QUALITATIVE AND QUANTITATIVE ANALYSIS OF CHLORPYRIFOS, CYPERMETHRIN PESTICIDES IN VEGETABLES

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INTRODUCTION

The term pesticide is used to describe all sorts of products which control pests. These range from synthetic chemicals which are poisonous to weeds, to natural chemicals such as pyrethrums derived from chrysanthemums used to repel insects or larger animals.

Examples of pesticides include:

- Chemical insecticides (which kill insect pests).
- Herbicides which kill plants or weeds.
- Slug pellets containing chemicals such as metaldehyde.
- Rat poison (warfarin).

Because pesticides are selected for their ability to kill living things, whether plant, insect or animal, it's perhaps not surprising that they can also harm unintended targets such as people, wildlife and in general the environment. They have been associated with claims of many different health problems from cancer and infertility to Gulf War syndrome and eye abnormalities. In many cases there is evidence to back such concerns.

For this reason, some countries have banned the use of certain pesticides, especially if there is a risk that they could get into the food chain, and safety levels have been set for others which are deemed to protect the public.

However, the jury is still out on the risk of exposure to many pesticides on a long-term basis.

Pesticides are used both at home and in private gardens as well as by most farmers who, under pressure to provide food at low prices often rely on pesticides to maximise their harvest. The use of pesticides in farming often relies on the fact that levels used to kill insects are generally low enough not to affect humans.

But some people may be susceptible to these pesticides even though the dose is only low, or may be exposed to higher levels in certain situations. And there may be other potential damage to the food chain

Research has identified several pesticides that pose a risk to health over a prolonged period and even with small-scale exposure. The UK Environment Agency and the Health and Safety Executive monitor the use of pesticides in the UK and work to implement EU legislation. The Health and Safety Executive also keeps a Pesticides Register of UK Approved Products.

Farmers now use one third less pesticides chemicals than they did 30 years ago, and some pesticides are no longer used or even made any more, such as chlordecone (Kepone). Others such as Lindane are banned throughout the EU (including the UK) because of links to breast and other cancers, and fertility problems which result from their effect on hormone levels, but are still used in some developing countries.

Other examples include:

- Vinclozalin: a dicarboximide fungicide used mainly on oilseed rape and peas in the UK and on vines, fruit and vegetables worldwide. Regulators have been concerned about potential adverse effects on reproductive health impacts of vinclozolin because it disrupts hormone systems. The EU now appears to be satisfied about its use but the US is committed to phasing it out as far as possible.
- Carbendazim: The most commonly used fungicide in the UK. It's known to disrupt hormone systems in the body. It has been shown to disrupt sperm production in the testicles of adult rats, and to damage the development of mammals in the womb.
- DDT: one of nine persistent organic pollutants (POPs) which can bioaccumulate, DDT is highly fat soluble and high levels develop in fatty foods such as meat and dairy products. DDT has been linked to cancer and male infertility after it was shown to block the action of male hormones. It has been banned in the UK and elsewhere in the developed world since the 1980s. Since 2001, when the Stockholm Convention on Persistent Organic Pollutants was signed it has been banned worldwide except for some limited use in controlling insect borne illness such as malaria (DDT is used to kill the mosquitoes that carry the disease).
- Organophosphates: These are a large group of chemicals which form the basis of many insecticides and herbicides, and which can pass into the body through the lungs and skin or on food. They're of particular concern because they work by irreversibly blocking an enzyme that's essential to nerve function. Even at low levels, organophosphates may affect the brain development of fetuses and young children. They have also been linked to excessive tiredness, headaches, limb pains, disturbed sleep, poor concentration, mood changes, and suicidal thoughts. The European Protection Agency banned most residential uses of organophosphates in 2001. However they're still used in agriculture on fruit and vegetable crops and to control insect pests like mosquitos.

Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide[1]. It was introduced in 1965 by Dow Chemical Company and is known by many trade names , including Dursban and Lorsban[2]. It acts on the nervous system of insects by inhibiting acetylcholinesterase.

Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders. Exposure during pregnancy affects the mental development of children, and the use in homes in the U.S. has been banned since 2001.[4] In agricultural use, it remains "one of the most widely used organophosphate insecticides", according to theUnited States Environmental Protection Agency (EPA).[5]

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Chlorpyrifos is an organophosphate, with potential for both acute toxicity at larger amounts and neurological effects in fetuses and children even at very small amounts. For acute effects, the EPA classifies chlorpyrifos as Class II: moderately toxic. The oral LD50 for chlorpyrifos in experimental animals is 32 to 1000 mg/kg. The dermal LD50 in rats is greater than 2000 mg/kg and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC50 for chlorpyrifos in rats is greater than 200 mg/m3.[16]

Chlorpyrifos poisoning has been described by New Zealand scientists as the likely cause of death of several tourists in Chiang Mai, Thailand who developed myocarditis in 2011.[17][18][19] Thai investigators have come to no conclusion as to what caused the deaths,[20] but maintain that chlorpyrifos was not responsible, and that the deaths were not linked.[21]

Research indicated in 2006 that children exposed to chlorpyrifos while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD.[22] An earlier study had demonstrated a correlation between prenatal chlorpyrifos exposure and lower weight and smaller head circumference at birth.[23]

Among 50 farm pesticides studied, chlorpyrifos was one of two found to be associated with higher risks of lung cancer among frequent pesticide applicators than among infrequent or non-users. Pesticide applicators as a whole were found to have a 50% lower cancer risk than the general public, which is attributable to the nearly 50% lower smoking rate found among farm workers. However, applicators of chlorpyrifos had a 15% lower cancer risk than the general public, which the study suggests indicates a likely link between chlorpyrifos application and lung cancer.[24]

A 2010 study found that each 10-fold increase in urinary concentration of organophosphate metabolites was associated with a 55% to 72% increase in the odds of ADHD in children.[25]

Studies have shown evidence of "deficits in Working Memory Index and Full-Scale IQ as a function of prenatal [chlorpyrifos] exposure [as measured when the children reach] 7 years of age."[26] A 2012 study showed that the insecticide is more harmful to the mental development of boys than to that of girls.[4]

A 2011 study on the neurotoxic effects of chlorpyrifos showed that chlorpyrifos and its more toxic metabolite, chlorpyrifos oxon, altered firing rates in the locus coeruleus. These results indicate that the pesticide may be involved in Gulf War Syndrome and other neurodegenerative disorders.[26]

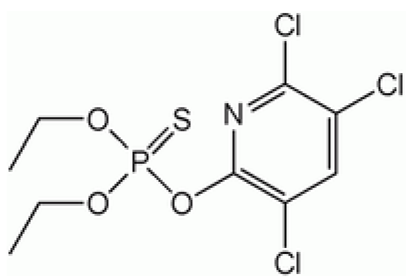

Figure. 6A Structure of Chlorpyrifos

Formula: $C_9H_{11}Cl_3NO_3PS$

Molar mass: 350.59 g/mol

Density: 1.40 g/cm³

Melting point: 42° C

Classification: Organothiophosphates

Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects[27]. It is easily degraded on soiland plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition [28]. Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN)[29]. It is found in many household ant and cockroach killers, including Raid and ant chalk.

Excessive exposure can cause nausea, headache, muscle weakness, salivation, shortness of breath and seizures. In humans, cypermethrin is deactivated by enzymatic hydrolysis to several carboxylic acid metabolites, which are eliminated in the urine. Worker exposure to the chemical can be monitored by measurement of the urinary metabolites, while severe overdosage may be confirmed by quantitation of cypermethrin in blood or plasma.[27]

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A recent study at Xuzhou Medical College in China showed that, in male rats, cypermethrin can exhibit a toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced. These data suggested that cypermethrin can induce impairments of the structure of seminiferous tubules and spermatogenesis in male rats.[28]

Long term exposure to cypermethrin during adulthood is found to induce the dopaminergic neurodegeneration in rats and postnatal exposure enhances the susceptibility of animals to dopaminergic neurodegeneration if rechallenged during adulthood.[29]

If exposed to cypermethrin during pregnancy, rats give birth to offspring with developmental delays. In male rats exposed to cypermethrin, the proportion of abnormal sperm increases. It causes genetic damage: chromosome abnormalities increased in bone marrow and spleen cells when mice were exposed to cypermethrin.[30] Cypermethrin is classified as a possible human carcinogen because it causes an increase in the frequency of lung tumors in female mice. Cypermethrin has been linked to an increase in bone marrow micronuclei in both mice and humans.[31]

One study showed that cypermethrin inhibits “gap junctional intercellular communication” which plays an important role in cell growth and is inhibited by carcinogenic agents.[32] Studies have shown that residue from cypermethrin can last for 84 days in the air, on walls, the floor and on furniture.[33]

Cypermethrin is a broad-spectrum insecticide, which means it kills beneficial insects and animals as well as the targeted insects.[34] Fish are particularly susceptible to cypermethrin.[35] Resistance to cypermethrin has developed quickly in insects exposed frequently and can render it ineffective.[36]

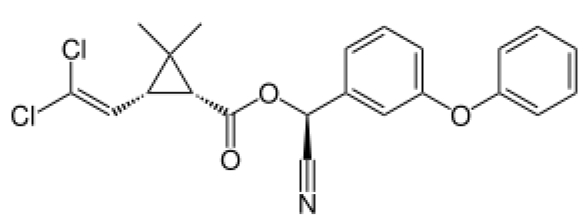

Figure. 6B Structure of Cypermethrin

MATERIALS AND METHODS

2.1. Instrumentation

For quantitative estimation of Cypermethrin, Chlorpyrifos in vegetables an isocratic peak hplc instrument with chromosil c18, c8 column (250 mm x 4.6 mm, 5µ), (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using PEAK software. techcomp UV 2301UV-Visible spectrophotometer (Hitach software) was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Denver balance was used for weighing of the materials.

2.2. Chemicals and Solvents

The Chlorpyrifos reference standard sample was obtained from Modern Insecticide Limited, PUNJAB, Cypermethrin standard solution was obtained from Paramount Pesticides Ltd meerut, Uttar Pradesh, India

The Vegetable samples were collected from the local markets, Crops, exporting companies. Acetonitrile, Methanol, Water used is HPLC grade are purchased from Merck Specialties Private Limited, Mumbai, India. M Phosphate Buffer, T.E.A of AR grade purchased from local market.

2.3 Sample collection

Vegetables are directly collected from local vegetables markets of Guntur, Tenali, Ongle, Vijayawada, early in the morning.

2.4. The Mobile Phase

Two different suitable mobile phases are prepared individually for analysis of target Pesticides in vegetable. The prepared mobile phases are sonicated up to 30 min, and filtered through 0.45 µ nylon filter paper.

2.5. Standard Solution of the Drug

For analysis of cypermethrin, chlorpyrifos 1000 ppm stock solutions are prepared with reference standards of cypermethrin, chlorpyrifos Pesticides with their mobile phases. From the stock solution calibration curves prepared to estimate target Pesticides.

2.6. Preparation of vegetable Samples:

Extraction of pesticides residues from vegetables [37]

For each vegetable sample 20g was taken to which 20ml of distilled water was added. The mixture was left to stand for 15minutes, after which 50ml of acetonitrile was added and the sample was homogenized by crushing in a pestal and mortar. The sample was then filtered by suction. To the remaining residue on the filter paper, 20ml of acetonitrile was added and again the sample was homogenized and filtered by suction. Both filtrates were combined together and the volume was increased to 100ml by the addition of acetonitrile. From this solution 20ml of sample was taken to which 10g of NaCl and 20ml of 0.5 mol/L of phosphate buffer was added and shaken. The solution was left to stand to allow for removal of the aqueous layer. The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was dried at 40_0C from which 2ml were added to a 3:1 mixture of pesticides. toluene/acetonitrile.

Optimization of HPLC methods from Standard Methods

During HPLC method optimization, a systematic study on effect of various factors was performed by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and chromatographic conditions like stationary and mobile phase. The following studies were conducted for this purpose.

3.1. Detection Wavelength: The proper wavelength was needed to determine maximum detector response. The first step was to run a UV-VIS spectrum (from 190-320 nm) using an HPLC system equipped with the Photo Diode Array Detector.

3.2. Choice of Stationary Phase: In general, develop all methods with HPLC columns from the same vendor. The preferred brand of HPLC column should be selected primarily based on the long term stability and lot-to-lot reproducibility. Preliminary development trials have performed with octadecyl columns form different manufacturers with different configurations.

3.3. Selection of the Mobile Phase: Liquid chromatography method development began with the optimizing mobile phase composition and column type. The feasibility of several mixtures of solvent such as acetonitrile, water and methanol using different buffers such as ammonium acetate, ammonium formate, acetic acid and formic acid with variable pH range 3–6 was tested for complete chromatographic resolution.
In order to get sharp peak and base line separation of the components, a number of experiments were carried out by varying the composition of various solvents and its flow

rate. Under isocratic conditions, mixtures of solvents like methanol, water and Acetonitrile with and without different buffers indifferent combinations were tested as mobile phase on a C18 stationary phase.

3.4. Flow Rate

Flow rate of the mobile phase was changed from 0.5 – 1.5 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents.

3.5 HPLC Conditions Optimization for Analysis of CHLORPYRIFOS (38-41)

For analysis of Chlorpyrifos in tissue samples, HPLC with UV-detector set at 230 nm was used, with low sensitivity and specificity. So, HPLC with UV detector is used to analysis of Chlorpyrifos. In this study C18 reversed phase thermo column was employed at Ambient temperature Acetonitrile and 1Mm Na_2HPO_4 buffer (85:15) pH (4.5) as the mobile phase. The isocratic elution under the condition employed allows the separation of Chlorpyrifos. Good separation and peak shape was obtained at flow rate of 1 ml/min.

S.No	Condition	Parameter
1	Mobile Phase	Acetonitrile and 1Mm Na_2HPO_4 buffer (85:15)
2	Column	C18, 100 mm×4.6 µm
3	Wave length	230 nm
4	Flow rate	1 Ml/Min
5	Column temperature	Ambient
6	Run time	10 min
7	Sample volume	20 µL
8	P^H	4.5

TABLE. 6.1 Chromatographic conditions of Chlorpyrifos

3.6 HPLC Conditions Optimization for Analysis of CYPERMETHRIN (42-43)

For analysis of cypermethrin in tissue samples, HPL with uv-detector set at 254 nm was used, but has low sensitivity and specificity. So, HPLC with U.V detector is used to analysis of cypermethrin. in this study c18 reversed phase thermo column was employed at 27c temperature, Cyclo Hexane and acetone 95:5 v/v Ph 6.8 as the mobile phase. The isocratic elution under the condition employed allows the separation of cypermethrin. Good separation and peak shape was obtained at flow rate of 1ml/ min.

S.No	Condition	Parameter
1	Mobile Phase	Acetone and Cyclo hexane 5:95
2	Column	C18, 250 mm×4.6 µm
3	Wave length	254 nm
4	Flow rate	1 Ml/Min
5	Column temperature	27 c
6	Run time	10 min
7	Sample volume	20 µL
	P^H	6.8

TABLE. 6.2 Chromatographic conditions of CYPERMETHRIN

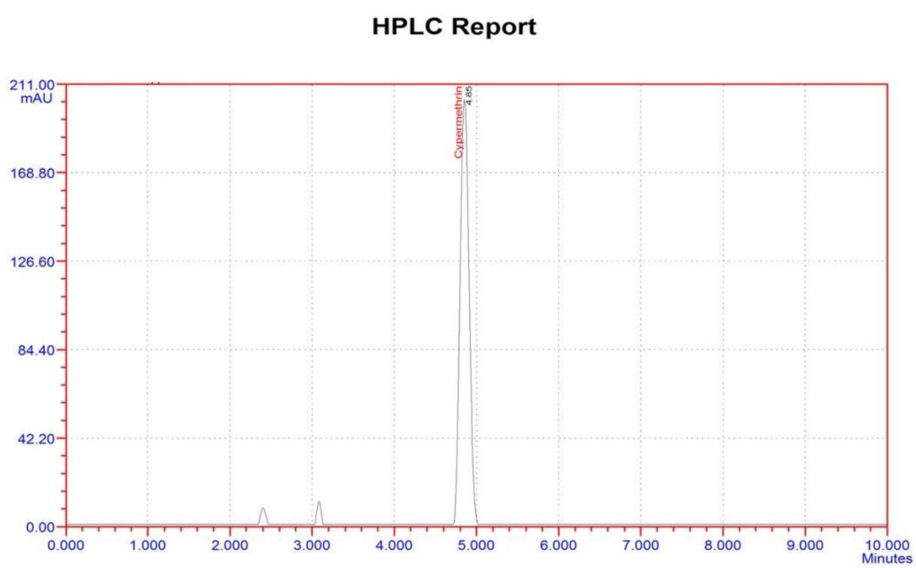

Figure: 6.C HPLC CHROMATOGRAM FOR CYPERMETHRIN

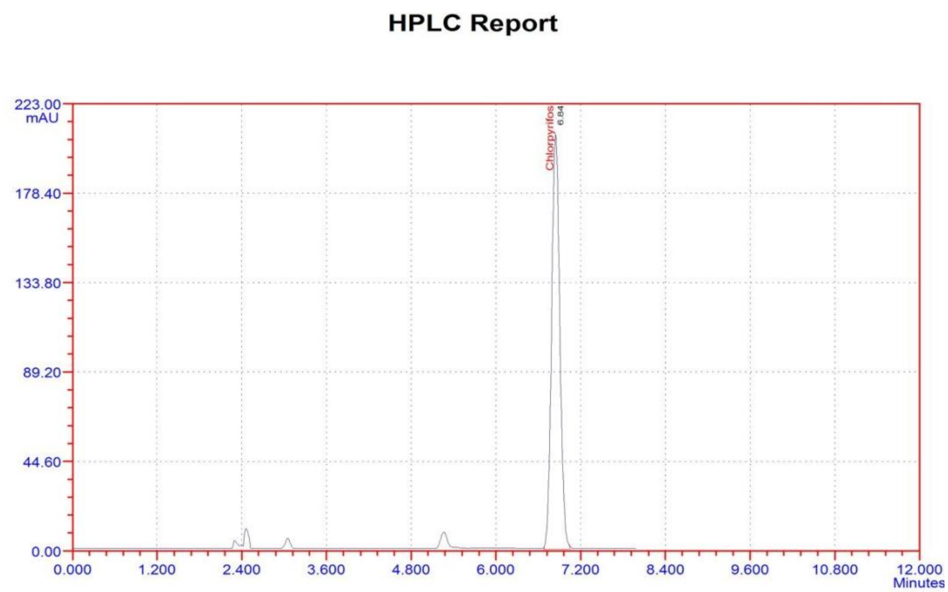

Figure: 6.D HPLC CHROMATOGRAM FOR CHLORPYRIFOS

RESULTS

1. Calibration curve with standard

From the stock solutions different concentrations chlropyrifos (2ppm-12ppm) cypermethrin (0.5ppm-3ppm) of standard pesticide solutions are injected in to HPLC at system suitable condition what are optimized from standard procedures. The calibration curves are plotted between area of peak and pesticide concentrations.

S.NO	Standard concentration (ppm)	Peak Area
1	2	1782
2	4	3416
3	6	5034
4	8	6598
5	10	8362
6	12	9836

7	Slope = 1645.55	Intercept = 156.1667

Table.6.3 Calibration Table for chlropyrifos

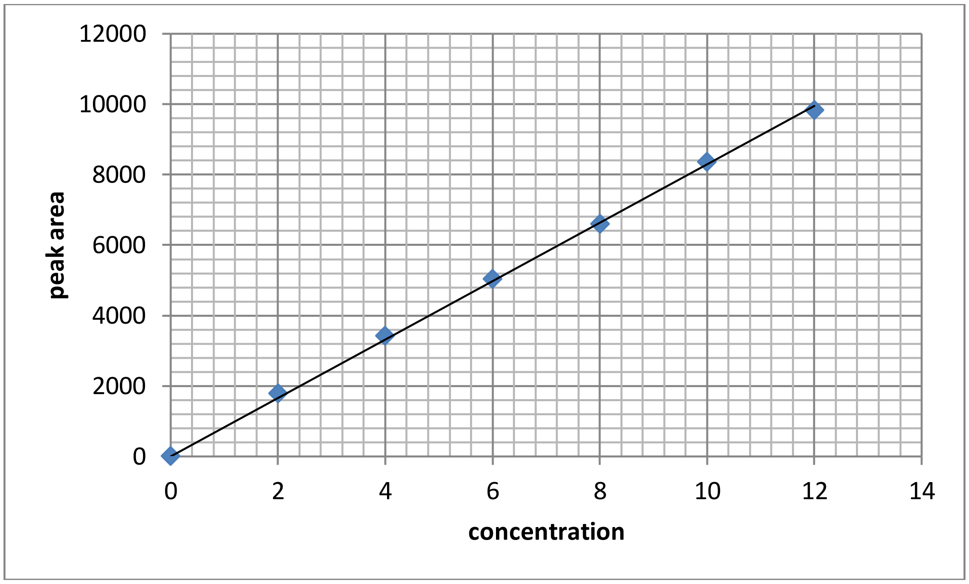

Fig 6.E Calibration curve chlropyrifos

S.NO	% OF RECOVERY	Fixed conc in ppm1	Spiked conc in ppm1	Total sample concentration	Amount of recovery	% of recovery	% of Average recovery
1	50%	4	2	6	5.98	99.66	99.40
2	100%	4	4	8	7.94	99.25	
3	150%	4	6	10	9.93	99.3	

Table 6.4 Recovery studies of chlropyrifos

S.NO	Parameter	Concentration in ppm
1	L.O.Q	1.5
2	L.O.D	0.8

Table.6.5 L.O.Q and L.O.D studies of chlropyrifos

S.NO	Standard concentration (ppm)	Peak Area
1	0.5	1173
2	1	2246
3	1.5	3297
4	2	4381
5	2.5	5502
6	3	6585
8	Slope = 2183.57	Intercept = 78

Table.6.6Calibration Table for Cypermethrin

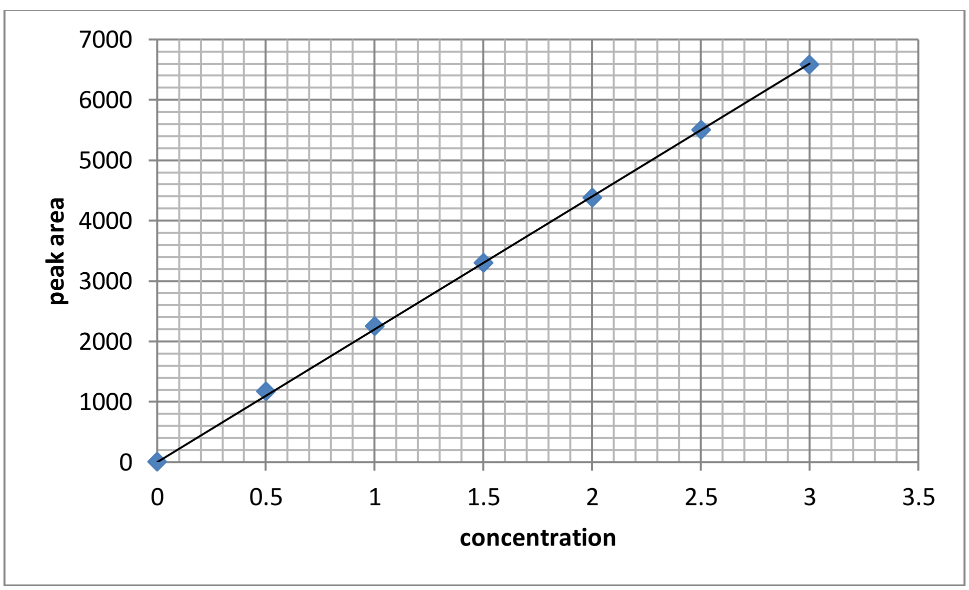

Figure 6.F Calibration curve Cypermethrin

S.NO	% OF RECOVERY	Fixed conc in ppm1	Spiked conc in ppm1	Total sample concetration	Amount of recovery	% of recovery	% of Average recovery
1	50%	1	0.5	1.5	1.49	99.33	99.14
2	100%	1	1	2	1.97	98.5	
3	150%	1	1.5	2.5	2.49	99.6	

Table 6.7Recovery studies of Cypermethrin

S.NO	Parameter	Concentration in ppm
1	L.O.Q	0.03
2	L.O.D	0.01

Table.6.8 L.O.Q and L.O.D studies of Cypermethrin

S.NO	Vegitable	Sample collection place	Concentration of chlorpyrifos in mg/kg	Cypermethrin in mg/kg
1	*Cabbage*	Guntur	5.63 ± 0.67	2.66 ± 0.18
2	*Cabbage*	Tenali	4.58 ± 0.35	7.53 ± 0.37
3	*Cabbage*	Vijayawad	6.37 ± 0.76	3.85 ± 0.29
4	*Cabbage*	Vizag	0.85 ± 0.95	3.02 ± 0.34
5	*Cabbage*	Hyderabad	3.26 ± 0.54	4.21 ± 0.12
6	*Cabbage*	Ongole	9.84 ± 0.31	3.86 ± 0.29
7	*Cabbage*	Rajamundry	6.37 ± 0.05	3.74 ± 0.34

Table No.6 9 Concentration of Chlorpyrifos and Cypermethrin in Cabbage

S.NO	Vegitable	Sample collection place	Concentration of chlorpyrifos in mg/kg	Cypermethrin in mg/kg
1	**cauliflower**	Guntur	10.52 ± 0.35	2.55± 0.52
2	**cauliflower**	Tenali	13.67± 0.46	6.37 ± 0.36
3	**cauliflower**	Vijayawad	18.25± 0.22	5.38 ± 0.44
4	**cauliflower**	Vizag	17.62± 0.14	10.4 ± 0.37

5	**cauliflower**	Hyderabad	14.25± 0.38	7.32 ± 0.69
6	**cauliflower**	Ongole	12.57 ± 0.47	6.41 ± 0.34
7	**cauliflower**	Rajamundry	13.58± 0.55	8.25 ± 0.12

Table No. 6.10 Concentration of Chlorpyrifos and Cypermethrin in cauliflower

S.NO	**Vegitable**	**Sample collection place**	**Concentration of chlorpyrifos in mg/kg**	**Cypermethrin in mg/kg**
1	**Capsicum**	Guntur	4.27 ± 0.38	2.44± 0.02
2	**Capsicum**	Tenali	5.36± 0.92	6.37 ± 0.35
3	**capsicum**	Vijayawad	4.71± 0.87	3.68 ± 0.63
4	**Capsicum**	Vizag	3.58± 0.34	1.85 ± 0.61
5	**Capsicum**	Hyderabad	2.64± 0.95	2.64 ± 0.05
6	**Capsicum**	Ongole	5.02 ± 0.32	3.33 ± 0.24
7	**capsicum**	Rajamundry	5.64± 0.15	5.29 ± 0.53

Table No. 6.11 Concentration of Chlorpyrifos and Cypermethrin mg/kg

DISCUSSION

For analysis of Chlorpyrifos in vegetables we developed a HPLC method with UV-detector at 230 nm, with C18 column, at Ambient temperature Acetonitrile and 1Mm Na_2HPO_4 buffer (85:15) pH (4.5) as the mobile phase. at flow rate of 1 ml/min. This method was showed maximum recovery i.e 99.40 %. The Limit of detection is 0.8 ppm and Limit of Quantification is 1.5 ppm The cypermethrin HPLC method conditions are uv-detector wavelength 254 nm, Column C18, at 27c temperature, Cyclo Hexane and acetone 95:5 v/v P^H 6.8 as the mobile phase, flow rate 1ml/ min. This method was showed maximum recovery i.e 99.40 %. The Limit of detection is 0.01 ppm and Limit of Quantification is 0.03 ppm

WHO and environment scientist are telling about the effect of pesticides on human health. From green revolution Indian formers are using pesticides very rapidly to control pest in all crops like paddy, Wheat, vegetables. The major way of pesticides to entering in to humans is food ezpecillay vegitables. We are analyzed few vegetable sample in AndraPradesh vegetables market. We found Chlorpyrifos and Cypermethrin in three vegetables such as *Cabbage,* cauliflower, Capsicum. In all places we found higher concentrations of target pesticides in Cauliflower. i.e 12.57 – 18.25 mg/kg, 2.55-10.54 mg/kg chlorpyrifos, Cypermethrin respectively. In Capsicum we found 2.64 – 5.64 mg /kg 1.85 – 6.37 mg/kg chlorpyrifos, Cypermethrin respectively. In cabbage we found 0.85-9.84 mg/kg, 2.66-7.53 mg /kg chlorpyrifos, Cypermethrin respectively. We confirmed the presence of target pesticides in these three vegetables.

Due to presences of pesticides the flowing health problems will arise in humans.

1. Asthma
2. Birth Defects
3. Neurological Effects
4. Cancer

We are encouraging our local formers about organic and eco pesticides to control the pest. In international market pesticide free vegetables and food items has very good value. Due to use of eco pesticides environment will protect from chemical pollution and maximum number of disease will prevent by this precaution.

REFERENCE

1. Muller, Franz, ed. (2000). Agrochemicals: Composition, Production, Toxicology, Applications. Toronto: Wiley-VCH. p. 541. ISBN 3-527-29852-5.
2. Lide, David R. (1998). Handbook of Chemistry and Physics Boca Raton, FL: CRC Press. pp. 3–126. .
3. Sangster J; LOGKOW Databank. Sangster Res. Lab., Montreal Quebec, Canada (1994)
4. Common Insecticide May Harm Boys' Brains More Than Girls". Scientific American. August 21, 2012.
5. "Reregistration Eligibility Decision for Chlorpyrifos" 31 July 2006.
6. "NASS Agricultural Chemical Database". Pestmanagement.info. Retrieved November 20, 2011.
7. http://www.cdms.net/ldat/ld0AT004.pdf
8. http://www.farmoz.com.au/label/farmoz/STRIKE_OUT_500WP_16103844.pdf
9. EPA administrator's announcement, June 9, 2000
10. EPA's Chlorpyrifos Page (archived on archive.org)
11. "Bhopal.net". Bhopal.net. June 8, 2000. Retrieved November 20, 2011.
12. "Dow AgroSiences agrees to pay $2M to state over pesticide ads". The Business Review. 15 December 2003.
13. Press Release: Lawsuit Challenges EPA on Deadly Pesticide, EarthJustice, July 31, 2007.
14. CBI raids Dow Chemical's Indian subsidiary for graft. Monsters and Critics, August 21, 2007.

15. "Federal Government Announces Plan to Protect Salmon from Pesticides". Earthjustice. Retrieved 2009-09-02.
16. "Chlorpyrifos". Pmep.cce.cornell.edu. Retrieved November 20, 2011.
17. "Toxin 'likely' cause of Sarah Carter's death". stuff.co.nz. Retrieved May 8, 2011.
18. "Sarah Carter's likely cause of death – insecticide". 3 News.
19. "Thailand death cover-up suspected". NZ Herald Online. May 9, 2011.
20. Hayden Donnell (May 11, 2011). "Thai experts: Bed bug spray didn't kill Kiwi tourist".The New Zealand Herald. Retrieved May 11, 2011.
21. "Thais deny tourists' deaths linked". Dominion Post. May 26, 2011. Retrieved May 26, 2011.
22. Rauh VA, et al. (2006). "Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life Among Inner-City Children". Pediatrics118 (6).
23. "Whyatt RM, et al., Environmental Health Perspectives, 2004, 112, 1125–32". Ehponline.org. Retrieved November 20, 2011.
24. "Lung Cancer in the Agricultural Health Study (IA)"http://aghealth.nci.nih.gov/pdfs/IALungCancer2006.pdf
25. "Access". Medscape. Retrieved November 20, 2011.
26. Rauh, V.; Arunajadai, S.; Horton, M.; Perera, F.; Hoepner, L.; Barr, D. B.; Whyatt, R. (2011). "7-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide". Environmental Health Perspectives.
27. R. Baselt, Disposition of Toxic Drugs and Chemicals in Man, 8th edition, Biomedical Publications, Foster City, CA, 2008, pp. 389-391.
28. Hu, JX; Li, YF; Li, J; Pan, C; He, Z; Dong, HY; Xu, LC (2011). "Toxic effects of cypermethrin on the male reproductive system: With emphasis on the androgen receptor". Journal of applied toxicology : JAT
29. ^ Singh, AK; Tiwari, MN; Upadhyay, G; Patel, DK; Singh, D; Prakash, O; Singh, MP (2012). "Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: Postnatal exposure enhances the susceptibility during adulthood". Neurobiology of Aging 33 (2): 404–15.
30. Amer, S.M., et al (1993). "Induction of chromosomal aberrations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin". Journal of Applied Toxicology 13:341-345.
31. Amer, S.M. and E.I. Aboulela (1985). "Cytogenetic effects of pesticides. III. Induction of micronuclei in mouse bone marrow by the insecticides cypermethrin and rotenone". Journal of Mutation Research 155:135- 142.
32. Tateno, C. et al (1993). "Effects of pyrethroid insecticides on gap junctional intecellular communications in Balb/c3T3 cells by dye-transfer assay.". Cell biology and toxicology journal 9:215-222.

33. Wright, C.G., R.B. Leidy, and H.E. Dupree, Jr. (1993). "Cypermethrin in the ambient air and on surfaces of rooms treated for cockroaches.". Bulletin of Environmental Contamination and Toxicology 51:356-360.
34. Pascual, J.A. and S.J. Peris. (1992). "Effects of forest spraying with two application rates of cypermethrin on food supply and on breeding success of the blue tit (Parus caeruleus)".Environmental Toxicology and Chemistry 11:1271-1280.
35. Stephenson, R.R. (1982). "Aquatic toxicology of cypermethrin. I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests.". Aquatic Toxicology 11:1271-1280.
36. Martinez-Cabrillo, J.L., et al. (1991). "Responses of populations of the tobacco budworm (Lepidopterea: Noctuidae) from northwest Mexico to pyrethroids.". Journal of Economic Entomology 11:1271-1280.
37. Determination of Pesticides in Fruits and Vegetables using Acetonitrile Extraction and GC/MS Technique, Zaib Hussain , Samia Siddique, Journal of Scientific Research, Vol. XXXX No. 2, December, 2010, 19-29
38. .Journal of Liquid Chromatography & Related Technologies , 29: 339–348, 2006, A Simple HPLC Method for the Determination of Chlorpyrifos pesticides , Richard E. Mauldin, Thomas M. Primus, Theresa A. Buettgenbach, and John J. Johnston
39. Analysis of chlorpyrifos and TCP residues in agricultural soil and apples by HPLC, Qingming Zhang 1, 2, Lusheng Zhu 1*, Chang Han 1, Jun Wang 1, Hui Xie 1, Jinhua Wang 1 and Shujuan Sun Received 14 November 2010, accepted 20 January 2011, Journal of Food, Agriculture & Environment Vol.9 (1): 659-662. 2011
40. J Anal Toxicol. 2001 May-Jun;25(4):275-9. Simultaneous determination of chlorpyrifos, permethrin, and their metabolites in rat plasma and urine by high-performance liquid chromatography. Abu-Qare AW, Abou-Donia MB.Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA.
41. Determination of cypermethrin residues in gingkgo biloba leaves by high performance liquid chromatography Jin-Jun Zhang1, Li-Guo Zang1, Jin-E Zhang1, Guan-Wei Cui1, Bo Tang1, Xiang-Yang Li 2 and Li Zhou, Bull. chem. soc. ethiop. 2009, 23(1), 97-10,
42. Epub 2012 Apr 12.A pretreatment method for HPLC analysis of cypermethrin in microbial degradation systems. Liu S, Yao K, Jia D, Zhao N, Lai W, Yuan H. J Chromatogr Sci. 2012 Jul, 50(6):469-76
43. Determination of cypermethrin content in oscillating pressure treated rubberwoodsalamah Selamat, Wan Asma Ibrahim, Habibah Mohamad, Journal of Tropical Forest Sciences 5 (3): 342-352