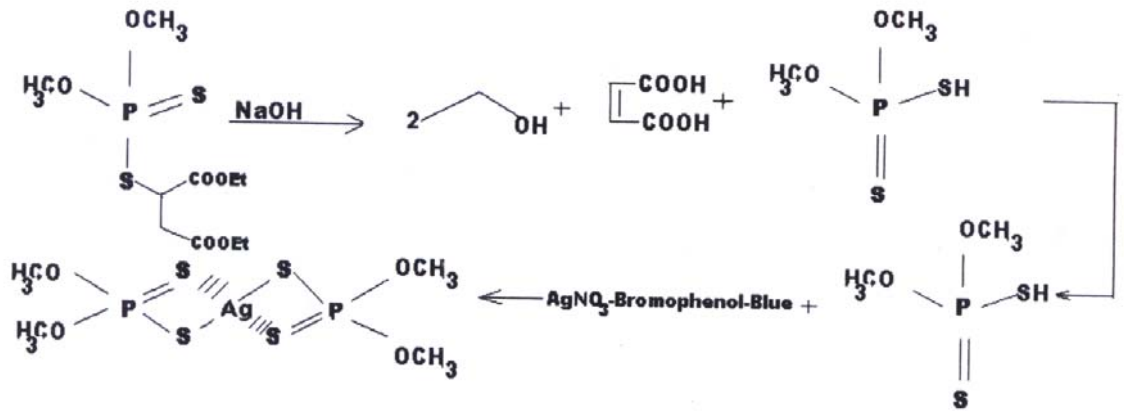
Keywords: suicidal, Homicidal, Postmortem Toxicology, Insecticides TLC, Bromophenol blue silver nitrate reagents.

Introduction

Various kind of substances are used in agriculture for crop protection and pest control. Owing to their easy availability these substances are misused in criminal poisoning cases. In such poisoning cases medical officer preserves the postmortem samples for routine toxicological examination and submit it to forensic science laboratory. In such situation toxicologist play a vital role in identification of the toxic substances at low concentration.

Number of chromogenic reagents is reported in the literature for detection of different class of insecticides[3-10].Various analytical and advance instrumental methods viz. gas chromatography[11-14] and mass spectroscopy[13] are also reported in the literature. Since in toxicology, generally no clue about the nature of poison is available and the poison extracted from the biological material is only in microgram level, TLC screening using numbers of reagents are in use. This may lose the quantity of poison. Hence, it has been taken to develop unique chromogenic reagent for all class of insecticides by reacting them in alkaline media with bromopheno[1] in presence of silver nitrate on thin layer chromatography plate.

Bromophenol blue is an acid-base indicator and its useful range lies between pH 3.00 and 4.6 at this pH it changes its colour from yellow to purple[2] The hydrolysis of insecteticide or its isomers produce some acids having pH between 3 to 4.6 which reacts with silver nitrate bromophenol blue reagent give different colored spots. The characteristic of this reaction is used to identify the commonly used insecticides like organophosphorous, organochloro, pyrethroids, plant growth regulator by using single screening test. Following probable reaction may be taking place which is as follows.



Experimental

Chemical and reagents:

Sodium hydroxide (AR grade):-10% w/v in distilled water.

Bromo phenol Blue-Silver Nitrate reagent: - 0.1% w/v Bromo Phenol Blue in 1% w/v Silver nitrate solution in a absolute alcohol.

Thin Layer Chromatography:

Standard glass plates (10 x 20 cm) are coated with a 0.25 mm layer of silica gel-G in water (1:2) and activated at 110°C for 1 hour before use. Technical grade samples of insecticide mentioned in table were procured from ITC pune and 1mg/ml solutions were prepared from it respectively. These samples were spotted by using linomate applicator of Camag make. The plate was developed to a distance 10 cm. in a presaturated chamber containing Hexane: Acetone (8:2) as mobile phase. (Plant growth regulator plate was developed in water as mobile phase). The plate was removed, air dried and uniformly sprayed with 10% Sodium hydroxide and then with Bromo- phenol Blue -Silver nitrate reagent. The intense yellow, purple, grey, blue, black, green, spots were developed at different Rf. values for different insecticides are observed and compared with reported values.

Table 1 Rf values for different insecticides.

Sr.No.	Name of Insecticide	R_f values	Colour of spots
1	Dichlorovos	0.95,0.90,0.75,0.65, 0.55,0.45, 0.18	Purple, Black, Gray
2	Chlorpyriphos	092,0.75,0.60,0.35	Purple ,Gray
3	Propoxur	0.70,0.45	Black
4	Thiodan	0.86,0.72,0.65	Blue, Yellow, Purple
5	Monocrotophos	0.87,0.70,0.45	Yellow, Black
6	Cypermethrin	0.72	Purple
7	Dimethoate	0.76, 0.55, 0.45, 0.23	Purple, Black Blue, Yellow
8	Temphos	0.62	Gray- Black
9	Triazophos	0.79, 0.62, 0.55	Purple, Dark Blue
10	Profenophos	0.75	Gray -Black
11	Phenthoate	0.72,0.50	White, Gray, Purple
12	Phorate	0.95,0.54,0.37	Purple, Black ,Gray
13	Methyl parathion	0.65,0.35	Yellow, Gray

14	Malathion	0.78,0.65,0.20	Yellow, Gray , Black
15	Alfacyper	0.80, 0.55	Blue, Yellow
16	Alfamethrin	0.80	Blue
17	Lamda cyhalothrin	0.80	Blue
18	Cypermethrin	0.80,0.55,0.45,0.38	Blue, Yellow, Purple
19	Transfluthrin	0.90, 0.70, 0.45	Green, Yellow, Purple
20	Deltamethrin	0.95, 0.75, 0.54, 0.48	Green, Blue, Gray, Purple
21	Fenvalerate	0.80,0.60, 0.55,0.35	Blue, Yellow, Purple
22	Deltamethrine + Triazophos	0.95,0.80, 0.55,0.25	Purple, Blue
23	Chloropyriphos+ Cypermethrin	0.85,0.74, 0.54,0.38	White, Blue, Purple
24	Chloropyriphos	0.90,0.60,0.52	Blue, Purple
25	Triazophos	0.90,0.80,0.55	Blue, Purple

Recovery of Insecticides from Biological samples

Experiments for recovery of some representative insecticides of different group are carried out by adding 10 mg of insecticide to 100 gm minced post mortem samples (Stomach, intestine etc.) was kept over night. Approximately 10 gm Ammonium sulphate was added to this mixture with thorough stirring and the insecticide were extracted separately with diethyl ether. The extracted volume is evaporated to room temperature. The residue were dissolved in 10 mL methanol and 10 µL of this solution of each representative insecticide spotted on activated TLC plate with 2.0 µg, 4.0 µg, 6.0 µg, 8.0 µg and 10 µg. The plates were developed and sprayed with chromogenic reagent as described above. The intensity of the spots developed for the visceral extract was compared with standards.

Results and Discussion

Insecticides are often misused in the suicidal or homicidal cases. The detection of these insecticides by using different chromogenic reagents has been reported in the literature. But in this

examination some reagents are only specific with some class or group of insecticides and fail to identify the other insecticide. The present Bromo phenol Blue-Silver nitrate reagent is found to be superior to the earlier spray reagents in terms of prominent intense spot formed by the complex and stabel up to one week with the different classes of insecticide or the combinations of insecticides. The sensitivity which is found to be very low that is up to 10ug. The screening of all groups of insecticide can be possible by using this simple spay reagent.

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