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Detecting Cypermethrin Pesticide as Analyte by Capillary Action on Adsorbent Phase of Silica Gel G by Thin Layer Chromatography: A Reinstate Method for Complex and Costly techniques

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Abstract

Pesticide poisoning is one of the most common instances of poisoning deaths in every agriculture-dependent country may it be in form of suicidal or homicidal cases. Traditional classes of pesticides like the Organophosphates, Organochlorides and Carbamates have given way to the newer class of pesticides known as synthetic Pyrethroids. Some of the synthetic Pyrethroids like Cypermethrin, Deltamethrin and Fenavalerate are increasingly being encountered as routinely used poisons in rural India. In the instance of poisoning detecting the class of Pyrethroids uses a complex and costly instruments which is not very user friendly and also expensive. In the present article Thin Layer chromatography which is classical method of seperation science has been used as tool to identify the class of Pyrethroids, identification of Cypermethrin pesticide is analyzed using different solvent system for qualitative analysis with suitable spray reagent to identify the movement of the spot.

Keywords: Cypermethrin, Pyrethroid, Pesticide, Thin Layer Chromatography, Pesticide poisoning

Introduction

In India more than 126 million population rely on farming for their livelihood and these farmers own 74.4 million hectares of arable land and average land that they hold is less than 0.6 hectares each which is not sufficient enough to upkeep their livelihood. Semi medium and medium land holding farmers own between 2-10 hectares of land which are almost 13% of total farmers and own up to 43.6% of crop area.[1] This research has shown that many of the farmers are poor and hold small piece of land and therefore to increase the yield of their crop farmers use chemical and its derivatives in different forms to increase the output of their crop in fields. These chemical may be in form of Pesticides, Insecticides or Herbicides and majority of them are chemically synthesized which are often poisonous in nature to human beings and if proper guidelines are not followed as to how to use them they can be fatal. Pesticides are the chemicals which are being used regularly by farmers in India to stop the manifestation of pests on crops and Cypermethrin is one such pesticide. These pesticides at times result in occupational and accidental poisoning. When such instances of poisoning happen to farmers

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either accidental or intentional, Investigation and Judicial agency comes into action and probe has to be done as to what could be the poison consumed by the deceased and to answer that there has to be performed chemical analysis of visceral organs which are preserved by the doctor during the post mortem. To identify the type of poison which the deceased has consumed there are sophisticated instruments which are routinely used like Gas Chromatography, Mass Spectrometry and High Performance Liquid Chromatography which are actually very costly and to procure these instruments for every laboratory it is not easy. Another drawback of working with these instruments is that cost for each sample to be analyzed is very high and the technique is not very user friendly. Therefore convenient and easy to use method should be developed in order to identify the Cypermethrin pesticide for which till date only the costly and complex instruments have been used. In the present study authors have made an effort to develop simple, user friendly and convenient method to detect Cypermethrin by using Thin Layer Chromatography.

Cypermethrin is a synthetic Pyrethroid having high insecticidal activity and research for the same has been done on cucumber which has shown decreased fungal and bacterial growth[2] and enough photo-stability to use in fields[3]. Cypermethrin poisoning in humans can have a devastating neurological consequence as reported by doctors.[4] Study has also revealed that combination of Organophosphate and Pyrethroid is more lethal as compared to any other pesticide poisoning alone.[5] Cypermethrin is not only used with suicidal intent but there has also proved to be as an accidental poisoning to the farmers in India as reported in the case study done.[4] In India acute poisoning have been reported by researchers[6] in farmers who are growing cotton crop. Ample of awareness program and effort is being made to educate farmers about the pest management so as to overcome the act of pesticide poisoning.[7] One of the study conducted in Poland on mice has shown that there is hematological changes after Pyrethroid poisoning that is increased number of leukocytes.[8] As far as treatment is considered efforts have been made to use hyosciane and chloropheniramine maleate in the treatment of pyrethroid poisoning.[9] Oral intake of Cypermethrin exhibits the neurotoxin and gastrointestinal effects in human beings.[10] Studies have shown that when there is increase in the occupational exposure of pesticide with farmers, the rate of suicide attempts also increases.[11] Cases have been reported where there is combination of two different type of pesticide used which has led to increased toxicity.[12] According to one of the research conducted in China it has been reported that Cypermethrin, Deltamethrin and Fenvalerate all three have more number of accidental poisoning as compared to any other occupational poisoning.[13]

Complex and high end techniques have been routinely used to identify the Cypermethrin in the laboratory and for the same many attempts have been done. In one of the study conducted by[14] has devised a High Performance Thin Layer Chromatography system for Cypermethrin detection using HPTLC-densitometry method. In another study done by[15] devised a Gas Chromatography-Electron Capture Device system for Cypermethrin detection in vegetables. Thin Layer Chromatography technique cited by[14] in his research article for the detection of Pyrethroid pesticides in general was able to detect Pyrethroids like Cypermethrin, Deltamethrin and Fenavalerate using solvent systems like methyl acetate and formic acid in ratio of 80:20, ethanol and hexane in ratio of 50:50, acetone and cyclohexane in 60:40 by using solution of iodine and o-tolidine as spray reagent. All the pyrethroid pesticides reacted the same way and produced pink spots with retention factor (Rf) value of 0.63. In another attempt of Thin Layer Chromatography technique developed by[16] for the detection of pyrethroid pesticides in general was able to detect Pyrethroids like Cypermethrin, Deltamethrin and Fenavalerate using solvent system of acetone, methanol and water in ratio of 30:30:30 and spray reagent 1 gm % alcoholic p-nitrobenzaldehyde followed by 10% sodium hydroxide. All the pyrethroid pesticides reacted the same way and produced pink spots with Rf value of 0.67. As per the Dept. of statistics, Government of India, among the 3 common pyrethroid pesticides, Cypermethrin seems to be the greater cause of suicidal poisoning deaths in India. In 2019 Cypermethrin has accounted for 874 deaths while Deltamethrin accounted for 162 and Fenavalerate accounted for 185 deaths.[17]

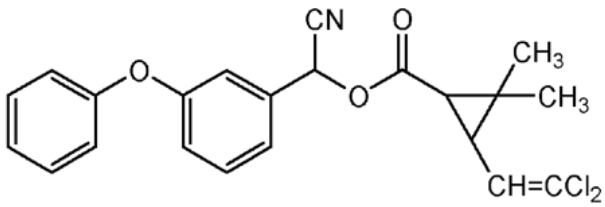

Figure 1: Chemical structure of Cypermethrin.

All the above quoted techniques are complex and time taking a simple, convenient and user friendly method has still not been developed for analysis of the Cypermethrin which can be done in less time and cost for each samples examination is less. There is an urgent need for a simple, cost-effective system for detection of Cypermethrin both in clinical and forensic contexts. With this study an effort would be made to develop new solvent systems and spraying reagents and also to standardize the same.

Materials and Method

Authors have adopted Thin Layer Chromatography technique to perform the experiments; Chromatography is a technique which allows separation, identification and determination of closely related components in complex solution which are non-volatile. The method of chromatography involves two phases firstly *mobile phase* and secondly *stationary phase*. Stationary phase here was the thin layer of silica gel G which was spread on the glass plate. Mobile phase is the sample to be analyzed here in our case it is Cypermethrin. The principle of chromatography lies in the fact that mobile phase is made to run on the stationary phase and during the process components of mobile phase which has more affinity to bind with stationary phase is held on the stationary phase surface and lag near to their source whereas those components which have less ability to bind with the stationary phase will travel for longer distance thus resulting in separation of different components in the complex mixture making a unique pattern for every sample.

Silica gel G has chemical composition of Silicon dioxide and G stands for Gypsum which is binder and the primary role of this binder is to hold the silica paste over the glass plate on which it is applied and the chemical composition of gypsum is Calcium Sulfate. Preparation of Thin Layer Chromatography plate was done by dissolving Silica gel G in distilled water in the ratio of 1 : 2 to make slurry and was evenly spread on the glass plate to make sure that it spreads with uniform thickness of around 0.5mm on the plate later leaving the plate at room temperature to dry. The plate once dried is kept in the hot air oven for activation at 110 degree centigrade for 30 minutes. The sample Cypermethrin standard 10% used for testing was provided by M/s. Anu Products Limited, Ahmadabad, Gujarat, India. Sample was taken in its pure form about 0.1ml using micro-capillary tubes there was no need for any solvent because the analyte was in liquid state.

Observation

Authors used seven different reagents to make six solvent systems with different volumetric ratios. Solvent systems like Methanol: water 65:35, Benzene: Acetone: Ammonia 50:30:20 and Ethyl Acetate: Cyclohexane 70:30 were used. Twenty five solvent systems were taken with its unique spray reagent for each combination and was used to identify if there is any positive observation on the TLC plate? For all twenty three solvent system the observation was negative except in two solvent system which gave a positive result on the TLC plate in form of white and black spot. The first solvent system which gave positive result was Benzene, Acetone and Ammonia in the ratio of 50: 30:20 when 5% Rhodamine B in Ethanol was used as sprayed over the developed plate it gave white spot with Rf value 0.92. The second solvent system which gave positive result was Benzene, Acetone and Ammonia in the ratio of 50:30:20 when the plate was treatedwith spray reagent 10% Molybdic Acid in 150ml Sulfuric Acid there was black spot observed with Rf value 0.89. Rf (Retention Factor) was calculated by using the formula that Rf is the ratio of distance travelled by the solute from the origin line is to distance traveled by the solvent from the origin line. After the development of TLC plate with the respective solvent systems, TLC plates were treated with total eighteen different types of spray reagents. Different spray reagents used in the experiment were namely $AgNO_3$ in Ethanol, $AgNO_3$ in Ammonia, $FeCl_3$ in butanol, Rhodamine B in Ethanol, Thymol Blue in water, Coomasie brilliant Blue in water, Cotton blue in water, Amido black

in water, Stannous Chloride in HCl, Ammonium Molybdate in HNO_3, Perchloric Acid in Acetone, Bromine in CCl_4, Dragendorff's reagent, Molybdic Acid in Sulphuric Acid, Para-dimethyl Amino Benzaldehyde and HNO_3 in Ethanol.

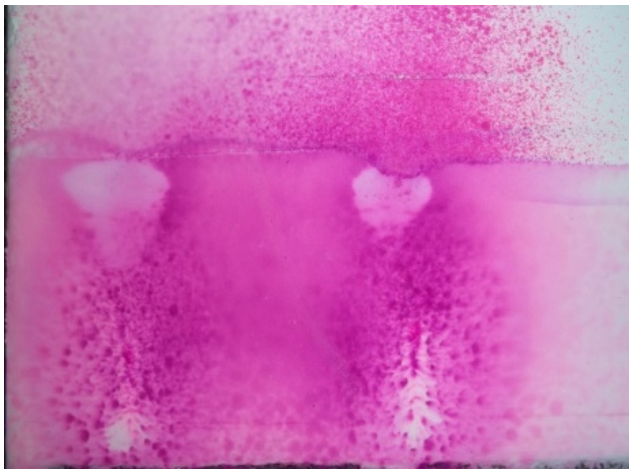

Figure 2: White spots visualized after spraying with Rhodamine B as spray reagent, by using Benzene: Acetone: Ammonia 50:30:20 as solvent system for detection of Cypermethrin by TLC

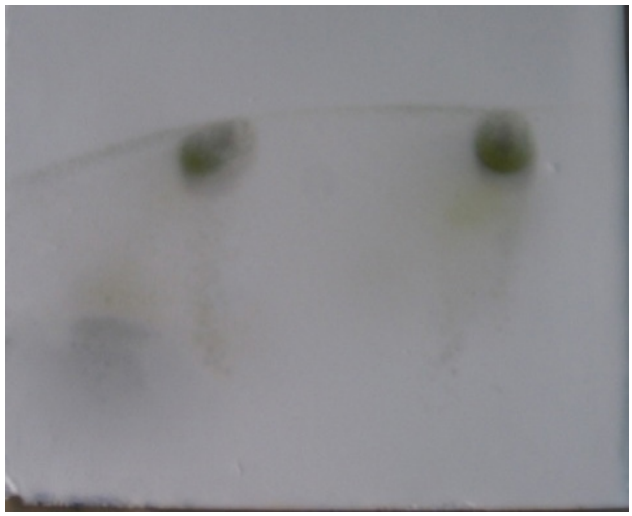

Figure 3: Black spots visualized after spraying with Molybdic Acid as spray reagent, by using Benzene: Acetone: Ammonia 50:30:20 as solvent system for detection of Cypermethrin by TLC.

The detailed observation all twenty five solvent systems used are shown below in tabular manner.

Table 1: Primary solvent systems and spray reagents

S. No.	Solvent System	Spray Reagent	Result
1	Methanol: water 65:35	1gm $AgNO_3$ in 90ml Ethanol and 5ml Ammonia	No Spot
2	Ammonia: Acetone 70:30	1gm AgNO3 in 90ml Ethanol and 5ml Ammonia	No Spot
3	Acetone: Acetic Acid 80:20	2% $AgNO_3$ in Ammonia	No Spot
4	Acetone: Acetic Acid 80:20	2% $FeCl_3$ in n-butanol	No Spot
5	Benzene: Acetone: Ammonia 50:30:20	5% Rhodamine B in Ethanol	White Spot Rf=0.92
6	Ethyl Acetate: Cyclohexane 70:30	10% Thymol blue in water	No Spot
7	Ethyl Acetate: Cyclohexane 70:30	10% Coomasie brilliant blue in water	No Spot
8	Cyclohexane: CCl4 60:40	10% Thymol blue in water	No Spot
9	Cyclohexane: CCl4 60:40	10% Coomasie brilliant blue in water	No Spot
10	Ethyl Acetate: Cyclohexane 70:30	0.5% Cotton Blue in water	No Spot
11	Ethyl Acetate: Cyclohexane 70:30	0.5% Amido Black in water	No Spot
12	Benzene: Acetone: Ammonia 50:30:20	0.5% SnCl2 in HCl	No Spot
13	Benzene: Acetone: Ammonia 50:30:20	5% Ammonium Molybdate in 35 ml HNO3 and 65 ml Water	No Spot

14	Benzene: Acetone: Ammonia 50:30:20	4% Perchloric Acid in Acetone	No Spot
15	Benzene: Acetone: Ammonia 50:30:20	SnCl2 followed by Ammonium Molybdate followed by Perchloric Acid reagents	No Spot
16	Ethyl Acetate: Cyclohexane 70:30	0.5% SnCl2 in HCl	No Spot
17	Ethyl Acetate: Cyclohexane 70:30	5% Ammonium Molybdate in 35 ml HNO3 and 65 ml Water	No Spot
18	Ethyl Acetate: Cyclohexane 70:30	4% Perchloric Acid in Acetone	No Spot
19	Ethyl Acetate: Cyclohexane 70:30	SnCl2 followed by Ammonium Molybdate followed by Perchloric Acid reagents	No Spot
20	Benzene: Acetone: Ammonia 50:30:20	Bromine: CCl4 1:8	No Spot
21	Benzene: Acetone: Ammonia 50:30:20	Dragendorff's reagent	No Spot
22	Benzene: Acetone: Ammonia 50:30:20	10gm Molybdic Acid in 150 ml H_2SO_4	Black Spot Rf=0.89
23	Benzene: Acetone: Ammonia 50:30:20	100mg $AgNO_3$ in 10ml water, 10ml Phenol, 200ml Acetone and 2 drops H_2O_2	No Spot
24	Benzene: Acetone: Ammonia 50:30:20	1% Para-Dimethyl Amino Benzaldehyde	No Spot
25	Benzene: Acetone: Ammonia 50:30:20	HNO_3:Ethanol 65:35	No Spot

Results

It was found that with two spraying reagents namely Rhodamine B in Ethanol and Molybdic Acid in Sulphuric Acid, the visualization of separated Thin Layer Chromatography plates were made possible. Spray reagents Rhodamine B in Ethanol and Molybdic Acid in sulphuric acid were replicated for experimentation thirty times each with the same solvent system to check the reproducibility of the test. The response of the separation of Cypermethrin with the same solvent system was checked and analysed. Following, the above set of results, the two different successful Thin Layer Chromatography systems were separately performed thirty times each to examine their reproducibility.

The Figure 4 below shows the reproducibility of the experiment while using solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 and using the different spray 5% Rhodamine B in Ethanol.

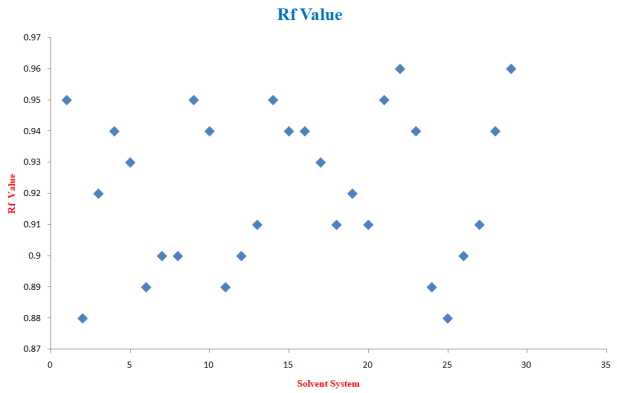

Figure 4: Graphical representation of Rf value when using solvent system Benzene, Acetone, Ammonia and Rhodamine B in Ethanol

The Figure number 5 below shows the reproducibility of the experiment while using solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 and using different spray reagent 10gm Molybdic Acid in 150ml H_2SO_4.

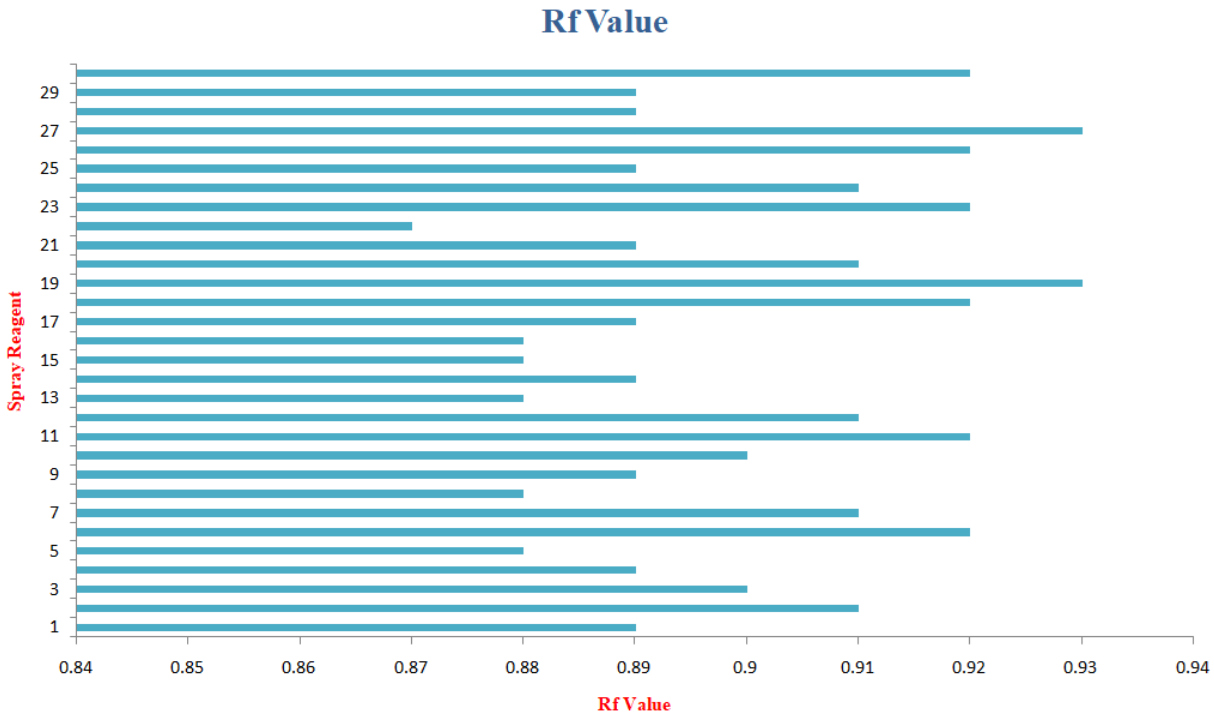

Figure 5: Graphical representation of Rf value using Solvent System Benzene Acetone Ammonia and Spray Reagent Molybdic Acid

In the end of the analysis it was found that 5% Rhodamine B in Ethanol was giving very promising results as visualized by white spots representing the separated Cypermethrin in the solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 as shown in Figure 2. Similarly, the spray reagent 10g Molybdic Acid in 150ml Sulphuric acid was also giving a very prominent result as visualized by black spots representing the already separated Cypermethrin with the solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 as shown in figure 3. The mean value of Rf values obtained with Rhodamine B was found to be 0.92 and for Molybdic Acid 0.90.

Table 2: Statistical Analysis of Data

Spray Reagent	Mean	Standard Deviation	Standard Error	t Value	p Value
Rhodamine B in Ethanol	0.92	0.025	0.005		
Molybdic Acid in Sulphuric Acid	0.90	0.017	0.003	3.45	> 0.0005

Standard deviation is a measure of the amount of variation or dispersion of set of values.

Standard deviation is calculated by using the formula:

$$\sigma = \sqrt{\frac{\sum xi - \mu}{N}}$$

σ= retention factor standard deviation

N= the size of the retention factor

X_i= each value of the retention factor

μ= the retention factor mean

Standard Error is defined as the measure of the statistical accuracy of an estimate, equal to the standard deviation of the theoretical distribution of a large sample of such estimates.

Standard Error was calculated using formula:

$$SE = \frac{\sigma}{\sqrt{n}}$$

SE= standard error of the retention factor

σ = retention factor standard deviation

n =number of retention factor

T-value measures the size of the difference relative to the variation in our sample data.

T-value is calculate using formula:

$$\frac{mean\ of\ 30\ solvent\ system - mean\ of\ 60\ solvent\ system}{Standar\ Error}$$

P-value is the probability of obtaining results at least as extreme as the observed results of a hypothesis test, assuming that the null hypothesis is correct.

Conclusion

By and large the Rf values for both Rhodamine B and Molybdic Acid trials range from 0.88-0.96 and 0.87-0.93 respectively. The difference in the mean Rf values of the Rhodamine B and Molybdic Acid trials range from 0.90 - 0.92 As expected the difference between Rf's in Rhodamine B and Molybdic Acid trials were satisfactorily significant. Having found that there is difference in Rf value and also the difference varied from 0.88-0.96 and 0.87-0.93, an attempt was made to see whether the Rf values of the Rhodamine B trial and the Molybdic Acid trial are varying and if so whether the obtained results were showing the Mean, Standard Deviation and if so whether the obtained results were showing significance in intra-comparison. After studying the Mean & Standard Deviation, the t-value was calculated and found to be 3.45 and the statistical significance through probability was found to be greater than 0.0005.

Therefore a Thin Layer Chromatography system using solvent system of Benzene, Acetone and Ammonia in ratio of 50:30:20 and spray reagents of either 5% Rhodamine B in Ethanol or 10gm Molybdic Acid in 150 ml H_2SO_4 is a reliable Thin Layer Chromatography system for the detection of cypermethrin pesticide. The technique is robust, reliable and easy to operate as compared to its counterparts which are difficult to operate and houses complex mechanism. Advantage of using Thin Layer Chromatography with the solvent system and spray reagent developed by author has wide scope which reduces the cost of per sample to be tested and even small laboratories can easy afford it as the techniques and requirement are not very expensive. More study is required to identify its applicability in detecting Cypermethrin in viscera samples in Forensic Toxicology.

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Conflict of Interest: Authors declare that there is no conflicts of ineterest between them

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References

1. Bisht IS, Rana JC, Pal Ahlawat S. The Future of Smallholder Farming in India: Some Sustainability Considerations. Sustainability. 2020 May 6;12(9):3751.
2. Zhang B, Zhang H, Jin B, Tang L, Yang J, Li B, et. al. Effect of cypermethrin insecticide on the microbial community in cucumber phyllosphere. Journal of Environmental Sciences. 2008 Jan;20(11):1356–62.
3. Yu-Tao T, Zhao-Wei L, Yang Y, Zhuo Y, Tao Z. Effect of alpha-cypermethrin and theta-cypermethrin on delayed rectifier potassium currents in rat hippocampal neurons. NeuroToxicology. 2009 Mar;30(2):269–73.
4. FAUTH. Toxic intracerebral demyelination in a case of suicidal Cypermethrin poisoning. [DATE] [cited 2021 Feb 3]; Available from: https://www.atmph.org/printarticle.asp?issn=1755-6783;year=2012;volume=5;issue=6;spage=615;epage=617;aulast=Majumdar
5. Iyyadurai R, Peter JV, Immanuel S, Begum A, Zachariah A, Jasmine S, et. al. Organophosphate-pyrethroid combination pesticides may be associated with increased toxicity in human poisoning compared to either pesticide alone. Clinical Toxicology. 2014 Jun;52(5):538–41.
6. Mancini F, Van Bruggen AHC, Jiggins JLS, Ambatipudi AC, Murphy H. Acute Pesticide Poisoning among Female and Male Cotton Growers in India. International Journal of Occupational and Environmental Health. 2005 Jul;11(3):221–32.
7. Mancini F, Jiggins JLS, O'Malley M. Reducing the Incidence of Acute Pesticide Poisoning by Educating Farmers on Integrated Pest

Management in South India. International Journal of Occupational and Environmental Health. 2009 Apr 1;15(2):143–51.

8. Haratym-Maj A. Hematological alternations after pyrethroids poisoning in mice. Ann Agric Environ Med. 2003 Dec 5;9(2):199–206.
9. Das RN, Parajuli S. Cypermethrin Poisoning and Anti-cholinergic Medication- A Case Report. Internet Journal of Medical Update - EJOURNAL [Internet]. 2007 Nov 1 [cited 2021 Feb 3];1(2). Available from: http://www.ajol.info/index.php/ijmu/article/view/39840.
10. Cypermethrin poisoning manifesting with prolonged bradycardia: A case report | Elsevier Enhanced Reader [Internet]. [cited 2021 Feb 3]. Available from: https://reader.elsevier.com/reader/sd/pii/S2214750020304479?token=58439BCF5909CC8D9CACFF5B5711C50280710F7097F8F5E877416E9905E693FC78728CB12BD315FFC7A504CB0BBDDCC4.
11. Abdullat EM, Hadidi MS, Alhadidi N, AL-Nsour TS, Hadidi KA. Agricultural and horticultural pesticides fatal poisoning; The Jordanian experience 1999–2002. Journal of Clinical Forensic Medicine. 2006 Aug;13(6–8):304–7.
12. Idris SB, Ambali SF, Ayo JO. Cytotoxicity of chlopyrifos and cypermethrin: The ameliorative effects of antioxidants. African Journal of Biotechnology. 2012;11(99):16461–7.
13. He F, Wang S, Liu L, Chen S, Zhang Z, Sun J. Clinical manifestations and diagnosis of acute pyrethroid poisoning. Arch Toxicol. 1989 Feb;63(1):54–8.
14. Sherma J. Recent advances in the thin-layer chromatography of pesticides: a review. J AOAC Int. 2003 Jun;86(3):602–11.
15. Chandra S, Mahindrakar A, Shinde L. Determination of Cypermethrin and Chlorpyrifos in Vegetables by GC-ECD. International Journal of ChemTech Research. 2010 Apr 1;2.
16. Mavle R, Katkar H, Daundkar B, Malve M, Krishnamurthy R. Thin Layer Chromatographic Technique for Detection and Identification of Endosulfan Insecticide with m-Dinitrobenzene Reagent. JPC-J Planar Chromat. 2008 Jun 1;21(3):197–8.
17. Sub section indiastat growth statistics details figures [Internet]. [cited 2021 Feb 12]. Available from: https://www.indiastat.com/data/health/death-rate