STUDIES OF PURITY OF SOME PESTICIDES APPLIED IN VEGETABLES AND FISHES

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To

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Abstract

Title: Studies of purity of some pesticides applied in vegetables and fishes

Human health is under threat due to the exposure to toxic chemicals used either in agricultural or industrial sectors. The use of toxic chemicals both in agriculture and aquaculture gradually increasing due to increasing food demand. Vegetables and fishes are important food stuff for local consumption as well as to earn foreign currency. The presence of residual amounts of these chemicals above the maximum residue limit (MRL) in food products is hazardous for local consumption as well as for export.

Vegetables are being consumed by the local people of Bangladesh almost every day. Pesticides are being used to protect the crops and there is no guide line about the safe harvesting period of the crops and MRL values for any pesticides in Bangladesh. Purity of selected commercial pesticides is found 87.01% which was applied in experimental field. Calibration curves of certified standard and commercial cypermethrin were constructed. The curves followed linear relationship with good correlation coefficients (R^2>0.95). Studies of dissipation pattern of pesticides in growing crops is necessary which will give a safe harvesting period as well as MRL value after final application. Dissipation patterns of cypermethrin on bean and cauliflower at recommended dose were studied after the applications in the respective experimental fields. Recoveries were found to be 105.95-106.89%, 75.71-82.54% for bean and cauliflower respectively. Amount of cypermethrin in edible part of bean and cauliflower was found to be below MRL (0.5 ppm) and (1.0ppm) on 10 and 7 days after application at the recommended dose respectively.

Monitoring system of POPs including DDTs in Bangladesh is very poor and hardly any expertise is available in the country. Our preliminary studies DDTs were found very poor amount in different kind of experimental fish samples like Gulsha and Taki fish. Due to bioaccumulation and biomagnification it is found in detected level, though it is far away of MRL (5.0 ppm for total DDT in fish) of Maximum Residue Limit (MRL) suggested by FAO/WHO (Codex, 1993). The calibration curves were linear over the range of the tested concentrations as shown by the fact that the correlation coefficients (r^2) for the linearity range were 0.995-0.999. The detection limit was found to be 5ppb for DDTs and the quantification limit was 6.5ppb. Recoveries were found (70-130%); this is acceptable for fish samples according to standard methodology. Taki fish was found to contain detectable amount of residual targeted pesticides DDTs (8.415 ppb in digestive tract, 4.037 ppb in gill, 56.442 ppb in gonad and 23.146 ppb in muscle) and (64.212 ppb in digestive tract, 75.234 ppb in gill, 119.819 ppb in gonad and 45.839 ppb in muscle) for Gulsha fish. Gonad is the main target of DDT and its metabolites. Because highest amount of DDT found in gonad 56.442 ppb, 119.819 ppb for taki and gulsha respectively. No detectable amount of residues of DDTs was found in liver of both experimental fishes.

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Chapter 1. Introduction

1.1 Background

Bangladesh is a small and developing country overloaded with almost unbearable pressure of human population. In the past, people of Bangladesh were mostly dependent upon land-based proteins. But, the continuous process of industrialization and urbanization consumes the limited land area (wiki. 2013). Large number of different types of water bodies both inland and marine makes Bangladesh one of the most suitable countries of the world for freshwater aquaculture. The freshwater inland aquaculture production in Bangladesh is the second highest in the world after China (FAO, 2009).

There are 260 freshwater and 475 marine fish species in the country. About 12 exotic species are being cultured in the country. The total annual fish production is estimated at 30.62 lakh Metric Ton in 2010-11 (Bangladesh fiscal year: 1 July-30 June), of which 14.61 Metric Ton (48%) are obtained from inland aquaculture, 10.54 Metric Ton (34%) from inland capture fisheries, and 5.46 Metric Ton (18%) from marine fisheries (DoF, 2011).

Fisheries sector contributes 4.43% to GDP and 22.21% to agricultural GDP and 2.73% to foreign exchange earnings by exporting fish products in 2010-11. Fish supplements to about 60% of our daily animal protein intake. About 10% of the population dependents directly and indirectly on the fisheries for their livelihood. Average annual growth rate of fish production in last 3 years is 6.11% (DoF, 2011).

In the ever-growing trend of environmental-concerned society, it is apparent that many countries are starting to apply strict environmental regulations in almost every aspect associated with human life (Sunarso and Ismadji, 2009). The increasing worldwide need for food demands a higher agricultural productivity, which can only be achieved by an extensive use of pesticides. Unfortunately pesticides contaminate the environment through intensive or inappropriate use. Although organochlorine insecticides like DDT and its metabolites, lindane, aldrin or dieldrin for instance have been banned years ago in many countries based on their mutagenic, carcinogenic and endrocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties (Lesueur et al., 2008). The degree of hazards depends on the amount of pesticides on crops and their toxicity. Since most of the pesticides are toxic in nature, their continuous intake by human even in trace amounts, can result in accumulation in body tissues with serious adverse effects on health (Handa *et al.,* 1999).

Pesticide use in crop production has been suspected of being a major contribution to environmental pollution. There are widespread and growing concerns of pesticide over-use, relating to a number of dimensions such as contamination of ground water, surface water, soils and food, and the consequent impacts on wildlife and human health (McLaughlin and Mineau, 1996). Farmers often spray hazardous insecticides like organophosphates and organochlorine up to five to six times in one cropping season while only two applications may be sufficient. The usual practice of draining paddy water into irrigation canals may cause river and lake contamination. Residues carried by the water can be taken up by non-target flora and fauna, leach into soil, and possibly contaminate groundwater or potable water. A greater problem lies in the bioaccumulation of pesticides in beneficial organisms like fish. Residues in food pose to consumers if the maximum residue limit set by Food and Agriculture Organization (FAO) and World Health Organization (WHO) is exceeded (Pingali and Roger, 1995). For evaluating safety to concern about human health and environment pesticide application must be come in regulations. So governments and international organizations have compiled and published a list of pesticides, which includes their tolerances and maximum residue limits (MRLs) (Codex Alimentarius Commission, 2010; KHIDI, 2011).

The primary regulatory standard employed to control pesticides residues in food is the maximum residue limit or MRL. The MRL has been defined as "the maximum concentration of pesticide residue that is legally permitted or recognized as acceptable on a food, agricultural commodity, or animal feed" (Holland, 1996). The MRL is intended primarily as a check that use of pesticide is occurring according to authorized labels and GAP. Detection of residues at or below the MRL implies that label directions and GAP have been properly followed. MRLs are not set on the basis of toxicology data, but once proposed based on GAP they must be evaluated for safety. This generally accomplished through a risk assessment process that compares dietary intakes estimated from expected residues concentrations in foods consumed with the relevant health-related regulatory endpoints, the acceptable daily intake (ADI) and the acute reference dose (ARfD) (Solecki et al., 2005).

Every pesticide has a Pre Harvest Interval (PHI) for residues to dissipate below the MRL established for that crop. Pre Harvest Interval (PHI) means difference between the date of final pesticides application and harvest. Food products become only safe after pre-harvest interval and it differs from pesticide to pesticide and crop to crop. Due to lack of education, farmers of our country do not follow the prescribed dosage and use pesticides at any stage of crop and harvest without following the time of PHI (Handa *et al.,* 1999). That is the main concerns over the possibility of excessive

residues on crops sold in local markets. So it has to need to determine the residues on fruits and vegetables.

1.2 Pesticide and Pest

A pesticide is any substance or mixture of substances intended to control, prevent, destroy, repel or attract or mitigate any pest in order to minimize their detrimental effects. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests (EPA, 2007). Any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant is also known as a pesticide. It may be a chemical substance, biological agent, antimicrobial, disinfectant or device used against any pest. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals.

Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals, which adversely affect our way of life. Pests can reduce the quantity and quality of produced food by lowering production and they destroy stored food. They compete with humans for food and affect health and the way of life. They are also cause of major cause of land degradation. Their activity greatly increases the cost of farming (Miller, 2004).

Pesticides can be derived from plants (e.g. pyrethrin, neem or azadirachta from neem seed oil) or they can be chemically manufactured (e.g. DDT, 2,4-D, Acephate, Aclonifen, cyprodilin etc.). Natural products or biological agents (e.g. pathogens, pheromones) and genetically engineered pesticides such as toxin-producing *Bacillus thuringiensis* are also used (Creanshaw, 2010).

Chemical insecticides are usually contact, stomach or fumigant poisons. Contact poisons may have immediate or delayed effects after physical contact with a pest. Fumigants, which may initially have the form of a solid, liquid or gas, but they kills pests in a gaseous state (Cornell Univ. Pest. Safe. Edu. Prog. 2007).

1.2.1. A Short History of Pesticide

At the ancient time the farmed crops suffered from pests and diseases causing a large loss in yield with consequence of famine for the population. Still today even though advances in agricultural sciences but losses due to pests and diseases range from 10-90%, with an average of 35 to 40%, for all potential food and fiber crops (Peshin, 2002).

So it was a great motivation to find the ways of overcoming the problems caused by pests and diseases. The first recorded insecticides were sulpher compounds to control insects and mites that were used by Sumerians about 4500 years ago. About 3200 years ago the Chinese were using mercury and arsenical compounds for controlling body lice (History of Pesticides, 2008). Copper sulfate and hydrated lime called Bordeaux mixture proved to be an effective fungicide and became widely used between 1860 and 1942. Pyrethrum derived from the dried flowers of *Chrysanthemum cinerariaefolium* "Pyrethrum daisies", has been used as an insecticide for over 2000 years (Pyrethrum.html., 2012). Nitrophenols, chlorophenols, creosote, naphthalene and petroleum oils were used for fungal and insect pests. Ammonium sulphate and sodium arsenate were used as herbicides.

The growth in synthetic pesticides such as DDT, BHC, aldrin, dieldrin, endrin, chlordane, parathion, captan and 2,4-D accelerated in the 1940s. These products were effective and inexpensive and DDT was the most popular, because of its broad-spectrum activity (History of Pesticides, 2008; Delaplane, 2000). Many persons predicted pesticides coupled with high-yield plant types, chemical fertilizers, irrigation technology, and mechanization would be a "Green Revolution" that would create an abundance of food for the world. The publication of *Silent Spring* by Rachel Carson in 1962 describes how DDT can enter the food chain, accumulate in the fatty tissue of all animals, including humans, and cause cancer and genetic damage. She concluded that DDT and other pesticides had irreversibly harmed birds and animals and negatively affected the world's food supply.

In the Stockholm convention on May 23, 2001 12 hazardous POPs (Aldrien, Chlordane, Dieldrin, DDT, Endrin, Heptachlor, Mirex, Toxaphane, Hexachlorobenzene, PCBs, Dioxins and Furans) were identified which are commonly known as 'Dirty Dozen'. The aim of the convention is to protect **H**uman **H**ealth and **E**nvironment by phasing out of these hazardous pollutants from the environment. These chemicals are of particular concern due to their four intrinsic characteristics, namely, wide spectrum persistency, bioaccumulation, transportability and toxicity.

1.2.2 Organochlorine pesticides

Organochlorine pesticides are insecticides composed primarily of carbon, hydrogen, and chlorine. They break down slowly and can remain in the environment long after application and in organisms long after exposure. DDT (Dichloro diphenyl trichloroethane) is one of the most notorious organochlorine pesticides. Promoted as a "cure all" insecticide in the 1940s, DDT was widely used in agricultural production around the world for many years. It was also the chemical of choice for mosquito control; until the 1960s, trucks sprayed DDT in

neighborhoods across the U.S. DDT was also the primary weapon in the global "war against malaria" during this period, and continues to be used for malaria control in a handful of countries. (chemicalbodyburden, 2013)

1.2.3 Green pesticides

Green pesticides, also called **ecological pesticides**, are pesticides derived from organic sources (Wikipedia, 2013b). Green pesticides refer to all types of nature-oriented and beneficial pest control materials that contribute to reduce the pest population and increase food production. They are safe and eco-friendly. They are more compatible with the environment components than synthetic pesticides. Eminent scientists have highlighted the importance of green pesticides (Ignacimuthu and Jayaraj, 2005).

1.2.4 Modern pesticides

In modern times, some sophisticated compounds, which are very carefully researched to ensure their effectiveness against target organisms, are safe to the environment and can be used without hazards to the operators or consumers are used as pesticides. These pesticides are called modern pesticides. Some of the modern pesticides are diazinon, malathion, cypermethrin, chlorpyrifos, acephate, fenitrothion, quinalphos and fenvalerate etc.

These pesticides belong to different classes of chemical action. Synthetic compounds predominate among them, especially derivatives of phosphoric, phosphorothioic acids. Pyrethroid pesticides are also modern pesticides. Basically the representatives of the same class are characterized by common specific properties and a single mechanism of their action on an organism (Ghosh, 1998). Organophosphate pesticides have increased in use, because they are less damaging to the environment and they are less persistent than organochlorine pesticides (Jaga and Dharmani, 2003). Many of these have been developed to target specific biochemical reactions within the target organisms, e.g. an enzyme necessary for photosynthesis within a plant or a hormone required for normal development in an insect. Modern chemicals are much safer, more specific and friendlier to environment than the older products they have replaced.

1.2.5 Continuing development and Alternatives

New pesticides are being developed, including biological and botanical derivatives and alternatives that are thought to reduce health and environmental risks. A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison is termed as a **Bio-**

pesticide. Bio-pesticides are more likely to be bio-degradable and they specifically interfere with the adsorption of food from the guts of some insects but are harmless to mammals (Rahman et al., 1995). Biological pesticides based on entomopathogenic fungi, bacteria and viruses cause disease in the pest species can also be used (Miller, 2004).

Integrated Pest Management (IPM), the use of multiple approaches to control pests, is becoming widespread and has been used with success in countries such as Indonesia, China, Bangladesh, the US, Australia, and Mexico (Miller, 2004).. IPM attempts to recognize the more widespread impacts of an action on an ecosystem, so that natural balances are not upset (Daly et al., 1998).

In addition, applicators are being encouraged to consider alternative controls and adopt methods that reduce the use of chemical pesticides. Alternatives to pesticides are available and include methods of cultivation such as polyculture (growing multiple types of plants), crop rotation, planting crops in areas where the pests that damage them do not live, time of planting according to when pests will be least problematic, and use of trap crops that attract pests away from the real crop and use of biological controls, such as pheromones and microbial pesticides, and genetic engineering, and methods of interfering with insect breeding. Interfering with insects' reproduction can be accomplished by sterilizing males of the target species and releasing them, so that they mate with females but do not produce offspring (Miller, 2004). This technique was first used on the screwworm fly in 1958 and has since been used with the medfly, the tsetse fly, and the gypsy moth (SP-401 Skylab, 2007). However, this can be a costly, time consuming approach that only works on some types of insects (Miller, 2004).These methods are becoming increasingly popular and often are safer than traditional chemical pesticides.

1.2.6 Types of Pesticides

There are multiple ways of classifying pesticides

According to chemical composition:

- **Inorganic compounds:** mercury, fluorine, barium, sulphur, copper, chlorates and borates.
- **Organic compounds:** organochlorine, organophorous, derivatives of carbonic, thio and di-thio acid, carbamides etc.
- **Synthetic and biological pesticides (biopesticides):** pyrethrines, bacterial and fungal preparations, antibiotics and phytocides.

According to function of different type of pest control:

- **Algicides** or **algaecides** for the control of algae
- **Avicides** for the control of birds
- **Bactericides** for the control of bacteria
- **Fungicides** for the control of fungi and oomycetes
- **Herbicides** (e.g. glyphosate) for the control of weeds
- **Insecticides** (e.g. organochlorines, organophosphates, carbamates, and pyrethroids) for the control of insects - these can be ovicides (substances that kill eggs), larvicides (substances that kill larvae) or adulticides (substances that kill adults)
- **Miticides** or **acaricides** for the control of mites
- **Molluscicides** for the control of slugs and snails
- **Nematicides** for the control of nematodes
- **Rodenticides** for the control of rodents
- **Virucides** for the control of viruses (e.g. H5N1)

According to mode of application:

- **Anti fouling agents:** Kills or repel organisms that attach to under water surfaces such as boat bottoms.
- **Attractants:** Attracts pests (for example to lure an insect or rodent to a trap).
- **Repellants:** Repel pests including insects (such as mosquitoes) and birds.
- **Insect growth regulators:** Disrupt the molting, maturity from pupal stage to adult or other life processes of insects.
- **Plant growth regulators:** Substances (excluding fertilizers and other plant nutrients) that alter the expected growth, flowering or reproduction of plants.
- **Desiccant:** Promote drying of living tissues.
- **Defoliants:** Cause leaves or other foliage to drop from a plant, usually to facilitate harvest.

1.2.7 Formulation

Formulation is the term used to describe the physical state of a pesticide and determines how it will be applied. The effective use of pesticide to control pests, plant diseases and weeds not only depends on their toxicity but also to a considerable extent on the form of pesticide. It also improves the properties of a chemical for handling, storage, application and safety. Common formulations are:

i) The active ingredient is mixed with an oil base i.e. forming an emulsion which is diluted with water (emulsifiable concentrate; EC or E) for application,

ii) A liquid, can be mixed with water to form a suspension in a spray tank (flowable; ForL),

iii) The active ingredient is made into coarse particles with inert material like fired clay particles (granules; G),

iv) The active ingredient is combined with a fine powder look like dusts and mix with water (wettable powders; WP),

v) The active ingredient is added to an edible or attractive substance and are often used to control slugs, snails, ground-dwelling insects, and rodents (baits; B) and

vi) an active ingredient in powder form is dissolved in water (Soluble powders; SP). (Cress, 1990)

1.2.8 Importance of pesticide use

Pesticides can save farmers' money by preventing crop losses to insects and other pests and they ensure a plentiful supply and variety of high quality, wholesome food at a reasonable price, nutritious food free from harmful organisms and blemishes (WRI, 1998-99). In the US, farmers get an estimated fourfold return on money they spend on pesticides (Kellogg et al., 2000). One study found that not using pesticides reduced crop yields by about 10% (Kuniuki, 2001). Another study, conducted in 1999, found that a ban on pesticides in the United States may result in a rise of food prices, loss of jobs, and an increase in world hunger (FAO, 1998). Pesticides are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grain. They are used to control harmful organisms. For example, they are used to kill mosquitoes that can transmit potential deadly diseases like west Nile virus, yellow fever, and malaria. They can also kill bees, wasps or ants that can cause allergic reactions. Insecticides can protect animals from illnesses that can be caused by parasites such as fleas (Purdue.edu, 2007). Pesticides can prevent sickness in humans that could be caused by mouldy food or diseased produce.

However, DDT use is not always effective, as resistance to DDT was identified in Africa as early as 1955, and by 1972 nineteen species of mosquito worldwide were resistant to DDT. A study for the World Health Organization in 2000 from Vietnam established that non-DDT malaria controls were

significantly more effective than DDT use (afronets.com 2007). The ecological effect of DDT on organisms is an example of bioaccumulation. Again, a study (October 2007) has linked breast cancer from exposure to DDT prior to puberty (sustainableproduction.org, 2007). Poisoning may also occur due to use of DDT and other chlorinated hydrocarbons by entering the human food chain when animal tissues are affected. Symptoms include nervous excitement, tremors, convulsions or death.

Each use of a pesticide carries some associated risk. Proper pesticide use decreases these associated risks to a level deemed acceptable by pesticide regulatory agencies. Uncontrolled pests such as termites and mould can damage structures such as houses (Purdue.edu, 2007)

1.2.9 Effects of pesticide use

1.2.9.1 Environmental effects

Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food (Miller, 2004). Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

Non target organisms, including predators and parasites of pests, can also be affected by chemical application. The reduction of these beneficial organisms can result in changes in the natural biological balances. Losses of honey bees and other pollinating insects can also be a problem (FAO, 1998).

1.2.9.2 Health effects

Pesticides can present danger to consumers, bystanders, or workers during manufacture, transport, or during and after use (US EPA, 2007).

Farmers and workers

There have been many studies of farmers with the goal of determining the health effects of pesticide exposure (McCauley et al. 2006). The World Health Organization and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly (Jeyaratnam, 1990).

These are associated with acute health problems for workers that handle the chemicals, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobichon, 1996). Additionally, many studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions (Arcury et al., 2003; O'Malley, 1997), cancer (Daniels et al., 1997), depression (Beseler et al., 2008), neurological deficits (Kamel et.al. 2003; Firestone et. al., 2005), miscarriages, and birth defects (Engel et al., 2000; Cordes and Foster, 1988; Das et al., 2001; Eskenazi et al. 1999; García, 2003; Moses, 1989; Schwartz et al., 1986; Stallones and Beseler, 2002; Strong et al., 2004; Van Maele-Fabry and Willems, 2003). Summaries of peer-reviewed research have examined the link between pesticide exposure and neurologic outcomes and cancer, perhaps the two most significant things resulting in organophosphate-exposed workers (Alavanja et al., 2004; Kamel and Hoppin, 2004).

Impact on Humans

Pesticides used to control pests on food crops are dangerous to people who consume those foods. The Bhopal disaster occurred when a pesticide producing plant released 40 tons of methyl isocyanate (MIC) gas, a chemical intermediate in the synthesis of some carbamate pesticides. The disaster immediately killed nearly 3,000 people and ultimately caused at least 15,000 deaths (BBC News, 1984). In China, an estimated half million people are poisoned by pesticides each year, 500 of whom die (Lawrence, 2007).

Children have been found to be especially susceptible to the harmful effects of pesticides (Noyes, 2007). A number of research studies have found higher instances of brain cancer, leukemia and birth

defects in children with early exposure to pesticides according to the Natural Resources Defense Council, U.S.A. (NRDC, 1998). Peer-reviewed studies now suggest neurotoxic effects on developing animals from organophosphate pesticides at recommended tolerable levels, including fewer nerve cells, lower birth weights, and lower cognitive scores. Some scientists think that exposure to pesticides in the uterus may have negative effects on a fetus that may manifest as problems such as growth and behavioral disorders or reduced resistance to pesticide toxicity later in life (Lorenz, 2006). Pyrethrins, insecticides commonly used in common bug killers, can cause a potentially deadly condition if breathed in (Young, 1986).

1.2.10 Toxicity

The toxicity of a pesticide is its capacity or ability to cause injury or illness and determined by subjecting test animals to varying dosages of the active ingredient (a.i.) and each of its formulated products. The active ingredient is the chemical component in the pesticide product that controls the pest. The two types of toxicity are acute and chronic. Acute toxicity of a pesticide refers to the chemical's ability to cause injury to a person or animal from a single exposure, generally of short duration and is determined by examining the dermal toxicity, inhalation toxicity, and oral toxicity of test animals. In addition, eye and skin irritation are also examined and is measured as the amount or concentration of a toxicant required to kill 50 percent of the animals in a test population. This measure is usually expressed as LD_{50} (lethal dose 50) or LC_{50} (lethal concentration 50) and it is recorded in milligrams of pesticide per kilogram of body weight (mg/kg b.w.) of the test animal or in parts per million (ppm). The chronic toxicity of a pesticide is determined by subjecting test animals to long-term exposure to the active ingredient. Any harmful effects that occur from small doses repeated over a period of time are termed as chronic effects. Some of the suspected chronic effects from exposure to certain pesticides include birth defects, production of tumors, blood disorders, and neurotoxic effects (nerve disorders).There is no term to express chronic toxicity. Pesticides are divided into four categories on the basis of their relative toxicity are given in Table 1 and also summarized values of LD_{50} and LC_{50} for each route of exposure for the four toxicity categories and their associated signal word (Rahman and Alam, 1997).

Table 1 Toxicity categories for active ingredients

	Toxicity category			
Routes of exposure	**I**	**II**	**III**	**IV**
Oral LD_{50} mg/kg	Up to and including 50 mg/kg	50–500 mg/kg	500–5,000 mg/kg	>5,000
Inhalation LC_{50}	Up to and including 0.2 mg/l	0.2–2 mg/l	2–20 mg/l	>20 mg/l
Dermal LD_{50} mg/kg	Up to and including 200 mg/kg	200–2,000 mg/kg	2,000–20,000 mg/kg	>20,000
Eye Effects irritation	Corrosive corneal Opacity not reversible; Within 7 days	Corneal opacity reversible within 7 days; irritation persisting for 7 days	No corneal opacity irritation reversible within 7 days	No
Skin Effects slight irritation	Corrosive	Severe irritation at 72hours	Moderate irritation at 72 hours	Mild or at 72 hours
Signal Word	DANGER	WARNING	CAUTION	CAUTION	POISION

1.2.11 Persistence

Persistence describes how long a pesticide remains active. Half-life is one of the terms of measure of persistence. The half-life of a substance is the time required for that substance to degrade to one-half its original concentration and it is not an absolute factor. The factors that influence the persistence of pesticides are the characteristics of the pesticide, including its over-all stability either as parent compound or metabolites, its volatility, solubility, formulation, and the method and site of application. It is also depend on environmental factors, particularly temperature, precipitation (humidity) and air movement (wind).The other factors depend on the properties of the plant or soil characteristics influencing the persistence of pesticides in plants include the plant species involved,

the nature of the harvested crop, the structure of the cuticle, the stage and rate of growth and the general condition of the plant. Corresponding soil characteristics are the soil type and structure, its organic matter content, clay content, acidity or alkalinity, mineral ion content and degree of aggregation and its microbial population. Of these factors, the most important seem to be related to the chemical stability and physical characteristics of the pesticide; its stability exerting the greatest influence, otherwise volatility being more important in soil and solubility in plants. In general, the longer a pesticide persists in the environment, the more likely it is to move from one place to another and be a potential source of pollution (Rahman and Alam, 1997; Aziz, 2005).

1.2.12 Uses of pesticides in Bangladesh

Bangladesh is predominantly an agricultural country with over population and agriculture plays an important role in the lives of its people. The use of pesticides, in Bangladesh started during the middle of the 1950s to promote crop production (Rahman and Alam, 1997). Major crops of the country are rice, wheat, pulses, jute, oilseed, vegetables, potatoes, sugarcane, cotton and tea of which rice accounts for 80% of the total cultivated area. The warm and humid climatic conditions of the country increased modern high yielding varieties of crops and more use of chemical fertilizers are highly favorable for development and multiplication of pests and diseases. The estimated loss in yields due to attacks from pest and diseases annually ranges from 15 to 25 percent (Aziz, 2005). Usually farmers get information about pesticides from traders. More than 47% of farmers in Bangladesh use more pesticides than needed to protect their crops, according to a recent survey of 820 boro (a variety of rice), potato, bean, eggplant, cabbage, sugarcane and mango growers (Rahman, 2002).

1.3 Cypermethrin, A Synthetic pyrethroid

Cypermethrin is a synthetic compound primarily used as an insecticide. It acts as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. It is a synthetic pyrethroid (Abou-awad and El-banhawy, 1985).

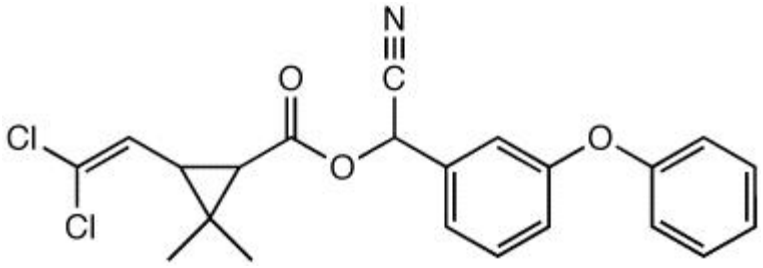


Figure 1: Structure of cypermethrin.

Cypermethrin was initially synthesized in 1974 and first marketed in 1977 as a highly active synthetic pyrethroid insecticide, effective against a wide range of pests in agriculture, public health, and animal husbandry. In agriculture, its main use is against foliage pests and certain surface soil pests, such as cutworms, but because of its rapid breakdown in soil, it is not recommended for use against soil-borne pests below the surface (Ahmed et al., 1985).

1.3.1 Physical properties of cypermethrin

Most technical grades of cypermethrin contain more than 90% of the active material. The material varies in physical form from a brown-yellow viscous liquid to a semi-solid (Tomlia, 14th ed.). Cypermethrin has a very low vapour pressure and solubility in water, but it is highly soluble in a wide range of organic solvents. Cypermethrin is highly stable to light and at temperatures below 220 °C. It is more resistant to acidic than to alkaline media, with an optimum stability at pH 4.

1.3.2 Environmental Levels and Residues in food

Cypermethrin is formulated as emulsifiable concentrates (100 and 250 g/litre), ultra-low-volume concentrate (10 - 50 g/litre), wettable powder (125 g/kg), and animal dip concentrate (5 - 15%).

Cypermethrin is used in a wide range of crops. In general, the maximum residue limits are low, ranging from 0.05 to 2.0 mg/kg in the different food commodities. The residues will be further reduced during food processing. In food of animal origin, residues may range between 0.01 and 0.2

mg/kg product. Residues in non-food commodities are generally higher, ranging up to 20 mg/kg product [FAO/WHO (1982a); FAO/WHO (1985c)].

Table 2 shows some of the maximum residue limit (MRL) values of cypermethrin in different foods according to Codex Alimentarius Commission (FAO, 1986).

Table2: Codex limits for cypermethrin residues in treated crops.

Commodity	**Maximum Residue Limit (mg/kg)**
Brassica leafy vegetables	1.0
Citrus	2.0
Lettuce	2.0
Oil seeds except peanuts	0.2
Peas	0.05
Root and tuber vegetables	0.05
Tomatoes	0.5
Wheat grain	0.2
Carcass meat (carcass fat)	0.2
Meat products	0.2
Eggs	0.05
Milk (whole milk)	0.01

Acceptable Daily Intake (ADI) of cypermethrin = 0.05 mg/kg body weight.

1.3.3 Environmental Fate of cypermethrin

1.3.3.1 Breakdown in soil and groundwater

Cypermethrin has a moderate persistence in soils. Under laboratory conditions, cypermethrin degrades more rapidly on sandy clay and sandy loam soils than on clay soils, and more rapidly in soils low in organic material (US EPA, 1989). In aerobic conditions, its soil half-life is 4 days to 8 weeks. It photodegrades rapidly with a half-life of 8 to 16 days. Cypermethrin is also subject to microbial degradation under aerobic conditions. Cypermethrin is not soluble in water and has a strong tendency to adsorb to soil particles (Kidd and James, 1991).

1.3.3.2 Breakdown in water

In neutral or acid aqueous solution, cypermethrin hydrolyzes slowly, with hydrolysis being more rapid at pH 9 (basic solution). Under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life of greater than 50 days and to photodegradation with a half-life of greater than 100 days. In pond waters and in laboratory degradation studies, pyrethroid concentrations decrease rapidly due to sorption to sediment, suspended particles and plants. Microbial degradation and photodegradation also occur (Muir et al., 1985; Agnihotri, 1986).

1.3.3.3 Breakdown in vegetation

When applied to strawberry plants, 40% of the applied cypermethrin remained after one day, 12% remained after three days, and 0.5% remained after seven days, with a light rain occurring on day 3. When cypermethrin was applied to wheat, residues on the wheat were 4 ppm immediately after spraying and declined to 0.2 ppm 27 days later. No cypermethrin was detected in the grain. Similar residue loss patterns have been observed on treated lettuce and celery crops (Ruzo and Casida, 1980).

1.3.4 Degradation of cypermethrin

Cypermethrin is a modern pesticide and it can undergo photo degradation, microbial degradation and biological degradation. The *trans*- isomer is more degradable than the *cis*-isomer. The *trans*-isomer shows a much shorter half life.

1.3.4.1 Photodegradation: Cypermethrin is one of the pyrethroids which are more light-stable. No loss of solid cypermethrin was detected exposing it to sunlight for 30 h. When exposed in methanol solution to light of wavelength > 290 nm for about 2 days, 55% of cypermethrin was recovered, the reaction quantum yield at 300 nm in methanol was low (Lauren and Henzel, 1977). Cypermethrin is more susceptible to radiation of lower wavelengths; under ultra-violet radiation, 90% of cypermethrin on a glass petri dish was decomposed after 3 days, but only 45% was decomposed after 3 days when the cypermethrin was deposited on grass and placed under an UV-lamp (Takahashi et al., 1985). The most important photodegradation products are, 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane-carboxylic acid **(CPA)**, 3-phenoxybenzoic acid **(PBA)**, the amide analogue of cypermethrin and various phenoxybenzyl derivatives, such as the alcohol, aldehyde, and acid (Day and Leahey, 1980; Leahey, 1979). These metabolites further degrade to smaller fractions. In studies on the 2 metabolites (PBA and CPA), PBA was quicker to degrade than CPA (Miyamoto and Mikami, 1983).

1.3.4.2. Biological Degradation: Cypermethrin degrades relatively quickly in soils, primarily by biological processes involving cleavage of the ester linkages, to give the two main degradation products, CPA and PBA. These products are themselves subsequently mineralized. There is also evidence for the formation, as an intermediate, of the amide of the intact molecule and occasionally the 4-hydroxy phenoxy analogue. Neither of the latter products appears to persist in the soil (Sakata et al., 1986; Roberts and Standen, 1981).

1.3.5 Toxicity of Cypermethrin

Cypermethrin is a moderately toxic material by dermal absorption or ingestion. Symptoms of high dermal exposure include numbness, tingling, itching, burning sensation, loss of bladder control, seizures, and possible death. Pyrethroids like cypermethrin may adversely affect the central nervous system. Symptoms of high-dose ingestion include nausea, prolonged vomiting, stomach pains, and diarrhea which progresses to convulsions, unconsciousness, and coma. Cypermethrin is a slight skin or eye irritant, and may cause allergic skin reactions (US NLM, 1995; Waller, 1988).

Some of the routes of exposure of cypermethrin, observed symptoms and their first aid treatments from the International Chemical Safety Cards (ICSC) are listed in **T**able 3.

Table 3: Symptoms of Cypermethrin Exposure.

Route of Exposure	Symptoms	First Aid
Inhalation	Burning sensation. Cough. Dizziness. Headache. Nausea. Shortness of breath.	Fresh air rest. Refer for medical attention.
Skin	Redness. Burning sensation. Numbness. Tingling. Itching.	Remove contaminated clothes. Rinse and then wash skin with water and soap.
Eyes	Redness. Pain	First rinse with plenty of water for several minutes (remove contact lenses if easily possible) then take to a doctor.
Ingestion	Abdominal pain. Convulsions. Vomiting.	Rinse mouth. Refer for medical attention.

1.4 DDT

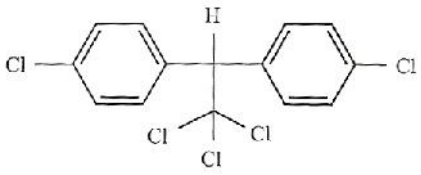


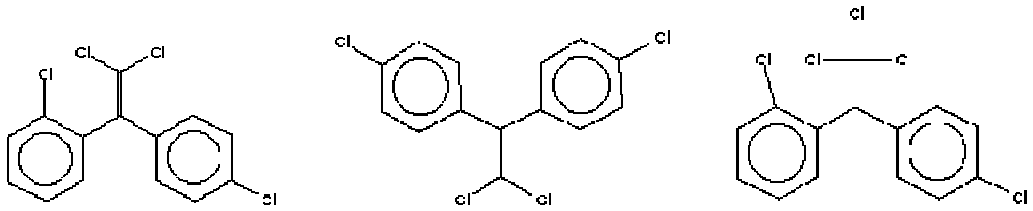
Figure 2: Structure of 4,4' DDT

DDT is perhaps the most infamous of the POPs. It was initially used during the Second World War to protect troops and civilians from malaria, typhus and vector-borne diseases. After the war, DDT was widely used for agriculture and disease control (Smith, 1991: IARC, 1974). It has a strong persistence in soil. Being the earliest, well known and one of the most widely used pesticides, DDT caused widespread contamination of water and soil resources, resulting in serious health effects in humans and animals (ATSDR, 2002). Its half life is ~15 years (PULSE, 2009). Food is the primary route of

exposure to DDT and its metabolites. They are fat soluble and accumulate well in adipose tissues and other organs of the body. In 1995, DDT has been banned in 34 countries and severely restricted in an additional 34 countries (Ritter *et al*., 1995). WHO Acceptable Daily Intake (ADI) allowance (0.02 mg/kg bw) (IPCS and IARC, 2009) and the Maximum Residue Limit (MRL) value is 0.05mg/kg (http//www, 2009). Estimated lethal dose for human is 500 mg/kg (ICPS, 1976).

1.4.1 Isomers and related compounds

The term "**total DDT**" is often used to refer to the sum of all DDT related compounds (*p,p′*-DDT, *o,p′*-DDT, DDE, and DDD) in a sample(wiki.).



DDE DDD 2,4′ DDT

Figure 3: Structure of DDE, DDD and 2,4′ DDT

1.4.2 Properties of DDT

DDT is a colourless crystalline substance, which is practically insoluble in water but highly soluble in fats and most organic solvents. It has potent insecticidal properties, which kills by opening sodium channels in insect neurons, causing the neuron to fire spontaneously. This leads to uncontrolled spasming and eventual death.

1.4.3 Toxicology of DDT and its metabolites

DDT was first synthesized in 1874 but its insecticidal properties were not discovered until 1939 (Smith, 1991), and large scale industrial production started in 1943.

DDT is given credit for having helped 1 billion people live free from malaria, thus saving millions of lives. In 1973, after 30 years of worldwide use of DDT, a World Health Organization (WHO) report concluded that the benefits derived from use of this pesticide were far greater than its possible risks (WHO, 1973). After 25 additional years, the benefits of DDT can be confirmed, but its stability, ubiquitous presence, and persistence in the environment, its accumulation in adipose tissues, and its estrogenic properties raise concern about its possible long-term adverse effects. In addition to a possible carcinogenic effect, DDT has been reported to affect neurobehavioral functions and to be associated with premature births (van Wendel *et al*., 2001 ; Longnecker *et al*., 2001). No living organism may be considered DDT free. DDT is stored in all tissues, but the highest concentration occurs in fat. It has been calculated that it would take between 10 and 20 years for DDT to disappear from an individual if exposure would totally cease, but that DDE would possibly persist throughout the life span (Smith, 1991).

1.4.3.1 Acute Toxicity of DDT

DDT is moderately to slightly toxic to studied mammalian species via the oral route. Reported oral LD50s range from 113 to 800 mg/kg in rats (1-3); 150-300 mg/kg in mice. Toxicity will vary according to formulation. DDT is readily absorbed through the gastrointestinal tract, with increased absorption in the presence of fats.

Acute effects likely in humans due to low to moderate exposure may include nausea, diarrhea, increased liver enzyme activity, irritation (of the eyes, nose or throat), disturbed gait, malaise and excitability; at higher doses, tremors and convulsions are possible. While adults appear to tolerate moderate to high ingested doses of up to 280 mg/kg, a case of fatal poisoning was seen in a child who ingested one ounce of a 5% DDT:kerosene solution. (pmep.cce.cornell.edu, 2013)

1.4.3.2 Chronic Toxicity of DDT

DDT has caused chronic effects on the nervous system, liver, kidneys, and immune systems in experimental animals. Effects on the nervous system observed in test animals include: tremors in rats at doses of 16-32 mg/kg/day over 26 weeks.

The main effect on the liver seen in animal studies was localized liver damage. This effect was seen in rats given 3.75 mg/kg/day over 36 weeks, rats exposed to 5 mg/kg/day over 2 years and dogs at doses of 80 mg/kg/day over the course of 39 months. Kidney damage was also seen in rats at doses of 10 mg/kg/day over 27 months.

Persons eating fish contaminated with DDT or metabolites may also be exposed via bioaccumulation of the compound in fish. Adverse effects on the liver, kidney and immune system due to DDT exposure have not been demonstrated in humans in any of the studies which have been conducted to date (pmep.cce.cornell.edu, 2013).

1.4.3.3 Reproductive Effects of DDT

There is evidence that DDT causes reproductive effects in test animals. In rats, oral doses of 7.5 mg/kg/day for 36 weeks resulted in sterility. It is thought that many of these observed effects may be the result of disruptions in the endocrine (hormonal) system.

Available epidemiological evidence from two studies does not indicate that reproductive effects have occurred in humans as a result of DDT exposure. No associations between maternal blood levels of DDT and miscarriage or premature rupture of fetal membranes were observed in two separate studies (pmep.cce.cornell.edu, 2013).

1.4.3.4 Teratogenic Effects of DDT

There is evidence that DDT causes teratogenic effects in test animals as well. In a two-generational study of rats, 10 mg/kg/day resulted in abnormal tail development. It seems unlikely that teratogenic effects will occur in humans due to DDT at likely exposure levels (pmep.cce.cornell.edu, 2013).

1.4.3.5 Mutagenic Effects of DDT

The evidence for mutagenicity and genotoxicity is contradictory. In only 1 out of 11 mutagenicity assays in various cell cultures and organisms did DDT show positive results. Results of in vitro and in vivo genotoxocity assays for chromosomal aberrations indicated that DDT was genotoxic in 8 out of 12 cases, and weakly genotoxic in 1 case.

In humans, blood cell cultures of men occupationally exposed to DDT showed an increase in chromosomal damage. Thus it appears that DDT may have the potential to cause genotoxic effects in humans, but does not appear to be strongly mutagenic (pmep.cce.cornell.edu, 2013).

1.4.3.6 Carcinogenic Effects of DDT

The evidence regarding the carcinogenicity of DDT is equivocal. It has been shown to cause increased tumor production (mainly in the liver and lung) in test animals such as rats and mice in some studies but not in others. In rats, liver tumors were induced in three separate studies at doses of 12.5 mg/kg/day over periods of 78 weeks to life, and thyroid tumors were induced at doses of 85 mg/kg/day over 78 weeks.

The available epidemiological evidence regarding DDT's carcinogenicity in humans, when taken as a whole, does not suggest that DDT and its metabolites are carcinogenic in humans at likely dose levels (pmep.cce.cornell.edu, 2013).

1.4.3.7 Neurochemical effects of DDT

The main target of DDT is the sodium channel. DDT delays the closure and prevents full opening of the channels in excited cells, leading to hyperexcitability. DDT also reduces potassium permeability through pores, inhibits neuronal ATPases, particularly the Ca^{2+}-ATPase and Na/K-ATPases, which are important for the neuronal repolarization, and inhibits the ability of calmodulin to transport Ca^{2+} (Echobichon, 1996). In agreement, Kodavanti *et al*., (1996) showed that DDT at relatively low concentrations (IC50, 4-5 μM) inhibits Ca^{2+} uptake in mitochondria *in-vitro*. Involvement of calcium may influence brain PKC activity and. Bagchi *et al*. (1997) reported increased brain PKC activity *in-vivo* in DDT exposed rats (40 mg/kg/body weight) and also in PC12 cells *in-vitro* (50-200 nM). Exposure to DDT at doses inducing tremors (25-100 mg/kg) also revealed region specific alterations in the levels of the rat brain biogenic amines, such as serotonin and noradrenalin (Hudson *et al*., 1985; Hong *et al*., 1986). Similarly, DDT has been shown to increase the levels of the amino acid neurotransmitters, glutamate and aspartate in brainstorm, and to stimulate release of acetylcholine at high concentrations (Hudson *et al*., 1985; Morio *et al*., 1985). The effects on neurotransmitters have been attributed to DDT's effect on sodium channels, promoting release of neurotransmitters (Hong *et al*., 1986). Eriksson and

coworkers found that DDT decreases the levels of the muscarinic cholinergic receptors in the brain of mice administered a single dose of DDT (0.5 mg/kg) (Eriksson *et al*., 1984; Eriksson and Nordberg, 1986; Johansson *et al*., 1995).

1.4.4 Ecological Effects

1.4.4.1 Effects on Birds

DDT may be slightly toxic to practically non-toxic to birds. Reported dietary LD50s range from greater than 2,240 mg/kg in mallard, 841 mg/kg in Japanese quail and 1,334 mg/kg in pheasant. In birds, exposure to DDT occurs mainly through the food web through predation on aquatic and/or terrestrial species having body burdens of DDT, such as fish, earthworms and other birds.

There has been much concern over chronic exposure of bird species to DDT and effects on reproduction, especially eggshell thinning and embryo deaths. There is evidence that synergism may be possible between DDT's metabolites and organophosphate (cholinesterase-inhibiting) pesticides to produce greater toxicity to the nervous system and higher mortality (pmep.cce.cornell.edu, 2013).

1.4.4.2 Effects on Fish Species

DDT is very highly toxic to fish species as well. Reported 96-hour LC50s are less than 10 ug/L in coho salmon (4.0 ug/L), rainbow trout (8.7 ug/L), northern pike (2.7 ug/L), black bullhead (4.8 ug/L), bluegill sunfish (8.6 ug/L), largemouth bass (1.5 ug/L), and walleye (2.9 ug/L). The reported 96-hour LC50s in fathead minnow and channel catfish are 21.5 ug/L and 12.2 ug/L respectively. Observed toxicity in coho and chinook salmon was greater in smaller fish than in larger. It is reported that DDT levels of 1 ng/L in Lake Michigan were sufficient to affect the hatching of coho salmon eggs.

In addition to acute toxic effects, DDT may bioaccumulate significantly in fish and other aquatic species, leading to long-term exposure. This occurs mainly through uptake from sediment and water into aquatic flora and fauna, and also fish. Fish uptake of DDT from the water will be size-dependent with smaller fish taking up relatively more than larger fish. A half- time for elimination of DDT from rainbow trout was estimated to be 160 days.

The reported bioconcentration factor for DDT is 1,000 to 1,000,000 in various aquatic species and bioaccumulation may occur in some species at very low environmental concentrations (pmep.cce.cornell.edu, 2013).

1.4.5 Fate in Humans & Animals

DDT is very slowly transformed in animal systems. Initial degrades in mammalian systems are DDE and DDD, which are very readily stored in fatty tissues. These compounds in turn are ultimately transformed into bis(dichlorodiphenyl) acetic acid (DDA) via other metabolites at a very slow rate. DDA, or conjugates of DDA, are readily excreted via the urine.

Blood samples collected in the latter half of the 1970s showed that blood levels were declining further, but DDT or metabolites were still seen in a very high proportion of the sample. Levels of DDT or metabolites may occur in fatty tissues (e.g. fat cells, the brain, etc.) at levels of up to several hundred times that seen in the blood. DDT or metabolites may also be eliminated via mother's milk by lactating women (pmep.cce.cornell.edu, 2013).

1.4.6 Environmental Fate of Pesticides

When a pesticide is released into the environment, whether through an application, a disposal or a spill, it influenced by many processes (Fishel, 1997). It may be taken up by a plant or ingested by insects, worms or microorganisms in the soil. It may stick to the soil particles or dissolve in water and move down through the soil to the water table. It may vaporize and enter the atmosphere or break down through microbial and chemical pathways into other less toxic compounds. Pesticides may dissolve in rain or irrigation water (Rao et al., 1983; Buttler et al., 1998). These processes determine a pesticide's persistence and movement and its ultimate fate. Pesticides fate processes fall into three major types namely adsorption, transfer and degradation (Fishel, 1997). [105]. All these processes are governed by the physico-chemical properties of pesticide and site on which it is applied (Buttler et al., 1998; Linde, 1994; Kerle et al., 2007).

1.4.6.1 Pesticides Adsorption

Pesticide adsorption is the binding of pesticides to soil or sediment particles (Linde, 1994; Devlin et al., 2008; Sprague, 2012). Pesticides adsorption often occurs because of the attraction between a

chemical and soil or sediment particles (Fishel, 1997). The mechanisms which operate when pesticides adsorb include strong or weak ionic attraction, hydrophobic attraction, and hydrogen bonding s. Factors which control pesticide adsorption includes polarity of pesticide itself, amount of moisture in soil or sediment, soil PH, organic matter content and soil texture (Fishel, 1997; Linde, 1994; Sprague, 2012).

Wet soils tend to adsorb less pesticide than dry soils because water molecules compete with the pesticides for the binding sites (Fishel, 1997; Jansma and Linders, 1995). A polar pesticide will be very water soluble and tend not to be adsorbed onto soil. Pesticides that are non-polar tend to be pushed out of water and onto soils which contain non polar carbon material (Linde, 1994). For pesticides that are weak acids or bases, adsorption is influenced by the pH of the soil; however pesticides that are not ionisable are generally not affected by pH (Linde, 1994). Some pesticides such as paraquat and glyphosate bind tightly to the soil while others bind only weekly and are readily desorbed or released back into the solution (Fishel, 1997).

The amount of organic matter in soil is the greatest factor influencing the amount of pesticides adsorbed. This is because organic matter is non polar and has a relatively light negative charge. Most pesticides are non-polar and will be attracted to the lightly charged surface (Linde, 1994). Besides, soils high in organic matter or clay are more adsorptive than sandy soils because clay or organic soil has more particle surface area or more sites into which pesticides can bind. Therefore sandy soil increase pesticides mobility while clay soil reduces pesticides mobility (Fishel, 1997).

1.4.6.2 Pesticides Transfer Processes

Transfer processes include processes that move the pesticide away from the target (Navarro et al., 2007). These processes include volatilization, spray drift, runoff, leaching, absorption and crop removal (Fishel, 1997; Navarro et al., 2007; Singh and Walker, 2006). Pesticides transfer process depends on its solubility in water, adsorption (retention) by soil and its persistence. It's also influenced by environmental and site characteristics including weather, topography, canopy, ground cover; and soil organic matter, soil texture and structure (Kerle et al., 2007; Estevez et al., 2008).

1.4.6.3 Pesticide Runoff

Runoff is the movement of pesticides in water over a sloping surface. The pesticides are either mixed in the water or bound to eroding soil. Runoff can also occur when water is added to a field faster than it can be absorbed into the soil. Pesticides may move with runoff as compounds dissolved in

the water or attached to soil particles. The amount of pesticide runoff depends on the slope, the texture of the soil, the soil moisture content, the amount and timing of a rain event (irrigation or rainfall) and the type of pesticide used (Kerle et al., 2007; Sprague, 2012).

1.4.6.4 Leaching

Leaching is the removal of soluble materials by water passing through the soil (Buttler et al., 1998; Bicki, 1989; Kerle et al., 1996). Leaching occurs downward, upward, or sideways. The factors influencing leaching of pesticides depend on both, the characteristics of the soil and pesticide. These include water solubility of the pesticide, soil structure and texture as well as the amount and persistence of pesticide adsorption to soil particles (Chilton et al., 1994; Holland and Sinclair, 2004).

Highly water soluble pesticides tend to move easier through the soil profile into groundwater (Kerle et al., 1996; Tharp, 2012). Pesticides which degrade easily the opportunity of leaching to occur is limited, on the other hand for pesticides which degrade slowly the chance of leaching to occur is maximum (Cardeal, 2011). Soil with high organic matter increases the capacity for adsorption of pesticides hence reduces leaching (CES. Univ Alaska, 2011). Leaching can be increased when a rain-event occurs shortly after spraying (Estevez et al., 2008; CES. Univ Alaska, 2011).

1.4.6.5 Pesticide Absorption or Uptake

Absorption or uptake is the movement of pesticides into and within the plant and animals (Fishel, 1997; Burner et al., 1997)]. Most pesticides break down once they are absorbed. Pesticide residues may be broken down or remain inside the plant or animal and be released back into the environment when the animal dies or as the plant decays. Some pesticides stay in the soil long enough to be absorbed by plants grown in a field years later. They may damage or leave residues in future crops (Tiryaki and Temur, 2010) 124]·

The most important factor governing sorption and movement within the plant is the solubility of the pesticide in water (Kerle et al., 2007; Burner et al., 1997). The content of the surrounding soil is also important to the plant uptake. For non-polar pesticides the volume of organic matter is particularly important. Other factors such as pH and clay and microbial activity are more important as the polarity of the pesticide increases (Burner et al., 1997). Plant uptake of pesticides prevents runoff or leaching (Kerle et al., 2007).

1.4.7 Pesticide Degradation Processes

Degradation or transformation of pesticides is the breaking down pesticides into simpler molecules than the parent compound, they can be none or less toxic compounds and, in some cases, they are also toxic and more persistence than the parent compound for example the degradation of endosulfan to endosulfan sulfate [(Cardeal, 2011; Vargas, 1975; NRCS, 1998; Shivaramaiah and Kennedy, 2006). Pesticides can be degraded by chemical (chemical degradation), sunlight (photo degradation) or by microbial activity (biological degradation) The rate of degradation depends on pesticide chemistry, environmental conditions, distribution between foliage and soil, as well as temperature, soil and water pH. The degradation of pesticides can occur in plants, animals, soil and water; or it can take place upon exposure to ultra-violet (UV) radiation (Kerle et al., 2007).

1.4.7.1 Chemical Degradation of Pesticides

Chemical degradation is the breakdown of a pesticide by processes not involving a living organism. Chemical processes generally occur in water or atmosphere and follow one of the three reactions: oxidation, reduction, hydrolysis, and photolysis. The rate and type of chemical reactions that occur are influenced by the adsorption, temperatures, pH, moisture and physical chemical properties of the pesticide (Fishel, 1997; Linde, 1994).

1.4.7.2 Biological Degradation of Pesticides

Pesticides biotransformation or biodegradation is the process by which pesticide substances are broken down into smaller compounds by the enzymes produced by living microbial organisms (Porto et al., 2011). The degradation of pesticides through microbial metabolic process may involve a three-phase process. In Phase I transformation, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar amino acid, or glutathione, which increases the water solubility and reduces toxicity compared with the parent pesticide. Generally, Phase II metabolites have little or no phytotoxicity and may be stored in cellular organelles. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also nontoxic (Hernandez et al., 2011; Chaplain et al., 2011; Eard et al., 2003). The processes responsible for biotransformation of pesticides include biodegradation, co-metabolism and synthesis (Chaplain et al., 2011).

Biodegradation process, the microbial organisms transform the substance through metabolic or enzymatic processes this occurs when micro organisms use pesticides as a food substrate. The

microorganisms participating in biodegradation include fungi, bacteria and other microorganisms that use pesticides as their substrate (Chaplain et al., 2011).

Synthesis includes conjugation and oligomerization. Pesticides are transformed into compounds with chemical structures more complex than those of the parent compounds. During conjugation, a pesticide (or one of its transformation products) is linked to hydrophilic endogenous substrates, resulting in the formation of methylated, acetylated, or alkylated compounds, glycosides, or amino acid conjugates. These compounds can be excreted from the living cells, or stored. During oligomerization, a pesticide combines with itself, or with other xenobiotic residues (proteins, soil organic residues). Consequently, they give high-molecular weight compounds, which are stable and often incorporated into cellular components or soil constituents (soil organic matter) (Chaplain et al., 2011).

The rate of microbial degradation depends highly on the amount and nature of pesticides present in the soil, the microbial population in the soil and soil conditions that favors microbial activities, such as warm temperature, favorable pH, adequate soil moisture, aeration and high organic matter content (Chaplain et al., 2011).

1.4.8 Bioaccumulation in fish

An important process through which chemicals can affect living organisms is bioaccumulation. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment (extoxnet.orst.edu, 2013). Pesticide Organic Pollutants (POPs) are highly hydrophobic chemicals that may accumulate in fishes depending on their lipid content. Knowledge regarding bioaccumulation and the levels of chemicals in biota is a prerequisite to understanding the adverse effects of the chemicals on ecosystems (Franke *et al*., 1994). POPs may accumulate in fishes via various pathways, for example, via direct uptake from water through gills or skin (bio-concentration), ingestion of particulate matter from water (ingestion) and/or consumption of contaminated food (bio-magnification).

Normally all these processes occur in varying degrees of combinations in all fishes. Even if acute or chronic effects are not detected in toxicity tests, accumulation of POPs in fish tissues should be regarded as a hazard criterion because some effects may be recognized only at a later stage of life, may be multigenerational (e.g. impact of PCBs on the egg hatching success (Tillitt *et al*., 1992), or may manifest themselves only in higher members of the food web. Even contaminant

concentrations in fishes may vary depending on species, age groups, reproductive status and various other parameters.

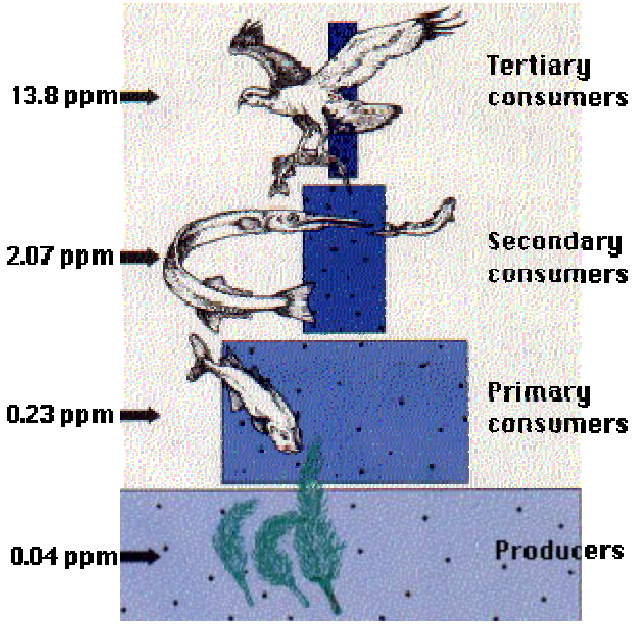
1.4.9 Bioconcentration in fish

Bioconcentration is the specific bioaccumulation process by which the concentration of a chemical in an organism becomes higher than its concentration in the air or water around the organism (extoxnet.orst.edu, 2013). Bioconcentration is the specific bioaccumulation process by which the concentration of a chemical is an organism becomes higher than its concentration in the air or water around the organism. for fish and other aquatic animals, bioconcentration after uptake through the gills (sometimes the skin) is usually the most important bioaccumulation process.

1.4.10 Biomagnification in fish

Biomagnification, also known as bioamplification or biological magnification, is the increase in concentration of a substance that occurs in a food chain.

Biological magnification often refers to the process whereby certain substances such as pesticides or heavy metals move up the food chain, work their way into rivers or lakes, and are eaten by aquatic organisms such as fish, which in turn are eaten by large birds, animals or humans. The substances become concentrated in tissues or internal organs as they move up the chain.



The numbers are representative values of the concentration in the tissues of **DDT** and its derivatives (in parts per million, ppm)

The following is an example showing how bio-magnification takes place in nature: An anchovy eats zoo-plankton that has tiny amounts of mercury that the zoo-plankton has picked up from the water throughout the anchovies lifespan. A tuna eats many of these anchovies over its life, accumulating the mercury in each of those anchovies into its body. If the mercury stunts the growth of the anchovies, that tuna is required to eat more little fish to stay alive. Because there are more little fish being eaten, the mercury content is magnified. (Wiki/Biomagnification, 2013)

1.5 The way of reducing the use of pesticides

By using suitable methods of cultivation, biological pest controls (such as pheromones and microbial pesticides), genetic engineering and methods of interfering with insect breeding might reduce the usage of pesticides. Application of composted yard waste has also been used as a way of controlling pests. These methods are becoming increasingly popular and often are safer than traditional chemical pesticides. In addition, EPA is registering reduced-risk conventional pesticides in increasing numbers.

Cultivation practices include poly-culture (growing multiple types of plants), crop rotation planting crops in areas where the pests that damage them do not live, time of planting according to when pests will be least problematic, and use of trap crops that attract pests

away from the real crop. In the U.S., farmers have had success controlling insects by spraying with hot water at a cost that is about the same as pesticide spraying. Release of other organisms that fight the pest is another example of an alternative to pesticide use. These organisms can include natural predators or parasites of the pests. Biological pesticides based on entomoapthogenic fungi, bacteria and viruses cause disease in the pest species can also be used (Banglapedia, 2010).

1.7 Objective of the present work

POPs, accumulated in fish, domestic animal's fat, eggs, meat, milk etc. can be exposed to human subjects through food chain. Therefore, the present study was aimed to

i) Determine organchlorine (DDTs) pesticide residues in different local fresh fish samples of different area

ii) Understand the uptake and bioaccumulation of POPs in two species of fishes such as gulsha and taki are local name and their scientific name is *Mystus cavasius L. & Channa punctatus B*. respectively.

iii) To study the rates of dissipation and safe harvesting period of one pyrithroids (cypermethrin on Bean and Cauliflower), based on the prescribed dosage and MRL value of the respective authority.

iv) Create a baseline for future studies in which health related issues should be included as well as to determine the feasibility and need of large scale monitoring within Bangladesh.

The aim of the present investigation was to determine the degradation kinetics of cypermethrin on bean and cauliflower after application of cypermethrin. These data are needed for establishing proper management practices in terms of harvest times and waiting periods to ensure food safety and environmental sustainability in vegetable production systems in the humid tropics.

2 EXPERIMENTAL

2.1 General

2.1.1 MATERIALS AND METHODS

This chapter gives description on the materials and methods concerning the study. The chapter consists of three parts, the first part describe sampling which involves sampling sites and collection, transport and storage of samples. The second one describes sample preparation; it involves extraction, clean-up and other laboratory works. The last part describes sample analysis.

2.1.1.1 Glass apparatus

All required glass apparatus including graduated test tubes were cleaned with water using detergent, rinsed about six times with water, then twice with distilled water and finally with redistilled acetone. All glassware was heated at 120^0C before use, cooled and stored by covering with aluminum foil prior to use. Short glass columns with stopper were used for clean up. Screw cap vials (1.5 mL) having PVDE septum (Shimadzu, Japan) was used to store cleaned sample extract for analysis by Gas chromatograph-Electron captured detector (GC-ECD) having an auto injector.

2.1.1.2 Reagents, chemicals and solvents

Analytical or reagent grade solvents purchased from Sigma, E. Merck or BDH were used in most experiments. Analytical grade ethyl acetate, n-hexane and dichloromethane were used for extraction, clean up column packing and as eluting agent from cleanup column respectively. Sulfuric acid (98%, w/w) {bought from BDH (Poole, UK)} was also used to carry out clean up of extracts. Extra pure hexane was used to prepare final sample to run in GC.
Anhydrous Magnesium sulphate, sodium chloride (analytical grade) of Merck, Germany, florisil from ACROS organics, USA, aluminium oxide (alumina) from Merck, Germany, Charcoal from Uni-Chem, China were used for this analysis. Florisil and alumina were activated by heating at 105^0 C for 3 hrs, charcoal was activated by washing with distilled

water, n-hexane, methanol, acetone in Buchner funnel & then dried at 105^0C and all kept in desiccators.

2.1.1.3 Methods

2.1.1.3.1 Activation of chemicals

Alumina & florisil were activated by heating for 12 h at 105ºC in an oven (Eyela , Japan) and charcoal was activated by washing with distilled water, n-hexane, methanol, acetone in Buchner funnel & then dried at 105^0C and all kept in desiccators.

2.1.1.3.2 Evaporation

All the evaporations were carried out under reduced pressure using rotary vacuum evaporator at water bath temperature not exceeding 40^0C.

2.1.2 Pesticide Standards

The standards of o,p´-DDT & p,p´-DDT (99% purity), p,p´-DDE (99% purity), p,p´-DDD (99% purity) and Cypermethrin (91% purity) purchased from Dr. Ehrenstorfer, Germany were used for analysis. All of the standards were stored at -20^0 C in a freezer and away from the experimental samples. Technical or commercial grade cypermethrin, named “Ripcord-10-EC” was obtained from Bangladesh Agricultural Research Institute (BARI).

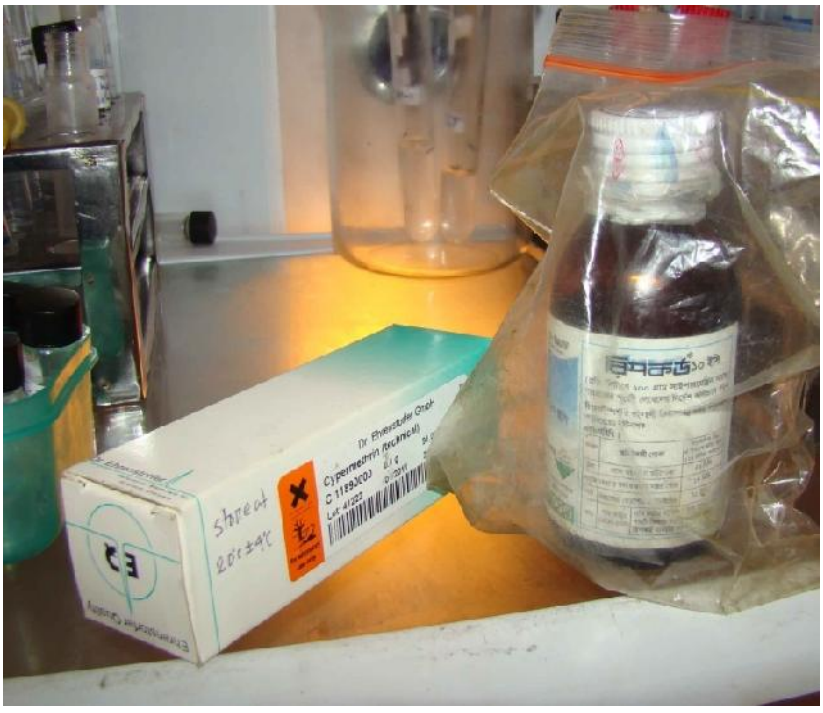


Figure 4 : Standard and commercial cypermethrin

Standard mixtures of various concentrations were prepared in n-hexane for calibraton curve and for identification of samples. All the solutions were kept or preserved in leveled screw cap test tubes with proper marking by permanent ink and stored in a refrigerator. During storing of standard sample if there was any loss of solvent due to evaporation more solvent upto the mark and made homogeneous by vortex.

2. 1.3 Instruments:

2.1.3.1 Homogenizer

Vegetables and fish samples were homogenized by normal kitchen blender.

2.1.3.2 Centrifuge machine

The samples were centrifuged by Hanil Science Industrial Co. Ltd., Model-Combi 514 R and cowbell from India.

2. 1.3.3 Gas Chromatography

Two gas chromatographs were used for analysis of modern pesticides. One was Shimadzu-2010 and another one was Shimadzu 17A. Both of them were equipped with electron capture detector (ECD) having split/splitless injector. The Shimadzu 2010 had auto injector whereas Shimadzu 17A has manual injector.

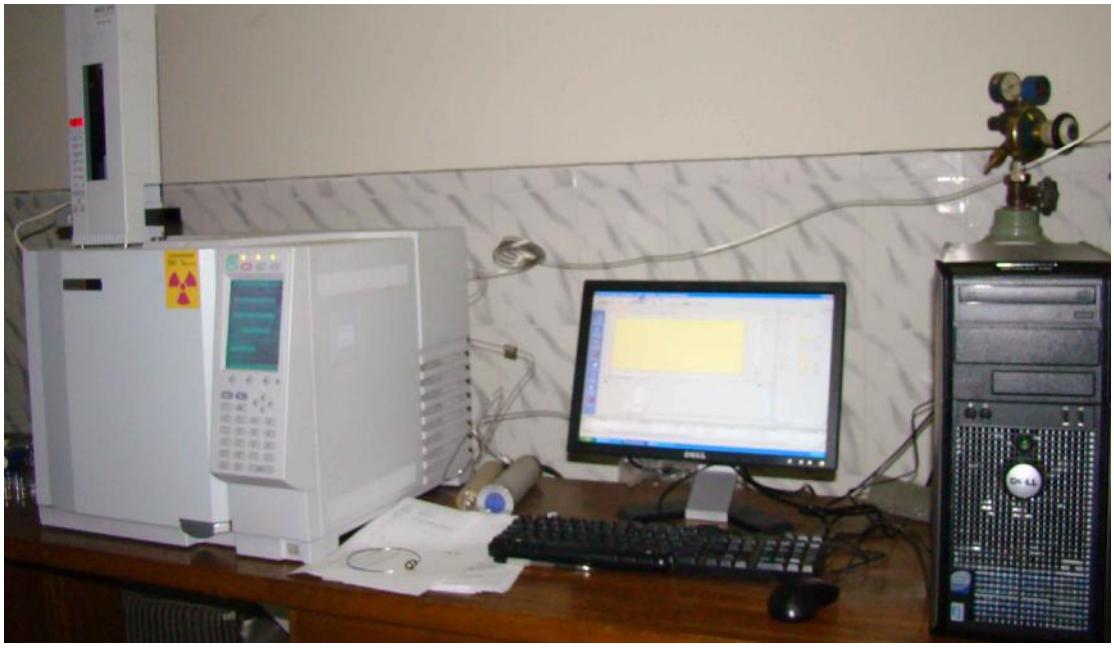


Figure 5: Gas chromatography (model: GC-2010)

2.1.4 Preparation of Standard Solutions

2.1.4.1 Preparation of primary standard solutions

The known amount of the analytical grade pesticide was dissolved in a definite volume of n-hexane and the concentration of the standard was calculated. Mass of the 91% certified standard was adjusted to 100% by calculation to determine the accurate concentration. Again, known volume of commercial pesticide was diluted in a definite volume of n-hexane and the concentration was calculated.

These stock solutions were transferred to different narrow test tubes, with PTFE lined screw caps. These solutions were labeled indicating name of the standard, solvent, concentration, date of preparation and signature. The meniscus were of the solutions were marked with permanent ink. These solutions were stored in the freezer of a refrigerator.

2.1.4.2 Preparation of secondary and working standard solutions

The stock solutions were kept outside the refrigerator to reach room temperature. Then from each stock solution, definite amount of the solutions was withdrawn after checking the meniscus mark. New marks were put after withdrawing. The withdrawn solutions were diluted to appropriate concentrations adjusting the volume of the solution by addition of n-hexane. These solutions were transferred to narrow test tubes, with PTFE lined screw cap and

labeled indicating substance, solvent, concentration, date of preparation and signature. The meniscus of the solution was marked with permanent ink.

Similarly, the working solutions were prepared from middle standard solutions. The commercial cypermethrin solutions for preparing calibration curve were made from a cleaned up solution. Known volume of the solution was cleaned by passing through floricil-alumina clean up column using dichloromethane as eluting agent. Then it was evaporated to dryness and the initial volume of the solution was reconstituted to maintain the concentration.

The middle and working standard solutions were kept in the residue laboratory**.**

2.1.5 Determination of limit of detection (LOD) and limit of quantification (LOQ)

To determine the LOD, working standard solutions were serially diluted to get desired concentration. The diluted standard solutions were injected one by one until the peak heights of the standards were same to the noise level. The limit of detection (LOD) of the test compounds was determined using a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample, whereas the limits of quantification (LOQ) were determined with a signal-to-noise ratio of 10. The LOD was determined by the equation(USP, 2009)

Sample LOD = machineLOD(MDA) x 1/sample weight or volume x final volume/ injection volume

The machineLODs of cypermethrin was found 0.025ppm. From the above equation the sample LOD were calculated 0.01mg/g for vegetables. Then the LOQ were found 0.033 for cypermethrin.

2.1.6.1 Identification by GC

The cleaned-up extract (1.0 μL) was injected into the GC-ECD (analysis conditions of GC-ECD mentioned in Section-2.8). In the same condition, the pesticide standard solutions were

also injected. Pesticide residues were identified by comparing the retention times of the different peaks in the sample with that of the retention time of different pesticide standards.

2.1.6.2 Quantification by GC

Quantitative determination was carried out by comparing the peak area of the each DDTs in the sample extract with that of the peak area of the respective DDTs in the external standard solution. From the calibration curve the amount of each DDTs present in the sample extracts were calculated by using the formula $y = mx + c$,

Here, y = Peak area

x = Concentration

m = Slope of the calibration curve

c = Intercept

2.1.7 Blank experiment

The known amount of control samples (three replicates of each sample) was extracted followed by respective extraction and cleaned up procedure to determine the matrix effect under analysis method. Reagent blank was done followed by extraction procedure and cleaned up method, using only solvent and reagents (in the absence of sample) to make the analysis realistic. In both cases no peak was observed at the retention time of standard.

2.2.8 Recovery

Recovery experiment for each standard in respective matrix individually were carried out, known amount of standard compounds were added drop by drop over to the control samples (known amount of analyte free samples for each type of sample) and allowed the sample to stand for 30 min to be adsorbed into the samples. Then the samples were extracted and cleaned up following same procedure as described in respective extraction and cleanup Section. The recovery of the each analyte was calculated by using the following formula.

$$\text{Recovery} = \frac{\text{Area}_{\text{Sample}} \times \text{Conc}_{\text{Std}}}{\text{Area}_{\text{Std}} \times \text{Conc}_{\text{Matrix}}} \times \frac{100}{\text{Known amount of Std}}$$

2.1.9 Method Validation

The extraction efficiency of the analytical procedure was evaluated via recovery experiments. Validation of the method was performed in terms of recovery studies before analysis of field samples. The extraction methods were validated by doing intra-day and inter-day recovery. The recovery experiments were conducted in three/four replicates at first day (intra-day recovery) at two/three fortification levels. Then the method was validated by recovery experiments that were performed in three/four replicates in another three days (inter-day validation) at the same fortification levels.

2.2 Analysis of residue of cypermethrin in Vegetable samples (Part-A)

2.2.1 Field Experiment for Residue Analysis

2.2.1.1 Location of the experimental field

The experiments was conducted at the experimental field of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur situated at latitude 23^0 46" N and longitude 90^0 23" E with an elevation of 8.45 meter the sea level.

Figure 6: Experimental plot of Bean

Figure 7: Experimental plot of Cauliflower

2.2.1.2 Soil

The soil of the experimental plots is silty-loam with fine textured having the pH. ranging from 5.5 to 6.2.

2.2.1.3 Land preparation

The land was well prepared by harrowing followed by ploughing, cross ploughing and leveling. Cowdung and other chemical fertilizers were applied as recommended dose for eggplant, bean, tomato and cauliflower cultivation (Rashid, 1993) at the rate of 15 tons of cowdung and 250, 150 and 125 kg Urea, TSP and MP, respectively per hectare. The full dose of cowdung, TSP and an half of MP was applied as basal dose during land preparation. The entire dose of Urea and the rest of MP were applied as top dressing. The first top dressing with one third of Urea was made at 20 days after transplanting followed by second top dressing comprising one third of urea and one fourth of MP at the time of flower initiation followed by last top dressing comprising rest of Urea and MP at the time of fruit initiation.

2.2.1.4 Raising of seedling and transplanting

Seeds of bean (variety IPSA Shem-2) and cauliflower (variety BARI-1) were used. Seeds were sown directly in the nursery bed at research field of Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. The plants were lightly irrigated regularly for ensuring proper growth and development of the seedlings. Forty days old seedlings were transplanted in the well prepared experimental plot in 28th October 2012. The total land area was 18 m^2 which was 3m x 6m in size. A Total of 42 seedlings were transplanted in the plot with 60 cm plant to plant distance. The standard cauliflower cultivation practices were followed.

2.2.1.5 Cultural operation

Pit having seedlings were irrigated lightly immediately after transplanting. Supplementary irrigation was applied at intervals of 2-3 days. Weeding was done as and when necessary. The MP and urea fertilizers were top dressed in 3 splits as prescribed earlier.

2.2.1.6 Design of experiment and layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The whole area of experimental field was divided into 3 blocks and each block was again divided into 6 unit plots.

2.2.2 Sampling of samples

Bean and cauliflower were grown in the experimental field of Entomology Division, Bangladesh Agricultural Research Institute (BARI). Two beds were selected for experiment of cypermethrin application. "Ripcord-10-EC" (100g cypermethrin per liter) was sprayed at recommended dose of (1 mL in 1 L water = 100 mg cypermethrin in 1 kg water) in experimental bed. The name of the vegetable samples and their family are given in **Table-2.**

Figure 8: Bean (*Phaseolus vulgaris)* and Cauliflower (*Brassica oleracea var.)*

Local name	English name	Scientific name	Family
Sim	Bean	*Phaseolus vulgaris*	Leguminosae
Fulkopi	Cauliflower	*Brassica oleracea var.*	Cruciferae

Table 4: List of the selected vegetable samples

Figure 9: a. The sprayer, b.commercial cypermethrin, c. measuring cylinder

2.2.2.1 Collection of samples

Cypermethrin was sprayed with the recommended dose in two different beds of bean and cauliflower. The samples were collected according to pre harvest intervals following the World Health Organization (WHO) guideline. Before spraying, blank/control samples were collected. Then two replicate samples from each bed were collected at the intervals of 0 (after 2 hours of spraying), 1, 3, 5, 7, 10 and 15 days after spraying.

Table 5: Sample collection of cauliflower at different date sprayed with recommended dose.

Time of collection	Site of Collection	Sample name	Sample ID	Date of collection
Before spraying		Cauliflower	CF (s) control 0 DAA-1	02-12-2012
			CF(s) control 0 DAA-2	

			CF(s) control 0 DAA-3	
After two hours		Cauliflower	CF(s) 0 DAA-1	02-12-2012
			CF(s) 0 DAA-2	
			CF(s) 0 DAA-3	
After 1 day		Cauliflower	CF(s) 1 DAA-1	03-12-2012
			CF(s) 1 DAA-2	
			CF(s) 1 DAA-3	
After 3 days		Cauliflower	CF(s) 3 DAA-1	05-12-2012
			CF(s) 3 DAA-2	
			CF(s) 3 DAA-3	
After 5 days		Cauliflower	CF(s) 5 DAA-1	07-12-2012
			CF(s) 5 DAA-2	
			CF(s) 5 DAA-3	
After 7 days		Cauliflower	CF(s) 7 DAA-1	09-12-2012
			CF(s) 7 DAA-2	
			CF(s) 7 DAA-3	
After 10 days		Cauliflower	CF(s) 10 DAA-1	12-12-2012
			CF(s) 10 DAA-2	
			CF(s) 10 DAA-3	
After 15 days		Cauliflower	CF(s) 15 DAA-1	17-12-2012
			CF(s) 15 DAA-2	
			CF(s) 15 DAA-3	

Table 6: Sample collection of cauliflower at different date sprayed with recommended dose.

Time of collection	Site of Collection	Sample name	Sample ID	Date of collection
Before spraying	Experimental field of BARI, Gazipur	Bean	bean (s) control 0DAA-1	18-12-2012
			bean (s) control 0DAA-2	
			bean (s) control 0DAA-3	
After two hours		Bean	bean (s) 0 DAA-1	18-12-2012
			bean (s) 0 DAA-2	
			bean (s) 0 DAA-3	
After 1 day		Bean	bean (s) 1 DAA-1	19-12-2012
			bean (s) 1 DAA-2	
			bean (s) 1 DAA-3	
After 3 days		Bean	bean (s) 3 DAA-1	21-12-2012
			bean (s) 3 DAA-2	
			bean (s) 3 DAA-3	
After 5 days		Bean	bean (s) 5 DAA-1	23-12-2012
			bean (s) 5 DAA-2	
			bean (s) 5 DAA-3	
After 7 days		Bean	bean (s) 7 DAA-1	25-12-2012
			bean (s) 7 DAA-2	
			bean (s) 7 DAA-3	
After 10 days		Bean	bean (s) 10 DAA-1	28-12-2012
			bean (s) 10 DAA-2	
			bean (s) 10 DAA-3	

After 15 days		Bean	bean (s) 15 DAA-1	02-01-2013
			bean (s) 15 DAA-2	
			bean (s) 15 DAA-3	

2.2.2.2 Preservation of samples

Each sample packet contained 50-200g of bean and cauliflower respectively. They were kept in a freezer by wrapping with clean airtight polythene bag (jeeper lock) at temperature below -15^{0}C. Each bag was labeled according to sample name, sample ID and date of collection with permanent marker.

2.2.3.1 Extraction

Edible part of each vegetable sample (20 g) was cut into small pieces and homogenized by means of a kitchen blender. 20 mL ethyl acetate was added to 10 g homogenized sample in 50 mL Teflon tube and shaken for 1 minute in hand & vortex for 1 min. 6 g anhydrous $MgSO_4$ & 1.5 g NaCl were added and vortexed for 1 min and then centrifuged for 5 minutes at 4000 rpm. 10 ml supernatant solution was taken in 100 mL RB flask, evaporated in rotary evaporator and then reconstituted in n-hexane (2 mL). This extract was kept in an airtight container and put in a freezer at temperature below -15^{0}C before clean up (Anastassiades et al., 2003) **(QuEChERS method)**.

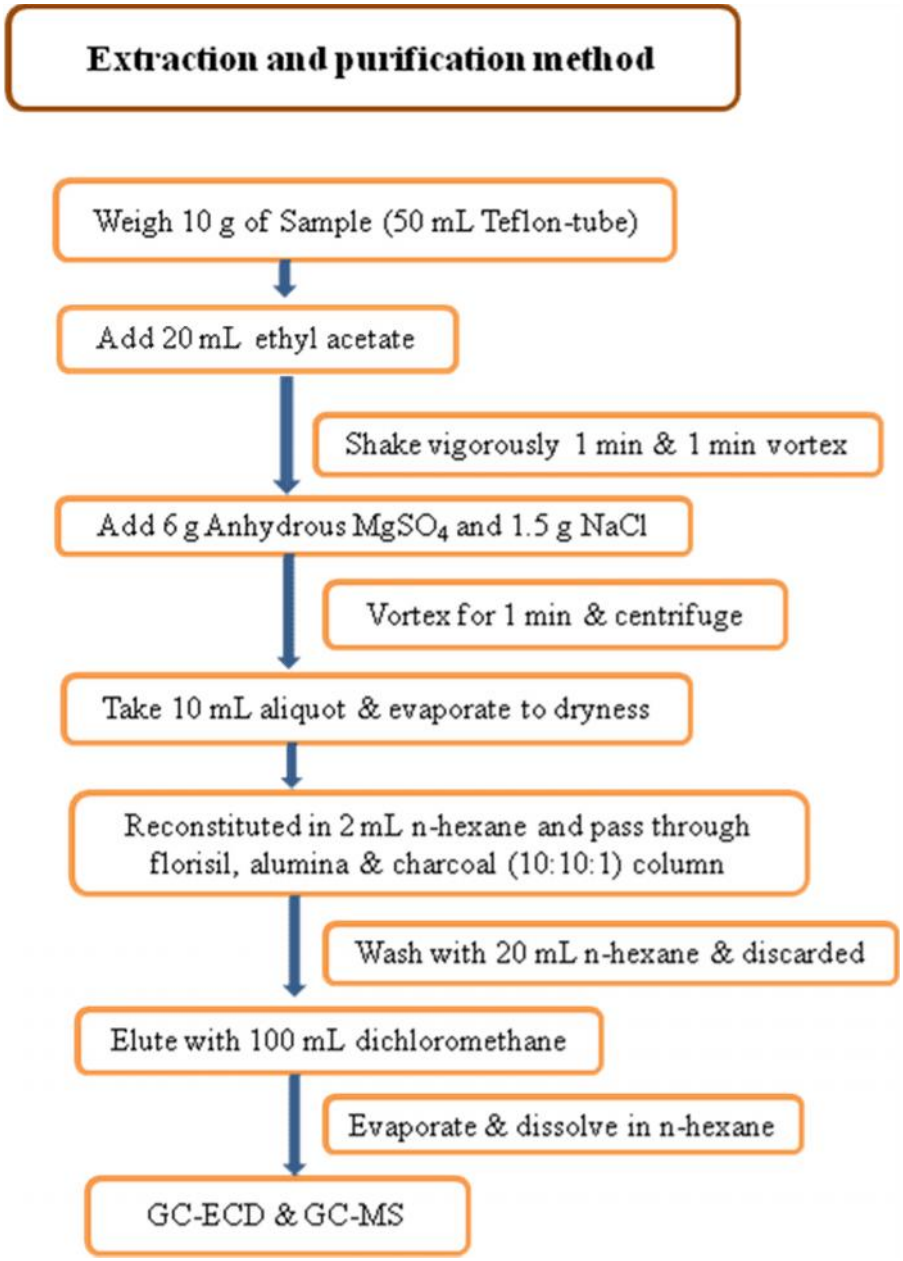


Figure 10: flow diagram of the extraction and purification procedure.

2.2.3.2 Purification

A glass column (40 cm long & 12 mm internal diameter) was packed with a 10.5 g mixture of aluminum oxide, florisil and charcoal (10:10:1) in n-hexane. The column was equilibrated with 50 mL n-hexane and then the sample extract in n-hexane (2 mL) was transferred to the column. The column was washed with 20 mL of n-hexane and eluted with 100 mL of dichloromethane at the rate of 1 $mLmin^{-1}$. The eluent was concentrated to dryness on a rotary evaporator & dissolved the residue in 2 mL of n-hexane and injected to GC-ECD.

2.2.3.3 Analysis and Quantification by GC-ECD to obtain dissipation pattern

The final cleaned extracts were analyzed in GC-2010. A Shimadzu 17A GC system equipped with Electron Capture Detector was used for the determination of pesticide residues in the samples. A HP-5MS fused silica capillary column (30 m long × 250 µm i.d. and 0.25 µm film thickness) from Agilent, USA was used for the separation. Nitrogen was used as a carrier gas. The injector temperature was 280^0C. The detector temperature was 300^0C for cypermethrin. All injections were made in split-less mode and injection volume was 1µL. The oven temperature was programmed as: For Cypermethrin - initial temperature of 120^0C held for 2 minute; increased at 10 ^{0}C min^{-1} to 270 ^{0}C; held for 1 min. and then another increased at 2^0C min^{-1} to 290 ^{0}C; held for 3 min. The column flow was 1.0 mL min^{-1} for cypermethrin. The four peaks of cypermethrin were obtained at a retention time of around 26 minute. The sum of the areas of the four peaks was used for calculation.

2.2.4.1 Preparation of standard solutions: Preparation of primary, secondary and working standard solutions

The known amount (0.0109g) of the analytical grade cypermethrin was dissolved in a definite volume of n-hexane (10.0 mL) and the concentration of the standard was calculated. Mass of the 91% certified standard was adjusted to 100% by calculation to determine the accurate concentration. Again, known volume (1.0 mL) of commercial cypermethrin was diluted in a definite volume (100.0 mL) of n-hexane and the concentration was calculated. Primary and secondary standard solutions of cypermethrin were prepared followed by Section 2.1.3.1 & 2.1.3.2. Secondary standard solutions were diluted to get 0.1, 0.25, 0.50, 1.00, 2.50, 5.00 and 7.50 ppm working standard solutions to make calibration curves.

2.2.4.2 Calibration Curve

Four different level concentrations of certified standard cypermethrin were prepared to obtain the standard calibration curve. Similarly, four same different level concentrations of cleaned up commercial cypermethrin were also prepared to obtain the calibration curve for commercial cypermethrin.

Different concentration solutions prepared for calibration curve are listed in Table 4:

Table 7: Concentrations of cypermethrin solutions prepared for calibration curve of purity analysis.

Initial oncentration, mg/kg	volume taken, mL	total volume, mL	final concentration, mg/kg
1000.0 (primary)	0.5	10.0	50.0 (working)
50.0 (cleaned up)	1.0	2.0	25.0
25.0	1.0	2.0	12.5
12.5	1.0	2.0	6.25
6.25	1.0	2.0	3.125

2.2.4.2 Standard calibration curves

The calibration curves were made by injecting solutions at concentrations 0.025, 0.050, 0.125, 0.050, 1.00 and 2.00ppm of the respective cypermethrin in the GC and plotting the integrated peak areas against the standard concentration using MS Excel software. The calibration curves of the cypermethrin standards are presented in the Figure 12. Peak areas below the detection limit were not included in the curves. Correlation coefficients (r^2) were found to be linear with 0.998 for cypermethrin.

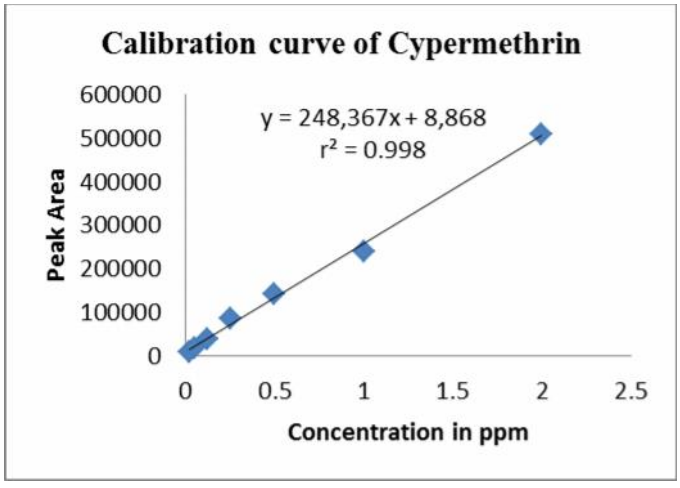


Figure 12: Calibration curves of analytical cypermethrin

2.2.4.3 **Identification** and **Quantification** of **Cypermethrin** by **GC**

For identification of cypermethrin present in the bean and cauliflower samples, reference standard sample was used. The four peaks of cypermethrin were obtained at a retention time

of around 23 minute. By comparing the retention time of peak present in the sample chromatogram with the chromatogram of standard solution, cypermethrin was identified. Cypermethrin shows a group of four close peaks in the chromatogram because of having different stereoisomers.

Solutions of different concentrations were made from the certified standard and commercial cypermethrin. These solutions were analyzed and using sigma plot software, calibration curves of cypermethrin were made. From the calibration curve, the amount of cypermethrin present in the sample was calculated. The sum of areas for all four peaks was considered to determine the concentration in the standards and samples.

2.2.5 Recovery, LOD & LOQ of vegetables samples

2.2.5.1 Recovery

The extraction efficiency was assessed by doing recovery experiment. The recovery experiments were done with control samples collected from field before pesticide spraying which were initially confirmed that there were no pesticides. The recovery experiments were conducted in three replicates at two fortification levels (0.25 mg/kg and 1.0 mg/kg). The spiked samples were allowed to stand for 1 h to allow for the adsorption of pesticide onto the samples. They were then extracted, cleaned-up and analyzed following the above-described procedures. The recoveries were 77.26 – 100.51% for cypermethrin in bean, 81.98 – 124.04% for cypermethrin in cauliflower with precision below than 13.88% .

2.2.5.2 Method justification

At first the control vegetables (samples collected before pesticide spraying) matrices were confirmed that there were no pesticides by doing blank experiments. The recovery experiments were done with these control matrices. The recovery experiments were performed in three replicates at three fortification levels. The fortified samples were left to stand for 1 h to allow for the adsorption of pesticide onto the samples. These samples were then extracted, purified and analyzed following the mentioned procedures.

2.2.5.3 Specificity

The unnecessary compounds interfering with the analytes were examined by comparing the chromatograms of the standard, blank sample and fortified sample. There were no interference peaks at the retention time of cypermethrin.

2.2.5.4 Linearity. In this study, calibration curves were prepared in matrices that extracted from control matrix (bean and cauliflower) and the linearities, limits of detection (LODs), and limits of quantification (LOQs) were calculated (table-16). Matrix matches calibration curves (cypermethrin in bean & cauliflower) were constructed in the range of 0.025–2 mg/L (table-2). The linearity was excellent with a correlation coefficient of $r^2 \geq 0.99$. The residual concentrations of cypermethrin in the treated samples were determined via the matrix matched calibration curves developed herein.

2.7.5 Limits of detection and quantification

The limit of detection (LOD) defined as the minimum concentration of analyte in the test sample that can be measured with a stated probability that the analyte is present at a concentration above that in the blank sample. The limit of quantification defined as the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test (Codex, 1993). The LOD and LOQ were found 0.01 mg/kg & 0.033 mg/kg for the cypermethrin in bean & cauliflower.

2.3 Calculations

2.3.1. Determination of purity of commercial cypermethrin

From the calibration curves of the commercial cypermethrin and 91% pure certified standard cypermethrin, the purity of commercial cypermethrin was calculated from the area ratio of a middle concentration taken arbitrarily. Using the “sigma plot” software, the slope (m) and intercept (c) of both calibration curves were obtained to perform the calculation.

The purity of commercial cypermethrin was obtained using the following equation.

Purity

$$= \frac{\text{Area for concentration X, calculated from commercial cypermethrin calibration curve}}{\text{Area for concentration X, calculated from analytical cypermethrin calibration curve}} \text{x} 100\%$$

Area of concentration X (Y) = slope (m) x concentration (X) + intercept (c)

2.3.2. Determination of recovery

A blank sample was spiked with a commercial cypermethrin solution of known concentration. After spiking, the sample was kept for 25 minutes before extraction to let cypermethrin penetrate the sample matrix. The observed area due to cypermethrin in the chromatogram for the spiked sample was compared with the area obtained by calculation from the calibration curve for the same concentration. Thus the recovery was calculated using the following equation.

Recovery

$$= \frac{\text{Area observed for spiking concentration X in blank}}{\text{Area calculated for concentration X from analytical cypermethrin calibration curve}} \text{ x } 100\%$$

2.8.3. Determination of concentrations of cypermethrin in cauliflower and bean samples:

Using the value of slope (m) and intercept (c) of the calibration curve concentration of cypermethrin in mg/kg was calculated using the following formula.

$$\textbf{Concentration=} \frac{\text{Area observed - intercept}}{\text{slope x relative recovery x amount of sample}} \text{ mg/kg}$$

2.4 Preparation of dissipation curves

There were two replicates for each sample. Mean concentration of two replicates were calculated for each sample. Mean concentrations were plotted VS day of collection after spraying for both recommended dose.

2.5 Analysis by Gas Chromatography

GC-2010 a SHIMADZU Gas Chromatograph (Figure 5) having Electron Capture Detector (ECD) was used for identification and quantification of cypermethrin. ECD was very sensitive and Limit Of Detection (LOD) was 1 fg level. Capillary column HP-5ms, of 30 meter length and 0.25 mm Inner Diameter (ID) was used for the analysis. 1micro liter of sample was injected to the column. To minimize the error the Standard reference standard sample was diluted first into stock solution which is primary standard and from the stock solution working standard was prepared.

All the solutions were kept and preserved in screw cap test tubes leveled with proper marking by permanent ink and stored in a refrigerator. To make sure the minimum evaporation of solvent all the test tubes were wrapped with parafilm. During storing of standard sample if there were any loss of solvent, more solvents were added up to the mark and made homogeneous by vortex.

2.5.1 GC Condition for GC-2010 to analyze cypermethrin

- Column: (HP-5ms) length-30m, Inner diameter-0.25mm
- Film Thickness-0.25µm.
- Carrier gas: Nitrogen
- Make-up gas: Nitrogen
- Injection mode: Splitless/split
- Injection volume: 1.0 µL
- Injector temperature: 280^0C
- Column flow: 1mL/min
- Column oven temperature program:

Rate, ^{0}C/minute	Temperature, ^{0}C	Hold time, minute
0.0	120.0	1.0
10.0	270.0	10.0

- Total program time: 26.00 minute
- Temperature of the detector: 300°C

2.6 Analysis of residual DDTs in fish samples

2.6.1 Sampling

2.6.1.1 Sampling sites of fish samples

fish were collected from Titas basin of Brahmanbaria and Modhukhali of Faridpur. The River Titas, is a trans-boundary river of south eastern Bangladesh (Fig. 1). It originates in the state of Tripura in India and flowing near Agartala (India), it enters Bangladesh through Akhaura Upazila in the Brahmanbaria District of Bangladesh, then merges with the Meghna River to the south of near Ashuganj. The length of the river is about 98 km and 50-80 m wide in the dry season. During the dry season, the upper reaches of the river become dry, except for some pools. After the onset of the monsoon, (July-November) this area floods and forms the floodplain. The present study was conducted from November 2002 to June 2003 at Goshaipur to Chitri about 12 km at lower reaches of the river.

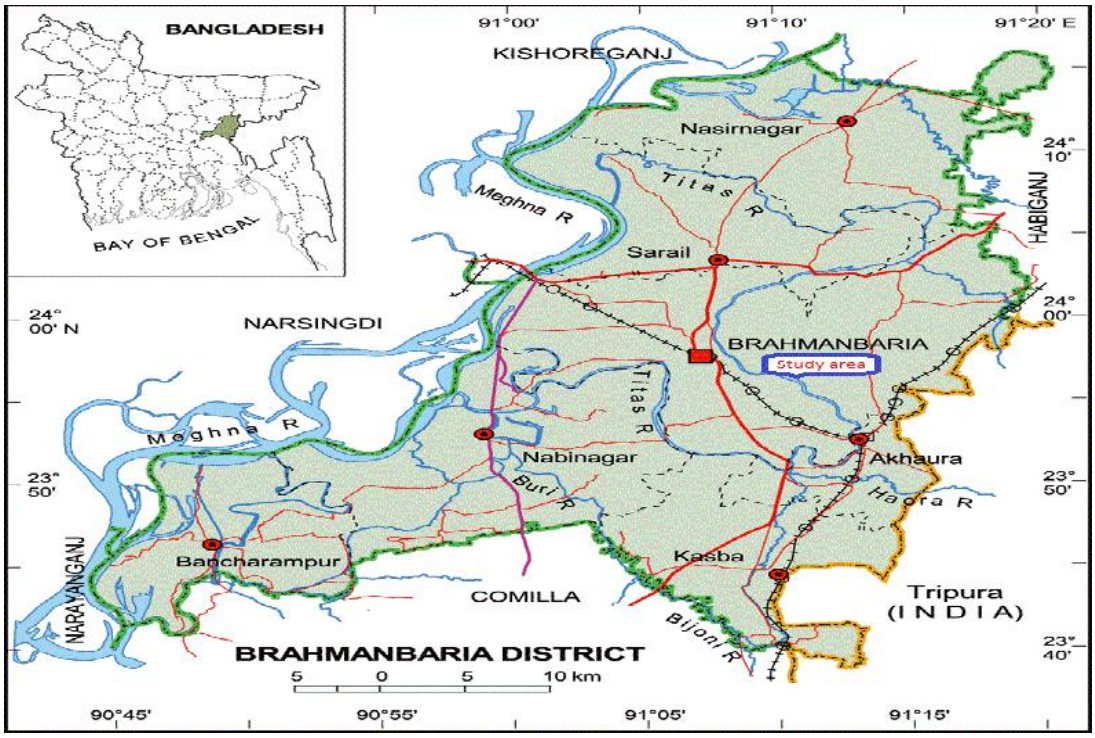


Fig. 13. Map of Brahmanbaria district showing the River Titas and study area

& F**aridpur District** (DHAKA division) with an area of 2072.72 sq km, is bounded by RAJBARI and MANIKGANJ districts on the north, GOPALGANJ district on the south, DHAKA, MUNSHIGANJ and MADARIPUR districts on the east, NARAIL, MAGURA and RAJBARI districts on

the west (Fig. 2) . Once upon a time the district consisted mainly of depression based marshland. But the alluvial soil of the PADMA made the soil fertile. Average highest temperature 35.8°C and lowest 12.6°C; annual rainfall is 1546 mm. Main rivers are Padma, Old Kumar, ARIAL KHAN, Gorai, Chandana, Bhubanshwar and Lohartek; main depressions are Dhol Samudra, Beel Ramkeli, Shakuner Beel, Ghoradar Beel. Both the area include fishes and invertebrate organisms, including gastropods, bivalves, ostracods, decapods, copepods, leeches, sponges (Banglapedia, 2010).

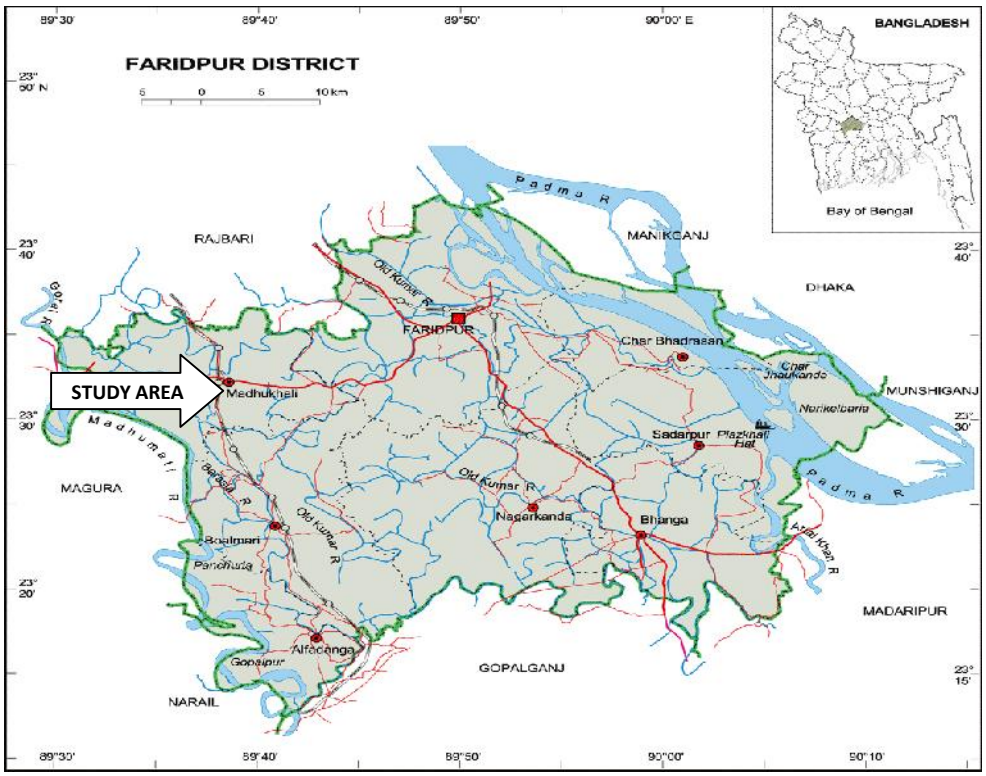


Fig 14 : Map of Faridpur district including Modhukhali area

2.6.1.2 Experimental fishes

There are Two fresh water fish (5-10 kg) samples such as *Mystus cavasius* (Hamilton, 1822) and *Channa punctatus* (Bloch, 1794) were collected from Modhukhali, Faridpur and Titas Basin of Brahmanbaria (fish market) on 12th May and 23th May respectively and the efishes were identified by Shafi and Quddus (1982). After collection of the fish samples, each of the vital organs such as muscle, gills, livers, digestive tract and gonad etc. were chopped and homogenized in kitchen blender and taking extraction and clean up the samples separately. The name of the fish samples, their family and nature are given in **Table-8**.

Scientific name	Local name	English name	Family	Size (cm) in total length	Weight (g)	Nature
Fresh fish samples						
Mystus cavasius (Hamilton,1822)	Gulsha	Cat fish	Bagridae	20-30	70-140	Carnivore
Channa punctatus (Bloch)	Taki	Snake head	Channidae	15-25	90-170	Carnivore

Table 8: List of the experimental fish samples

Fig 15: Gulsha (*Mystus cavasius* Hamilton, 1822) and Taki (*Channa punctatus* Bloch, 1794)

2.6.1.3 Sample storage

All the collected fish samples was wrapped with aluminium foil and kept in a chilled box and transferred immediately to the laboratory. All the collected fish samples were stored in a freezer at a temperature below -15 ^{0}C until dissection.

2.6.2 Extraction procedure of fish samples

Each of the collected fish sample was taken out from the freezer. Scales and bones of the fish samples were removed. Then separate vital parts of body i.e. muscle, gill, liver, digestive tract and gonad collected in different petri-dish. The muscle of the fish was chopped into

small pieces and homogenized by a kitchen blender. The homogenized samples were divided into several portions of 10g for replicate analysis. Representative homogenized sample (10 g) was taken in a Teflon tube (50ml volume). Then, it was extracted by vortex mixing for 1 minute successively with 10mL ethyl acetate. Add 6g MgSO4 and NaCl as water remover. The extracts were centrifuged for 5 minutes and the filtrates in a round volumetric flask. The solvent was exchanged from ethyl acetate to n-hexane by evaporation and the volume of the extract was adjusted up to 2mL. From this 2mL of the extract was transferred quantitatively in a vial for analysis of DDTs **(QuEChERS method,** Anastassiades et al., 2003**)**. The extraction procedure is presented in **Scheme-1.**

Scheme-1 Extraction procedure of fish samples (QuEChERS method)

Homogenized fish flesh (10 g)

↓

Chopped into small pieces

↓

Homogenized by a kitchen blender

↓

10 g homogenized sample

↓

Add 10mL Ethyl Acetate

↓

Vortex for 1 minute

↓

Add 6g MgSO4 and 1.5g NaCl

↓

Vortex for 1 minute

↓

Centrifuge for 5 minutes

↓

Collect 5mL supernatant and evaporate

↓

Change of ethyl acetate into n-hexane

2.6.3 Clean-up

2.6.3.1 Saturation of sulphuric acid

Concentrated Sulphuric acid (80 mL, 98%) was taken in a reagent bottle and 20 mL of n-hexane was added to the acid. It was shaked about one minute and kept for 5-6 minutes to separate the two phases. The lower sulphuric acid phase, saturated with n-hexane was collected and the upper phase was discarded.

2.6.3.2 Clean-up procedure of fish samples

The extract (~2 mL; in a graduated test tube) was treated with concentrated sulphuric acid (2 mL) saturated with n-hexane and the test tube was inverted by an inverter carefully ~40 second (not shaked) and vortex for 30 seconds. Then the content was centrifuged for 5 minutes at a rate of 4000rpm to separate the two layers. The upper clean organic phase was taken into a clean and dried a vial for analysis with GC-ECD **(Åkerblom, 1995).**

The clean-up procedure is demonstrated in **Scheme-2**.

Scheme-2 Clean-up procedure for fish sample extra

2 mL sample extract + 2 mL saturated conc. H_2SO_4

↓

Inverted 30 times

↓

Centrifuged (5 min)

↓

Organic phase

↓

Volume adjusted to ~ 1 mL

↓

GC-ECD analysis

2.6.4 Equipment

For extraction of DDTs from all kind of samples a centrifuge machine (Hettich Universal 2S, Germany) was used. For clean-up, a small centrifuge machine, model 800, Xiangshui Fada Medical Apparatus Factory, China was used in the present work.

All glass apparatus were dried after cleaning in an oven (EYELA, Rikakikai Co. Ltd. NDO-450 ND, Tokyo Japan) at 105 °C. Mixing of small amount of solvents were done by a Vortex machine purchased from VWR International, Germany. Vegetable samples were extracted with Ultra Turrax, purchased from Sweden (IKA-WERK).

2.6.5 Analysis of Organohalogenated compounds by Gas Chromatograph with an electron-capture detector

GC-2010 a SHIMADZU Gas Chromatograph (Figure 5) having Electron Capture Detector (ECD) was used for identification and quantification of cypermethrin. ECD was very sensitive and Limit Of Detection (LOD) was 1 fg level. Capillary column HP-5ms, of 30 meter length and 0.25 mm Inner Diameter (ID) was used for the analysis. 1μL of sample was injected to the column. To minimize the error the Standard reference standard sample was diluted first into stock solution which is primary standard and from the stock solution working standard was prepared.

All the solutions were kept and preserved in screw cap test tubes leveled with proper marking by permanent ink and stored in a refrigerator. To make sure the minimum evaporation of solvent all the test tubes were wrapped with parafilm. During storing of standard sample if there were any loss of solvent, more solvents were added up to the mark and made homogeneous by vortex.

2.6.5.1 GC Condition for GC-2010 to analyze organohalogenated compounds

- Injection volume: 1.0 uL
- Injection mode: splitless
- Injector temperature: 220^0C
- Career gas: He
- Column flow: 1.00 mL/min
- Make up gas: N_2/Air
- Column oven temperature program:

Rate, ^{0}C/minute	Temperature, ^{0}C	Hold time, minute
0.0	120.0	1.0
10.0	285.0	4.0

- Total program time: 16.00 minutes
- Temperature of the detector: 290^{0}C
- Flow rate: 2 ml/min

2.6.6.1 Preparation of primary standard solutions

A known amount of the standard (10 mg)4,4′-DDE, 4,4′-DDD 2,4′-DDT and 4,4′-DDT were dissolved in definite amount of solvent (100 mL; *n*-hexane) in a volumetric flask so that the concentration each solution becomes 30ppm. Equal volumes of all the three solutions are then taken and mixed together to form 10 ppm solutions. These solutions were transferred to different narrow test tube, with PTFE lined screw cap. These solutions were labeled indicating name of the standard, solvent, concentration and date of preparation and signature. The meniscuses of the solutions were marked with permanent ink. These solutions were stored in the freezer (-20 ^{0}C) remote from the pesticide residue laboratory.

2.6.6.2 Preparation of secondary and working standard solutions

The primary standard solution was taken out from the freezer, allowed to come at room temperature. Then a definite amount of the solution was withdrawn after checking the meniscus mark and put a new mark in the stock solution after withdrawing. The withdrawn solution was diluted serially with the appropriate solvent to prepare secondary and working standard solutions. This solution was labeled indicating substance, solvent, concentration, date of preparation and finally the meniscus of the solution was marked with permanent ink.

2.6.7 Calibration curve

Working standard solutions were serially diluted from 0.05 to 0.0025 ppm. Each of the diluted standard solution was injected to GC-ECD. The calibration curve of each of the standard was made by plotting area vs concentrations. By using the formula $y = mx + c$, the concentrations of samples can be calculated from these calibration curves.

Here, y = Peak area

x = Concentration

m = Slope of the calibration curve

c = Intercept

The calibration curves for standard DDE, DDD, 2,4 DDT and 4,4 DDT are given below **(Figure-16, 17, 18 & 19)**.

Table 9: The concentrations of standard solutions of DDE and their relative peak areas.

Concentrations (ppm)	Peak Area
0.05	556729
0.025	224640
0.01	113237
0.005	45097
0.0025	22840

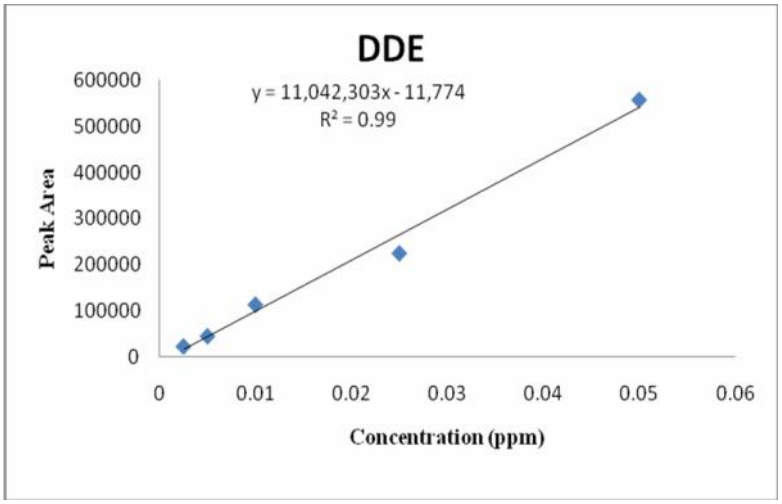


Figure 16: Calibration curve of standard DDE

Table 10: The concentrations of standard solutions of DDD and their relative peak areas.

Concentrations (ppm)	Peak Area
0.05	390359
0.025	166404
0.01	90378
0.005	39818
0.0025	20949

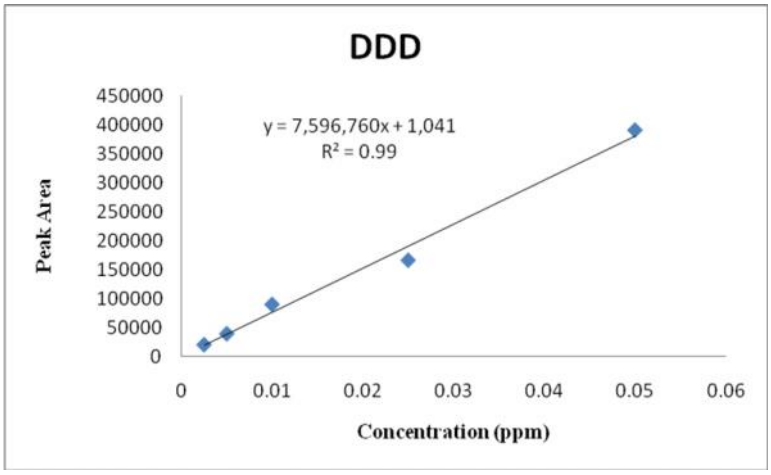


Figure 17: Calibration curve of standard DDD

Table 11: The concentrations of standard solutions of 2,4′ DDT and their relative peak areas.

Concentrations (ppm)	Peak Area
0.05	255248
0.025	112610
0.01	61381
0.005	27607
0.0025	17408

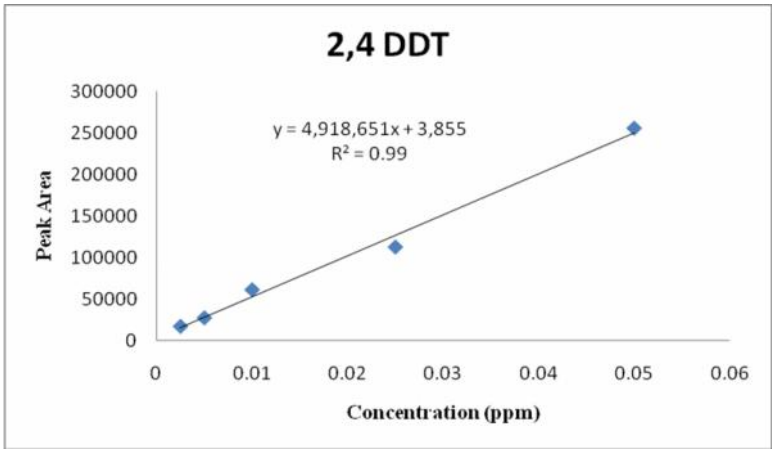


Figure 18: Calibration curve of standard 2,4′ DDT

Table 12: The concentrations of standard solutions of 4,4′ DDT and their relative peak areas.

Concentrations (ppm)	Peak Area
0.05	417662
0.025	162961
0.01	86156
0.005	26169
0.0025	13716

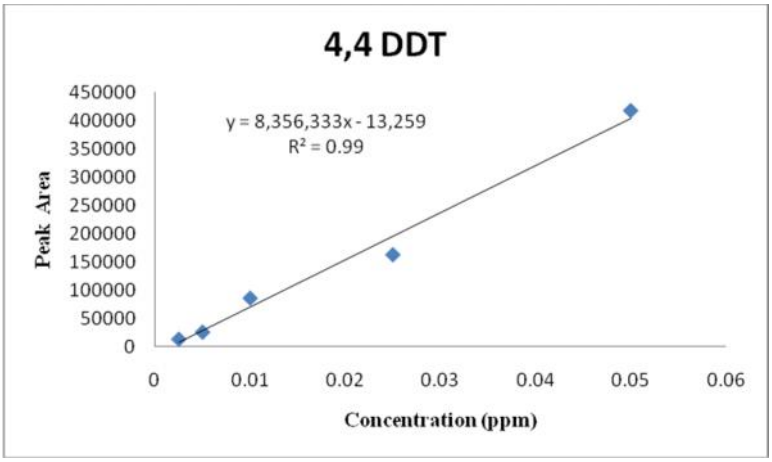


Figure 19: Calibration curve of standard 4,4′ DDT

2.6.8 Recovery experiments of fish samples

Known amount of pesticide standards (0.25 μg) were added drop by drop over to the fish control sample (10 g) and allowed the sample to stand for 30 min to let the pesticides be absorbed into the samples. The samples were extracted and cleaned-up by following the same procedure as described above. Reagent blank samples were also analyzed. The recovery of each of the pesticide was calculated by using the following formula.

$$\textbf{Recovery} = \frac{\text{Area}_{\text{Sample}} \times \text{Conc.}_{\text{Std.}}}{\text{Area}_{\text{Std.}} \times \text{Conc.}_{\text{Matrix}}} \times \frac{100}{\text{Known amount of Std}}$$

2.6.9 **Identification** and **Quantification** of **DDTs** by **GC**

For identification of DDTs present in the snakehead and gulsha samples, reference standard sample was used. The four peaks of DDTs (DDE, DDD, 2,4′ DDT & 4,4′ DDT) were obtained at retention time of around 11-12 minute. By comparing the retention time of peak present in the sample chromatogram with the chromatogram of standard solution, DDTs was identified. DDTs shows a group of four compounds in the chromatogram because of breakdown of DDT.

Solutions of different concentrations were made from the certified standard. These solutions were analyzed and using sigma plot software, calibration curves of DDTs was made. From the calibration curve, the amount of DDTs present in the sample was calculated. The sum of areas for all four compounds was considered to determine the concentration in the standards and samples.

3. Review of Literature

3.1 Pest and pesticide

Pesticides play an important role in increasing agricultural productivity. Most of them constitute serious environmental threats due to their high toxicity and persistence, leading to the complete banning or restriction on the use of some organochlorine pesticides (OCPs) during the 1970s (Herbert et al., 2006).

Pesticides can produce negative impacts, both private and social (Antle and Pingali 1994). For example, when farm workers are exposed to dangerous pesticides, this exposure can reduce farmproductivity through effects on farmer health. Excessive application of pesticide also can be ecologically damaging. Low dosages can lead to development of resistance in target populations and pesticide runoff can reduce the productivity of aquatic ecosystems.

Pesticide use in crop production has been suspected of being a major contribution to environmental pollution. There are widespread and growing concerns of pesticide over-use, relating to a number of dimensions such as contamination of ground water, surface water, soils and food, and the consequent impacts on wildlife and human health (McLaughlin and Mineau, 1996).

The usual practice of draining paddy water into irrigation canals may cause river and lake contamination. Residues carried by the water can be taken up by non-target flora and fauna, leach into soil, and possibly contaminate groundwater or potable water. (Parveen, and Nakagoshi 2001)

The third world's use of pesticides increased greatly during the Green Revolution in the 1960`s and beyond, and it is related to the changed growing conditions which was brought about by the use of green revolution varieties and technologies. (Parveen, and Nakagoshi 2001)

Increased use of chemicals on vegetables started gaining momentum and continued its up-trend in Bangladesh. Wide spread use of pesticides in agriculture concern of residue accumulation, which may remain in food and agricultural environment causing concern of human health and risking ecological balance. Attempt made to ensure that their applications were correct and safe and result in no residues in food beyond codex developed maximum residue limits. (Islam et al., 2009)

Pesticide application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhance agricultural productivity and crop yield. According to the United Nations Food and Agricultural Organization, the world's potential human food losses are about 55 percent that include pre-harvest (35%) and post-harvest (20%). In the developed countries, crop losses are estimated to be they are as high as 75% (Khan et al., 2011)

Pesticide use has resulted in acute and chronic ecological damage, either by direct injury to non-target organisms such as birds and fish, or by indirect effects such as elimination of natural enemies. Pesticides are a singular form of environmental hazard in that they are actually designed to harm living things. Many organochlorine compounds are very persistent in the environment and have a tendency to bioaccumulate significantly through food chains (UNEP, 1993).

The widespread use of pesticides not only contaminates water, soil, and air, but also causes them to accumulate in crops (e.g., fruit and vegetables). Pesticides are transported mainly by rain and wind from their points of application to neighboring crops and land, where their presence may be undesirable or harmful(Fenik et al., 2011).

3.2 Pesticides Use in Bangladesh

Pesticide as agricultural input was introduced in Bangladesh in 1957 and mainly DDT and BHC was distributed by the Government to the farmers free of cost until 1973. The pesticides become very popular to the farmers for two reasons; firstly quick and visible effect on pest and secondly, no cost involvement. In 1974, the subsidy was reduced to 50% and in 1979 it was withdrawn completely (Islam, 2000). As a result at first pesticide use declined and again gradually increased and in 1999 the amount reached 15000 metric tons. At present 84 pesticides with 242 trade names have been registered in Bangladesh (PAB, 2000).

The use of pesticides in Bangladesh is less in comparison to other developing countries. It is 0.03kg/ha compared to 0.3 kg/ha in India, 0.4 kg/ha in Sri Lanka and 0.8 kg/ha in Indonesia (Karim, 1998). Currently, 14,340.40 metric tons of commercial pesticides are used annually, primarily in the cultivation of rice, tea, jute, sugarcane and vegetables. About 70% of pesticides are used on rice. Pesticides used on rice consist almost exclusively of insecticides, but fungicides are used occasionally. In 1989-90 almost 90% of pesticides were used on rice. In Bangladesh, insect pests' outbreak is frequent in rice and crop losses occurred due to rice insect pest attack up to 80% (Kalam, 1998).

The intensification of agriculture has been accompanied by the rapid increase of insecticide use.Increased use of pesticides leads to two primary concerns (Kalam, 1998):
1) Adverse effects on the health of farm workers as well as others exposed to the pesticides.
2) Polluted ground water and surface water, causing harm to the water users as well as inland fisheries and other aquatic animals.

Biodiversity is declining due to the effect of pesticide and fertilizer use. Population of native fish species (*Channa* spp., *Heteropneustesclarias*, and *Anabas testudineus*) is now endangered and the traditional rice-fish systems have disappeared. The bird and other small wild animals are in threat of wide spread because of the use of pesticides in rice and vegetables. The rice-based agro-ecosystem is showing signs of unsustainability. (Parveen and Nakagoshi 2001)

Most of the farmers of Bangladesh are not capable of taking decisions on pest management and pesticide application. Often they apply pesticides when there is no real need or they use wrong chemicals at wrong doses, methods and times. As a result they kill the beneficial organisms easily and create pest resistance causing the greater problems and crop losses. (Parveen and Nakagoshi 2001)

3.3 Agriculture and environment in Bangladesh context

Agriculture is the most important sector of the Bangladesh economy. Approximately 84 percent of the people are directly or indirectly dependent on agriculture for the major source of their livelihoods. It provides about 32 percent of the gross domestic product (GDP) and employs 63 percent of the country's labour force (Weinberger and Genova, 2005). The sustainability of agriculture is in a threatened state from the continuous degradation of land, water and other resources basically due to application of unfriendly technologies (Rasul and Thapa, 2003).

Farmers in Bangladesh are highly dependent on pesticide application to protect their crops, without considering its detrimental effects on environment (Rahman and Hossain, 2003).
Use of broad spectrum pesticides has posed serious risk to the environment, leading to diminishing bio-diversity, hampering the growth of aquatic habitats, disrupting natural pest control, reducing earthworms, causing toxicity in soil, developing resistance among target pests and creating potential hazards to human health (Islam, 2005; Ibiayo, 2006).

3.4 Contamination by chlorinated pesticides

POPs are already strictly regulated and most of them are not currently in production. These chemicalsare prone to long-range transport through the upper levels of atmosphere and can be deposited 1000 miles awayfrom the pollution source. Through atmospheric deposition and river inputs they have spread to all aquatic environments. POPs are characterized by being lipophilic (high octanol–water partition coefficient *K*ow) and hydrophobic. Due to the persistence and lipophilic properties of the POPs, they are able to accumulate in the ecosystem. In addition to carcinogenetic/mutagenic potential they may cause toxic effects on animal reproduction, development, and immunological function (Suchan et al., 2004).

3.5 Importance of promoting ecological agriculture in Bangladesh

people are encouraged to consume more vegetables and fruits, these being a good source of vitamins and fiber and also beneficial to their health - and on the other hand, the mass media have rightly created an awareness about, but wrongly magnified the environmental and health problems and the risk involved in the use of chemicals, especially pesticides, in agriculture. Consequently, this has created a certain apprehension and fear in the public as to the presence of pesticide residues in their daily food. The public is confused and alarmed about food safety. The results of a market survey carried out during the year 1997 to determine the level of residues of some insecticides in the seasonal vegetables and fruits available on the local markets are presented to give a truer picture of the situation(Porto, 2011)

The Bangladesh Environment Conservation Act, 1995 (Amended in 2002 followed by the Bangladesh Environment Conservation Rules, 1997) was made for the conservation of the environment and control and mitigation of environmental pollution (ESDO, 2005).
According to the United Nations Economic and Social Commission for Asia and the Pacific (UESCAP), the Integrated Pest Management (IPM) is a suitable tool of managing pests efficiently and safely leading to sustainable agriculture. Besides, IPM has the potential to integrate various eco-friendly techniques of producing healthy crops by minimizing pesticide use, conservation of bio-diversity, augmentation of biological control agents and use of pest tolerant crop varieties, thereby increasing cost-effectiveness (UNESCAP, 2002).

Unfortunately, farmers of the country are not following recommended pest management practices due to various reasons. Many empirical studies identified that the lack of organic fertilizers, poor level of farmer's knowledge of arthropods, lack of skills and motivations

behind farmer's pesticide use, lower yields of crops, complex nature of IPM, lack of evidence for the value of ecological agriculture and insufficient demonstration plots are the key roadblocks to adopt IPM practices widely (Datta and Kar, 2006).

In field studies conducted in India, the maximum deposits of cypermethrin, fenvalerate and deltamethrin applied to cabbages at 50, 50 and 12 g a.i./ha were 0.34, 0.96 and 0.25 mg/kg, respectively, on heads, 1.34, 0.08 and 0.30 mg/kg, respectively, on leaves. These values were within the maximum residue limits of 2 mg/kg cypermethrin and fenvalerate on lettuce, and 0.5 mg/kg for deltamethrin or: leafy vegetables. Most of the insecticide residues were found on the outer leaves and it was concluded that the residue levels found do not constitute a health hazard 10 consumers (Singh et al. 1992).

One potential drawback associated with a shift toward more intensive vegetable production is the common reliance of most vegetable producers on heavy application of pesticide (Hossain*et. al.*, 2000). Among the vegetables grown in Bangladesh, country bean is a valuable and very popular one. It has been reported that in this vegetable the attack of insect pests are severe and farmers sprayed insecticides quite frequently even every day (Khatoon*et. al.*, 2004).The detection, identification and quantification of pesticide in the food becoming the public interest. But very little references are available on the presence of pesticides in vegetables in Bangladesh (Khatoon*et. al.*, 2004).

3.6 Analyzing methods of pesticide

Anastassiadeset al.2003, described the ‘‘Quick, easy, cheap, effective, rugged, and safe’’ (QuEChERS) method for the multiclass, multiresidue analysis of pesticides from different food matrices with high water content.

This method is particularly popular for the deterination of polar, middle polar and non-polar pesticide residues in various food matrices, due to its simplicity, low cost, amenability to high throughput, and relatively high efficiency results, as well as the minimal number of steps it involves (Herrero Martín et al., 2010). Recently, the QuEChERS method for multiple pesticides in fruits and vegetables has received the distinction of the Official Method of AOAC International (Lehotay, 2007)

3.7 Pesticide Residue Tolerances (MRLs, ADI)

ADI values of a number of pesticides have been published jointly by the World Health Organization. FAO and WHO through their Codex Committee on Pesticide Residues (CCPR) have worked out international pesticide residue tolerances which are intended as guidelines for world-wide national legislation. These- tolerances (maximum residue levels, MRLs), are set according to the philosophy that no crop should be treated with pesticides at higher application rates than necessary (good agricultural practice). Essentially, the MRL for a named pesticide is the highest concentration that may be present on a commodity at the time of marketing(FAO, 1982a).

3.8 Analytical Facilities for Residue Measurement

The detection and analysis of most common insecticides and many herbicides and fungicides can be undertaken in an analytical laboratory having a minimum two gas chromatography (GC) with choice of appropriate detectors such as Electron capture and flame photometry detectors for OC and OP compounds respectively. Numerous minor but essential items for extraction and clean-up would be necessary back up apparatus. To address all relevant issues of monitoring and research on uses of pesticides in food and environment continuous development of laboratory infrastructure is essential. In view of the vast sum of money spent on pesticides and the danger foreign exchange earnings if food for export is condemned and for the safety of man and the environment, this sum of money spent on development of residue analysis infrastructure may be regarded as trivial. It may be stressed that adequately trained personnel and principle of Good Laboratory Practices (GLP) are vitally important for pesticide residue analysis laboratory which can only be gradually, developed through hard work and sincere but continuous efforts. Moreover, provision for adequate supplies of residue grade solvents, specialized glass wares are routinely required for such analytical work (FAO, 1990).

The world health Organization (WHO) and national government authorities such as United States Department of Agriculture, the Department of Health and Human Services, the National Cancer Institute and the American Cancer Society strongly recommend that people should eat plenty of vegetables, fruits, and grain products. Scientists believe that consuming such a diet will help lower the risk of heart disease, obesity, and some cancers. Fortunately, while health experts are urging us to increase our consumption of fruits, vegetables and grains than the food production system are contaminating day by day by residual effect of

insecticides (Codex, 1993).

3.9 Review of Insecticide Residues

Khan (2011) conducted an experiment during crop season 2000, the initial residues cypermethrin obtained by HPTLC were 0.67 mg/kg. After 10 days, it was dissipated to 0.10 mg/kg, thus representing a loss of 85%. The samples did not contain any detectable residues 15 days after application. However, analysis by HPLC gave initial residues of 0.86 mg/kg which were dissipated to 0.09 mg/kg in 15 days. The year 2001, the initial residues of cypermethrin on tomato fruits by HPTLC methods were found to be 0.87 mg/kg which were reduced to 0.10 mg/kg after 15 days. Half-life values of cypermethrin in tomato fruits varied from 3.63 to 4.50 and 5.90 to 6.84 days during crop season 2000 and 2001, respectively. The withholding periods from 6.82 to 8.59 days for cypermethrin during 2000. In the year 2001, the periods were 11.59 to 13.54 days for cypermethrin. This is still the main method used for the analysis of fruit and vegetables in Sweden (Åkterblom, 1995).

According to Singh and Walker (2006) Gas-liquid chromatography determination of Cypermethrin residues in tomato fruits, leaves and soil samples drawn at 0,1,2,5 and 10 days after treatment were analyzed. The analytical process was done by silica gel column clean-up and 63 Ni gas liquid chromatographic estimation. The minimum limits of cis-permethrin and trans-cypermethrin were 0.008 and 0.006 mg/kg. Initial deposit of Cypermethrin on fruit and was observed 0.73 mg/kg after eighty sprays at 50g/kg a.i/ha application rate, which declined to 0.61 mg/kg one day after treatment and then became 0.08 mg/kg after 10 days.

Following studies were conducted on the residues of commonly used pesticides in/on vegetables in India. Detectable levels or residues were observed in 33.3% of tomatoes (Diazion, Endosulfan, Dimethoate and Monocrotophos), 73.3% of eggplant (Endosulfan, Diazinon, Cypermethrin, Fenvalerate, Quinalphos, Dimethoate and Monocrotophos), 14.3% of okras (Endosulfan), 88.9% of cabbage (Endosulfan, Fenvalerate, Cypermethrin, Dimethoate and Monocrotophos). However, the levels of pesticide residues were lower than the maximum residue limits (MRL) prescribed (Datta and Kar,2006).

3.10 Bioaccumulation, Biomagnification and Bioconcentrationof pesticides

Chlorinated organic compounds have a wide range of industrial and agricultural applications, and include organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethane (DDT) and Lindane, as well as the polychlorinated biphenyls (PCBs). Moreover, these compounds are chemically and biologically recalcitrant and readily undergo bioaccumulation in both terrestrial and aquatic organisms. Introduction of these compounds into the marine environment via atmospheric deposition, oil spillages and sewage discharges results in their biomagnification in the food chain, ultimately posing a risk to human health. Indeed, POPs are now routinely detected in fish and wildlife, as well as human adipose tissue, blood and breast milk (Basheer et al., 2005).

The concentrations and biomagnifications of dichlorodiphenyltrichloroethane (DDT) and its metabolites were examined in four fish species (*Clariasgariepinus, Oreochromisniloticus, Tilapia zillii,* and *Carassiusauratus*) from Lake Ziway, Rift Valley, Ethiopia. Paired stomach content analysis, and stable isotope ratio of nitrogen (δ^{15}N, ‰) and carbon (δ^{13}C, ‰) were used to study the trophic position of the fish species in the lake. 4,4′-DDE, 4,4′-DDT and 4,4′-DDD were the main DDTs identified in the fish samples, with 4,4′-DDE as the most predominant metabolite, with mean concentration ranging from 1.4 to 17.8 ng g^{-1} wet weight (ww). The presence of DDT in all tissue samples collected from all fish species in the lake indicates the magnitude of the incidence (Deribe et al., 2013)

Organochlorine pesticides (OCPs) and other persistent organic pollutants are of great concern due to their longevity, endocrine disrupting effects, and bioaccumulation [1–3]. Despite a ban onOCP production effective since the mid-1970s, OCPs have recently been found in air [4], water [5], soil [6], and sediment [7,8]. In aqueous and marine environments, OCPs tend to have strong affinities for suspended particulates and accumulate in sediment. The determination of OCP levels in sediment can therefore indicate the level of contamination and bioaccumulation in aquatic organisms (Kim et al., 2008)

3.11 Degradation of pesticides

Many factors contribute to pesticide deposition and residue dissipation, e.g. themorphology of the crop, cuticle characteristics, the stage of growth at treatment, the growth rate, the pesticide application method (formulation, rate, nozzle type) and climate rates are crop

specific, and hence its residues must be examined individually according to the prevalent climatic conditions of a country (Chai et al., 2009).

According to Muir *et al*., 1985, In laboratory and field studies, cypermethrin concentrations decreased rapidly by adsorbing to sediment, suspended particulates and plants. Also, there was rapid photochemical and microbial transformation.In mammals and birds, cypermethrin is relatively non-toxic. Studies have shown that, when ingested, laboratory rats and dairy cattle rapidly excrete cypermethrin, posing a low toxicological threat to them. For aquatic organisms and fish, there is a much higher toxicological risk. Because of its high lipo-affinity and low solubility, cypermethrin has a strong potential to bio-accumulate in aquatic animals. However, as described above, in natural waters even small amounts of sediment will adsorb a significant amount of cypermethrin, reducing bioavailability and mitigating bio-concentration in aquatic animals.

The fate of pesticides in soil is controlled by chemical, biological and physical dynamics of this matrix. Theseprocesses can be grouped into those that affect persistence, including chemical and microbial degradation, and those that affect mobility, involving sorption, plant uptake, volatilization, wind erosion, run-off and leaching. Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactionssuch as photolysis, hydrolysis, oxidation and reduction. Biological degradation takes place when soil microorganisms consume or break down pesticides (Andrew and Yolanda, 2004).

4. Results and discussion

4.1 Dissipation of cypermethrin in selected vegetables(Bean and cauliflower)

Selected vegetables bean and cauliflower grow everywhere in Bangladesh seasonally. Pesticides are used extensively by the farmers in the production of vegetables to fulfill the need of food. Cypermethrin is a modern pesticide that undergoes degradation quickly. During the present study, Cypermethrin was applied to the bean and cauliflower in the experimental fields at the recommended dose in BARI. Bean and cauliflower were harvested at 0, 1, 3, 5, 7, 10 and 15 days after application of the pesticide following standard protocol of WHO guideline. The residue level was determined in brew of both of the vegetables. In case of cauliflower, only flowering parts were analyzed. Samples were extracted and cleaned up as described in the experimental Section 2.3.1 and analyzed by GC-ECD, results are given in Table 12-15. For each day three replicates were done. Statistical analysis were done i. e. mean, standard deviations, correlation coefficients (r^2), Regression, LODs, LOQs were also calculated.

Recoveries of the pesticide were carried out and LOD & LOQ were found out (Table 13).

Table 13. Name, retention times (RT), correlation coefficients (r^2), LODs, LOQs and recoveries of the tested pesticides.

Pesticides & Vegetables	Linear range (mg/L)	RT min.	Linearity r^2	LOD (mg/ kg)	LOQ (mg/ kg)	[a]Accuracy (% recovery), Precision (% RSD) (Spiking level, mg/kg)	
Cypermethrin in bean	0.025-2.0	24 -25	0.998	0.01	0.033	105.95, 6.78 (0.25)	106.89, 7.10 (1.0)
Cypermethrin in cauliflower	0.025-2.0	24 -25	0.998	0.01	0.033	75.71, 0.51 (0.25)	82.54, 13.32 (1.0)

[a] Mean of three replicates

4.2 Calibration curve preparation

4.2.1. Calibration curve of analytical grade cypermethrin

Standard cypermethrin solutions of seven different concentrations were prepared and analyzed by GC-2010. The observed sums of areas for each solution are tabulated in table 14.

Table14: Concentration and observed total area (analytical cypermethrin solutions)

Concentration (mg/Kg)	Area
0.025	8691
0.05	17339
0.125	38567
0.25	85679
0.5	141812
1	241175
2	509866

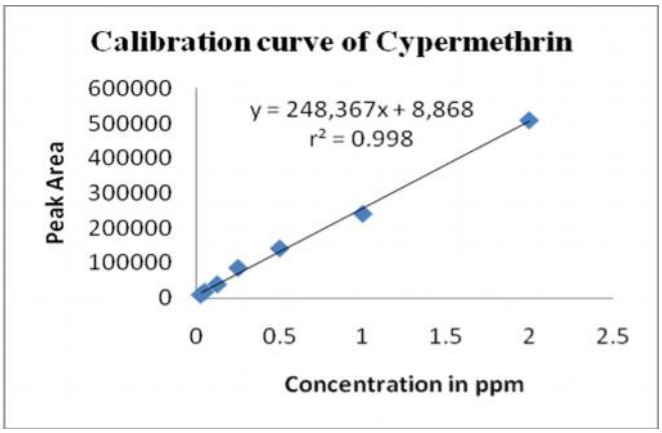


Figure18: calibration curve of standard analytical grade cypermethrin.

4.2.2. Calibration curve of commercial cypermethrin

Standard cypermethrin solutions of four different concentrations were prepared and analyzed by GC-2010. The observed sums of areas for each solution are tabulated in table 8 and calibration curve obtained by using these data is given in figure15.

Table8: Concentration and observed total area (commercial cypermethrin solutions)

Concentration (mg/kg)	**Total area**
00.000	000000
03.125	5124604
06.250	9910189
12.500	18857967
25.000	28946733

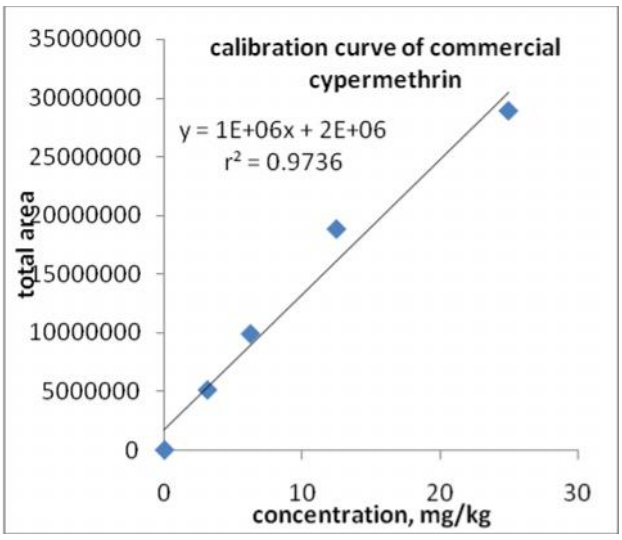


Figure20: Calibration curve of commercial cypermethrin

4.2.3 Calculation of purity of commercial cypermethrin

To determine the purity of commercial cypermethrin, areas for an arbitrary concentration,

15 mg/kg were calculated from the calibration curves of commercial and analytical cypermethrin.

Table 15: Calculated areas for concentration, 15 mg/kg

Area found for commercial cypermethrin	Area found for analytical cypermethrin
19022348.950	21862249.872

$$\text{Purity} = \frac{\text{Area for concentration X calculated from commercial cypermethrin calibration curve}}{\text{Area for concentration X calculated from analytical cypermethrin calibration curve}}$$

$$= \mathbf{87.01\%}$$

4.3Residual amount of Cypermethrin in vegetables

4.3.1 Results

Using the value of slope and intercept of the calibration curve concentration of cypermethrin in mg/kg was calculated in different samples from the values of observed area.

Average concentrations in mg of cypermethrin per kg bean and cauliflower with respect to commercial cypermethrin with recommended dose (1mL/L) in given below.

Table 16. Pesticide residues (Av. ± SD, mg/kg) in vegetables matrix at various time intervals following its application**.**

Samples	Day after spraying	Cypermethrin in Bean	Cypermethrin in Cauliflower
Control	-	ND	ND
Samples after pesticides spraying	0	3.77 ± 0.53	6.68 ± 1.86
	1	2.69 ± 0.19	2.94 ± 0.16

	3	1.13 ± 0.08	1.72 ± 0.05
	5	0.94 ± 0.11	1.12 ± 0.04
	7	0.89 ± 0.06	0.67 ± 0.05
	10	0.45 ± 0.11	0.17 ± 0.03
	15	0.32 ± 0.01	0.13 ± 0.01
MRL		0.5 mg/kg (Japan)	1.0 mg/kg (Codex)

In case of bean, the result (table 16) was found to be 3.769 ± 0.532 (mean & standard deviation), 2.689 ± 0.189, 1.131 ± 0.083, 0.938 ± 0.108, 0.892 ± 0.066, 0.446 ± 0.110 and 0.321 ± 0.012 ppm at 0,1,3,5,7,10 and 15 days after application, respectively, for recommended dose. On the other hand, for cauliflower, it was found 6.683 ± 1.857, 2.944 ± 0.164, 1.718 ± 0.051, 1.118 ± 0.046, 0.677 ± 0.055, 0.166 ± 0.029 and 0.123 ± 0.007 ppm at 0, 1, 3, 5, 7, 10 and 15 days after application, respectively.

From Table 16, it is found that the residue limit of cypermethrin in bean is 3.77 mg/kg for the sample sprayed according to recommended dose and residue limit of cypermethrin in cauliflower is 6.68 mg/kg for sample sprayed with recommended dose after two hours of spraying. It degrades rapidly and comes to almost zero at 15^{th} day after application. The dissipation curves in figure 19 and 20 shows the rate of dissipation. Both curves show a gradual declining trend.

4.4 Preparation of dissipation curves

Mean concentrations of each sample (two replicates) were obtained and plotted VS day of collection after application for both samples in recommended dose separately to prepare dissipation curves.

Figure 20 and 21 shows the two dissipation curves for recommended dose in bean and cauliflower respectively.

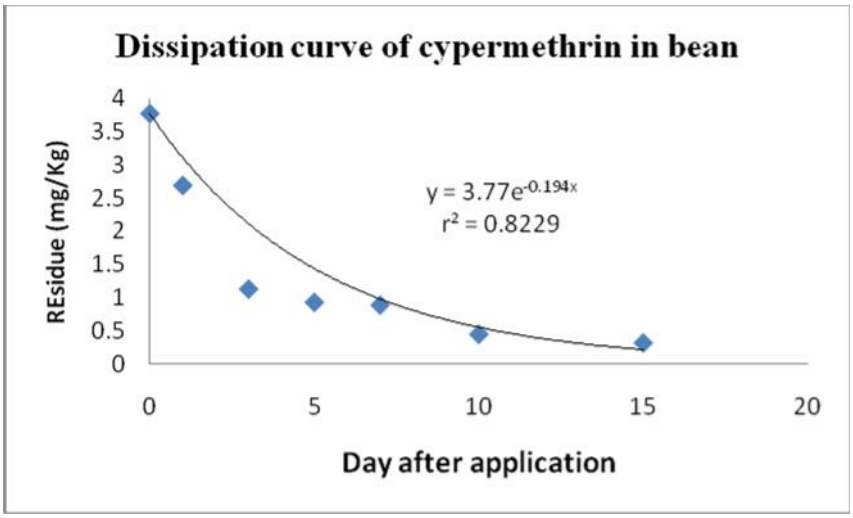


Figure 20. Dissipation curve (Residue VS day after application) for recommended dose in bean.

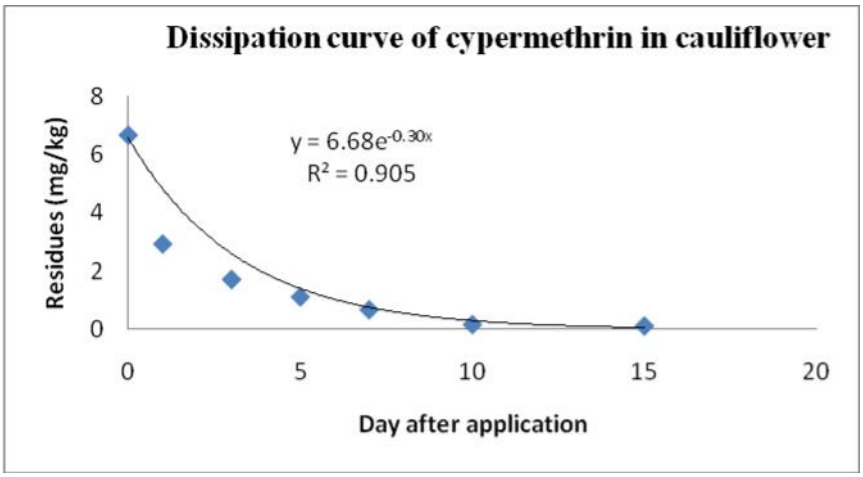


Figure 21. Dissipation curve (Residue VS day after application) for recommended dose in cauliflower.

4.5 Discussion

The Maximum Residue Limit (MRL) for cypermethrin in bean is 0.5 mg/kg according to Codex Alimentarius Commission (Codex, 1993) and the Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight. From the present study it was found that cypermethrin residue in bean is below the MRL value if it is sprayed in the recommended dose for all the samples. It takes 3 days to become the residue limit below MRL value as it is found that the residue limit is 0.44 at 10^{th} day but higher values than MRL are found for day 1 (2.69 mg/Kg) and day 0 (3.77 mg/kg).

Cypermethrin is used widely for bean and other vegetables in Bangladesh. Sometimes vegetable and fruit growers use a pesticide very frequently, even twice a day and harvest crop immediately after pesticide application. This is very dangerous in two ways. First, the pesticide residue limit is likely to be higher than MRL and hence harmful for human and environment. Secondly, pests can make some genetic changes to be resistant against the pesticide.So, farmer should know about these facts and control the use of pesticide. For cypermethrin in bean and cauliflower, they should apply cypermethrin as recommended and harvest after 2-3 days for consumer safety. They should not apply an overdose but if applied, the harvest period should be more than two weeks. And, they must never apply cypermethrin daily.

4.6 Determination of residual DDTs in different parts of Fresh Fish Samples.

Rice and fish dominate the diet of Bangladeshi to such an extent that the old proverb, "**macheebhateebangali**," which can be translated as "**fish and rice make a Bengali**," continues to hold true. Fish is an essential and irreplaceable food in the rural Bangladeshi diet. Paddy-cum fish culture is a common practice in South East Asia. Natural growth of fresh water fish decreased only due to shrinkage of surface water caused by extensive irrigation projects and river siltation but also probably due to extensive use of pesticides including organochlorine compounds (OC), other pollution. Contamination with toxic residual pesticides is at least partly responsible for fish mortality during hatching of eggs and growing of the post larvae (Hirose, 1975; Park et al., 2004; Singh and Singh, 2006).

For the purpose of boosting food production, the Government of Bangladesh supplied organochlorine pesticides including POPs free of cost to the farmers till 1970 and at reduced price up to 1980. But due to the worldwide understanding of serious adverse effects of POPs and other organochlorine pesticides these were banned in Bangladesh in phases and the industry producing DDT was closed down in 1993.

Aquatic ecosystems are the reservoirs of many contaminants. POPs enter into aquatic ecosystems either due to direct discharge or hydrologic and atmospheric processes. The use of fish for measurement of pollutants is common as fish bioaccumulate many pollutants, especially organochlorine substances thus a principal object for monitoring. Furthermore, fish

that is stationary will function as an integrator of the concentrations in water and its feed. Fish and fishery products are generally regarded as a high-risk food commodity in respect to pathogen contents, natural toxins and pesticide residues. Considering the impertinence of food safety, a number of fresh fish was analyzed to determine the Organochlorine pesticide residues.

4.6.1Recovery, LOD and LOQ

In order to make standard calibration curves, stock solution of standard reference certified samples were serially diluted to obtain five different concentrations. The calibration curves were obtained by plotting each peak area *vs*. amount of five concentrations of working standard solutions using GC-ECD. The calibration curves were linear over the range of the tested concentrations as shown by the fact that the correlation coefficients (r^2) for the linearity range were 0.995-0.999. The r^2 value was a bit higher in some cases than the value recommended by the Codex guideline ($r^2 = 0.95$). The percent recoveries for fish samples in digestive tract in case of lower recovery is 71.73% for DDE, 78.84% for DDD, 75.13% for 2,4 DDT and 94.49% for 4,4 DDT. And in case of higher recovery 73.91% for DDE, 79.70% for DDD, 72.63% for 2,4′ DDT and 92.99% for 4,4′ DDTwhich are in the range (70-130%) and acceptable for fish samples according to standard methodology(Codex, 1993).

To elucidate sensitivity of experimental method, limit of detection (LOD) and limit of quantification (LOQ) were determined by detecting analytes in blank samples. The limit of detection (LOD) is the smallest concentration from which it is possible to deduce the presence of the analyte in a blank sample, and the limit of quantification (LOQ, also called limit of determination) is the smallest measured content of the identified analyte in a sample that can be quantitatively determined with a specified confidence (Cinquina, 2003). The detection limit was found to be 0.39ppb for both DDE, DDD and for DDT it was 1.56ppb (signal: noise=3.1). The quantification limit was found 1.36ppb for both DDE, DDD and 4.89ppb for DDT (signal: noise=10:1).

Table 17. Name, retention times (RT), correlation coefficients (r^2), LODs, LOQs, accuracy, and precision of the tested pesticides.

Pesticides	Linear range (mg/L)	RT min.	Linearity r^2	LOD (mg/kg)	LOQ (mg/kg)	[a]Accuracy (% recovery), Precision(% RSD) (Spiking level, mg/kg)			
						Digestive tract	Gill	Gonad (in female)	Muscle
DDE	0.0025-0.5	12.173	0.998	0.005	0.0165	71.74 2.15 (0.02)	118.29 0.60 (0.02)	76.95 4.42 (0.02)	84.80 3.16 (0.02)
						73.91 4.82 (0.10)	91.04 7.38 (0.10)	103.28 6.99 (0.10)	70.01 0.84 (0.10)
DDD		12.813	0.999	0.005	0.0165	78.84 14.13 (0.02)	73.12 4.82 (0.02)	76.87 3.83 (0.02)	87.64 4.89 (0.02)
						79.70 5.53 (0.10)	103.26 2.39 (0.10)	89.23 5.87 (0.10)	71.00 1.58 (0.10)
2,4 DDT		12.899	0.995	0.005	0.0165	75.13 5.08 (0.02)	98.84 7.31 (0.02)	91.06 1.10 (0.02)	92.47 3.98 (0.02)
						72.63 5.12 (0.10)	94.60 1.38 (0.10)	106.52 3.93 (0.10)	70.00 1.80 (0.10)
4,4 DDT		13.426	0.998	0.005	0.0165	94.49 16.45 (0.02)	79.93 5.72 (0.02)	108.25 6.03 (0.02)	84.36 15.03 (0.02)
						93.00 4.36 (0.10)	132.80 2.62 (0.10)	138.38 1.34 (0.10)	75.50 1.22 (0.10)

4.6.1.1 Blank experiment: Take 10g control fish sample, extracted and cleaned by the QuEChERS method, then GC analysis.

4.7 DDTs in fresh fish samples

Two different fish (05-07 kg) samples were included in the study (**Table-8**) for analysis of organochlorinated pesticide residues. Selection of fishes includes carnivores like catfish Gulsha, *Mystuscavasius*(Hamilton,1822) and snakehead fishTaki, *Channapunctatus* (Bloch, 1794)were identified by Shafi and Quddus (1982).

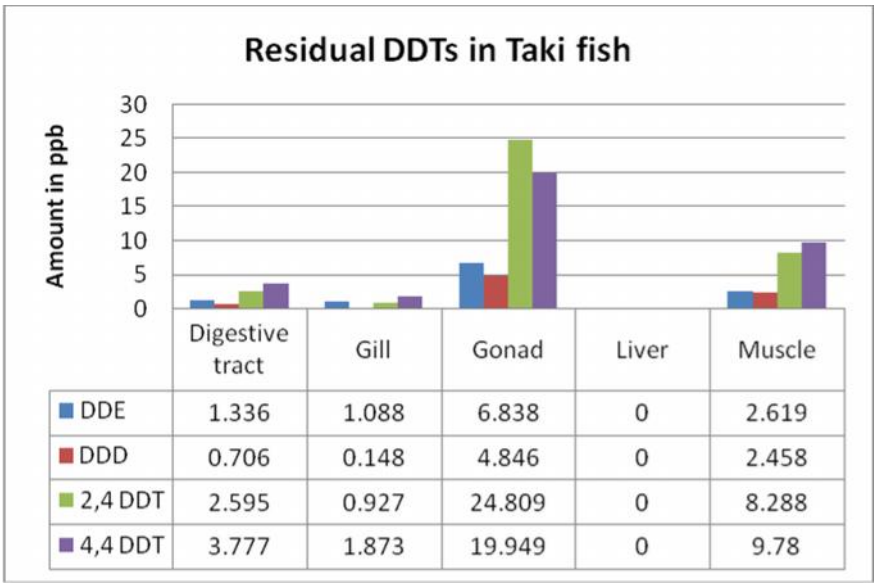
Organochlorine pesticideshave been used earlier in Bangladesh for pest control. Identification of the individual pesticides was done with respect to the retention time in gas chromatogram and quantification was done with reference to standard calibration curve of the individual OCPs. Replicate analysis were carried out in each case but in cases of Taki fishthree replicate studies were done and mean value was presented in Table 18 and in text. Recoveries were carried out by spiking with known amount of each organochlorine pesticide in three replicates of fish samples to check that the analytical procedure is working properly. The average lower recoveries in this study for digestive tract, gill, gonad and muscles were 80.05%, 92.54%, 88.28% and 87.31% respectively within a range of 71.73%-118.29%. And the average higher recoveries in this study for digestive tract, gill, gonad and muscles were 79.80%, 105.42%, 109.35% and 71.62% respectively within a range of 70.00%-138.36% ofDDTs in Taki fish.

The residual amounts of DDTs found in different vital organs (muscle, gill, digestive tract, liver and gonad) of Taki and Gulsha fish samples (expressed ppb) are presented in Table 18 and 19 as well as in **Bar diagram-1**.

DDTs in Taki fish												
Samples	DDE	Amount (ppb)	Average	DDD	Amount (ppb)	Average	2,4 DDT	Amount (ppb)	Average	4,4 DDT	Amount (ppb)	Average
Digestive tract-1	6659	1.335		6165	0.539		25251	3.480		23097	3.480	
Digestive tract-2	6627	1.333	1.336	9218	0.861	0.706	15554	1.903	2.595	28963	4.042	3.777

Digestive tract-3	6703	1.339		7854	0.717		18633	2.404		26532	3.809	
Gill-1	3249	1.088		2436	0.147		9565	0.929		6301	1.872	
Gill-2	3197	1.085	1.088	2491	0.153	0.148	9587	0.932	0.927	6342	1.876	1.873
Gill-3	3296	1.092		2422	0.145		9512	0.920		6289	1.871	
Gonad-1	41440	9.638		19252	4.794		61846	23.580		71269	20.231	
Gonad-2	32659	8.048	6.838	19348	4.820	4.846	67329	25.810	24.809	69053	19.700	19.949
Gonad-3	3841	2.828		19745	4.924		65429	25.037		69952	19.916	
Liver	0	0	0	0	0	0	0	0	0	0	0	0
Muscle-1	24321	2.615		24402	2.460		56335	8.536		90958	9.977	
Muscle-2	23842	2.580	2.619	24367	2.456	2.458	53880	8.136	8.288	87201	9.618	9.780
Muscle-3	24984	2.663		24381	2.457		54218	8.191		88537	9.745	

Table 18: The mean concentration of residue (in ppb) of DDTs in different vital organs (muscle, gill, digestive tract, liver and gonad) of Taki fish.

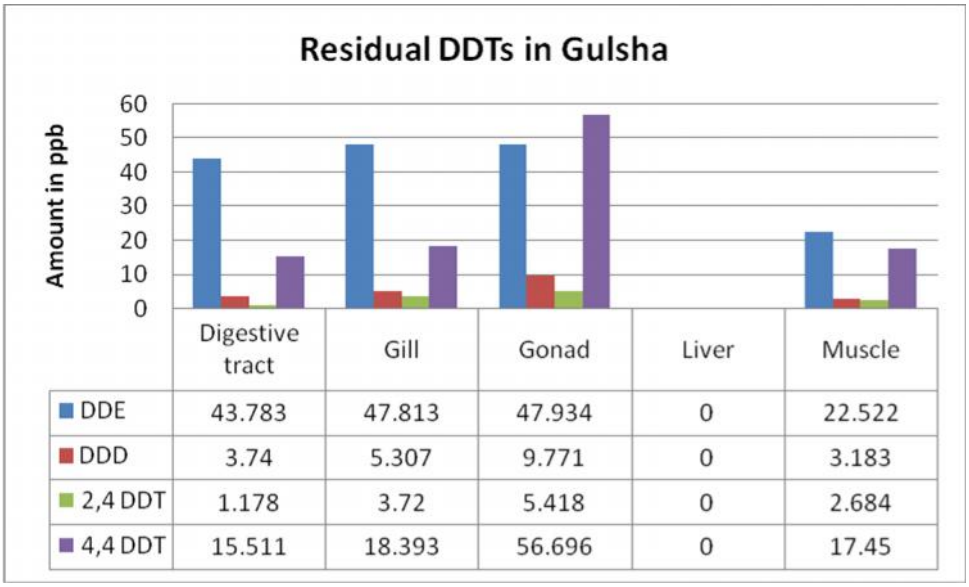


Bar diagram1:Level of residual DDTs in Taki fish.

Samples	**DDTs in Gulsha**											
	DDE	Ppb	Average	DDD	ppb	Average	2,4 DDT	Ppb	Average	4,4 DDT	ppb	Average
Digestive tract-1	14848	46.6111		16469	4.061		5098	0.505		47887	14.635	
Digestive	11757	41.199	43.783	14385	3.513	3.740	6404	1.036	1.178	54160	16.136	15.511

tract-2												
Digestive tract-3	13094	43.540		14887	3.645		8752	1.991		52603	15.763	
Gill-1	16431	49.383		21331	5.342		13271	3.829		86672	23.917	
Gill-2	14289	45.632	47.813	20742	5.187	5.307	12742	3.613	3.720	51208	15.429	18.392
Gill-3	15884	48.425		21529	5.394		13001	3.719		52887	15.831	
Gonad-1	15571	47.877		38350	9.822		17805	5.672		222844	56.509	
Gonad-2	15214	47.252	47.934	38838	9.951	9.771	16458	5.124	5.418	226034	57.272	56.695
Gonad-3	16026	48.674		37274	9.539		17278	5.458		221996	56.306	
Liver-1	0	0	0	0	0	0	0	0		0	0	
Muscle-1	20773	22.794		33101	3.376		22652	3.057		174968	18.020	
Muscle-2	19056	21.591	22.522	27722	2.810	3.183	15421	1.881	2.684	157817	16.378	17.450
Muscle-3	21325	23.180		32966	3.3612		22994	3.112		174265	17.953	

Table 19: The mean concentration of residue (in ppb) of DDTs in different vital organs (muscle, gill, digestive tract, liver and gonad) of Taki fish.



Bar diagram-2 Level of residual DDTs in Gulsha fish.

In experimental fish analysis, *p,p′*-DDE,*p,p′*-DDD, o,p′-DDT and*p,p′*-DDT were found in digestive tract, gill, gonad and muscles, but no pesticide residues were found to be present in liver of Taki fish and Gulsha. The total amounts of DDTs were measured to be 8.415, 4.037,

56.442 and 23.146 ppb respectively at digestive tract, gill, gonad and muscles of Taki fish and 64.212, 75.234, 119.819 and 45.839 ppb respectively at digestive tract, gill, gonad and muscles of Gulsha fish.

Table 20. Amount of DDTs in fish samples

Samples	**Taki fish**					
	DDE (µg/kg) Av. ± SD	DDD (µg/kg) Av. ± SD	2,4 DDT (µg/kg) Av. ± SD	4,4 DDT (µg/kg) Av. ± SD	DDTs	DDT/ DDTs
Digestive Tract	1.33 ±0.003	0.71 ±0.161	2.59 ±0.806	3.78±0.282	8.4147	0.44
Gill	1.09 ±0.003	0.15 ±0.004	0.93 ±0.006	1.87±0.003	4.0372	0.46
Gonad	6.84 ± 3.562	4.85 ± 0.069	24.81 ± 1.132	19.95±0.267	56.4422	0.35
Liver	BDL	BDL	BDL	BDL	BDL	BDL
Muscle	2.62 ± 0.041	2.46 ± 0.002	8.29 ±0.216	9.78±0.18	23.1456	0.42

Table 21. Amount of DDTs in fish samples

Samples	**Gulsha**					
	DDE (µg/kg) Av. ± SD	DDD (µg/kg) Av. ± SD	2,4 DDT (µg/kg) Av. ± SD	4,4 DDT (µg/kg) Av. ± SD	DDTs	DDT/ DDTs
Digestive Tract	1.33 ±0.003	0.71 ±0.161	2.59 ±0.806	3.78±0.282	8.4147	0.44
Gill	1.09 ±0.003	0.15 ±0.004	0.93 ±0.006	1.87±0.003	4.0372	0.46
Gonad	6.84 ± 3.562	4.85 ± 0.069	24.81 ± 1.132	19.95±0.267	56.4422	0.35
Liver	BDL	BDL	BDL	BDL	BDL	BDL

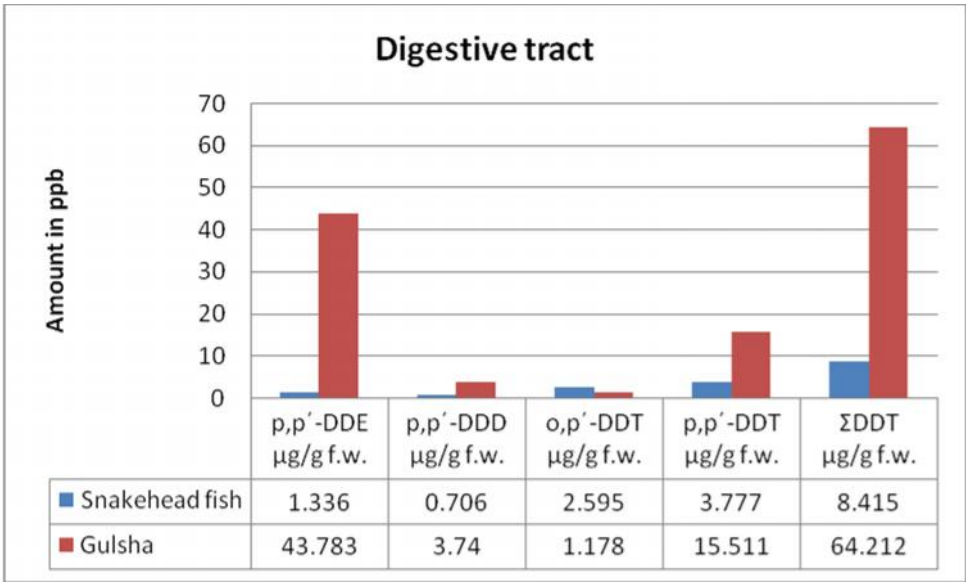
Muscle	2.62 ± 0.041	2.46 ± 0.002	8.29 ±0.216	9.78±0.18	23.1456	0.42

4.8Comparison between two fish species

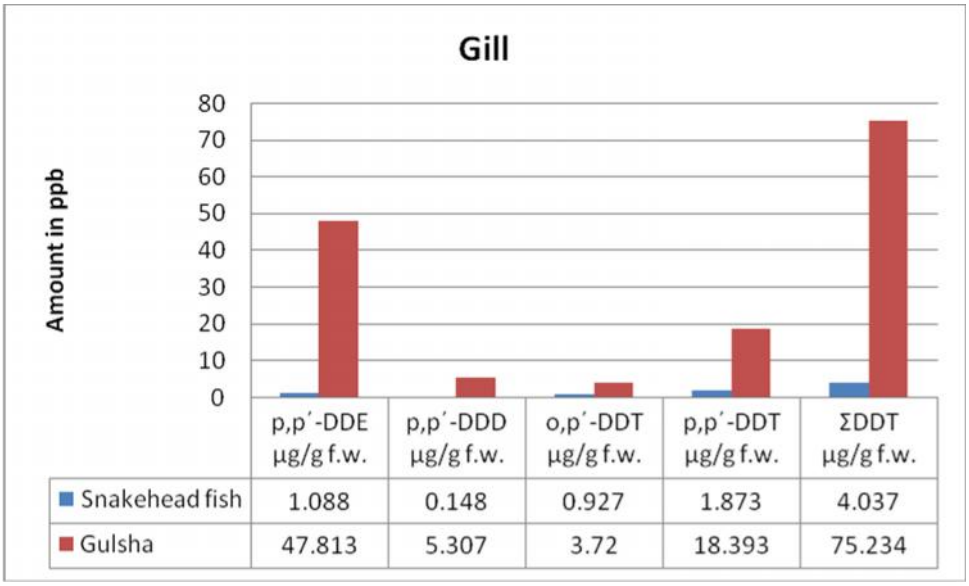
Local name	**Organs**	***p,p´*-DDE µg/g f.w.**	***p,p´*-DDD µg/g f.w.**	***o,p´*-DDT µg/g f.w.**	***p,p´*-DDT µg/g f.w.**	**ΣDDT µg/g f.w.**
Taki fish	Digestive tract	1.336	0.706	2.595	3.777	8.415
Gulsha		43.783	3.740	1.178	15.511	64.212
Taki fish	Gill	1.088	0.148	0.927	1.873	4.037
Gulsha		47.813	5.307	3.720	18.393	75.234
Taki fish	Gonad	6.838	4.846	24.809	19.949	56.442
Gulsha		47.934	9.771	5.418	56.696	119.819
Taki fish	Liver	n.d.	n.d.	n.d.	n.d.	n.d.
Gulsha		n.d.	n.d.	n.d.	n.d.	n.d.
Taki fish	Muscle	2.619	2.458	8.288	9.780	23.146
Gulsha		22.522	3.183	2.684	17.450	45.839

f.w. = fresh weight (as sold); n.d.= not detected

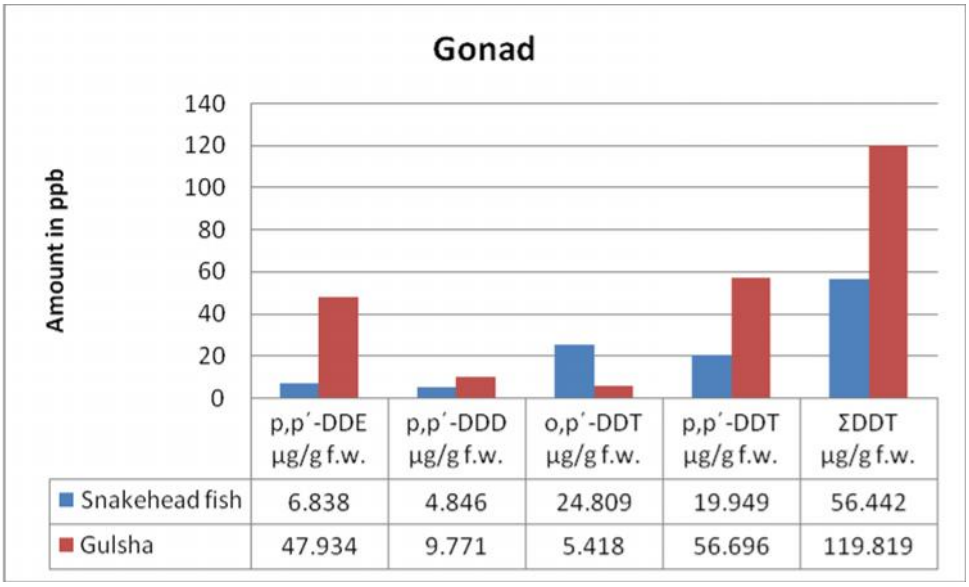
Table 22: Comparative study of residual DDTs in Taki and Gulsha fish.



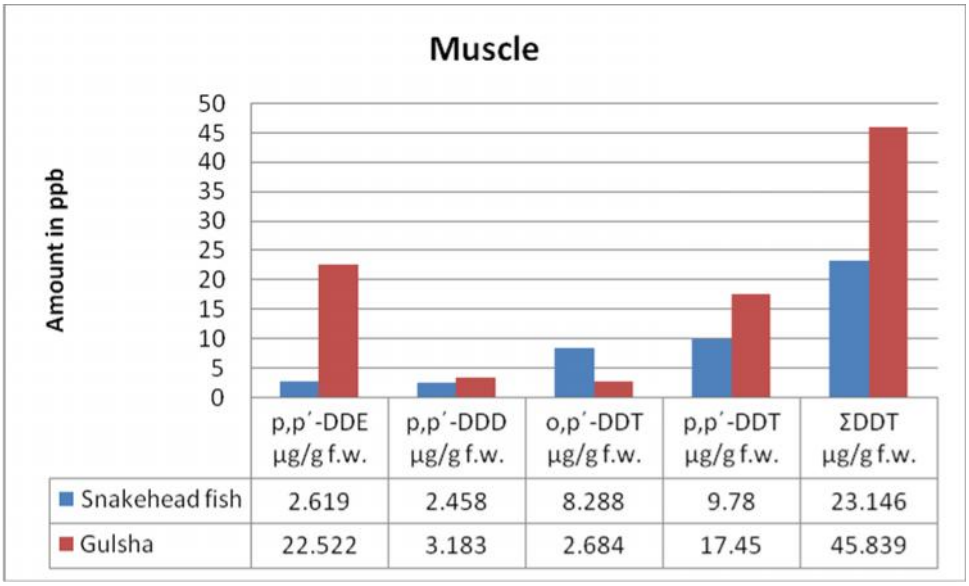
Bar diagram 3: Comparative study of DDTs at digestive tract in Takiand Gulsha fish.



Bar diagram 4: Comparative study of DDTs at gill in Takiand Gulsha fish.



Bar diagram5: Comparative study of DDTs at gonad in Takiand Gulsha fish.



Bar diagram 6: Comparative study of DDTs at muscle in Takiand Gulsha fish.

In comparison of two experimental fish analysis (Table 22 and Bar diagram 3, 4, 5 and 6), *p,p′*-DDE,*p,p′*-DDD,*p,p′*-DDT were found higher amount in Gulsha than that of Taki fish. In gonad of fishes, *o,p′*-DDT were found higher amount inGulsha than that of Taki fish.

The total amounts of DDTs were measured to be 8.415 µg/g f.w., 64.212µg/g f.w., at digestive tract of Takiand Gulsha fish respectively. In gill,4.037 µg/g f.w. and75.234µg/g f.w.respectively found inTaki and Gulsha fish. DDTs are found highest amount in gonad than the other organs of fish body, i. e. 56.442 µg/g f.w. 119.819 µg/g f.w. in gonad of Taki and Gulsha fish respectively. In muscles of Taki and Gulsha fish 23.146 µg/g f.w., 45.839µg/g

f.w. amounts of DDTs respectively.No pesticide residues were found to be present in liver of both fishes.

4.9Discussion

In the present study the highest amount of DDT and its metabolites (119.819 µg/g)were found in the gonad of Gulsha fish which might be due to its high lipid content (Mustafa, 2006) as the whole fish including gonad was taken for analysis. Taki showed small amount of DDT and its metabolites.

Carnivorous fishes are found in beels, haors, ditches and swamps of Bangladesh. The two species of fish analyzed, Takiand Gulshafish showed the presence of DDTs. Generally the carnivorous predator fish, Takiwas found to contain detectable amount of residual targeted pesticides DDTs (8.415 µg/g f.w. in digestive tract, 4.037 µg/g f.w. in gill, 56.442 µg/g f.w. in gonad and 23.146 µg/g f.w. in muscle.Medium size of Taki fish and Gulsha fish samples, purchased from the market might be from cultured pond and river. The Gulshafish which lives in river, lakes and canals, were found to contain DDT and its metabolites64.212 µg/g f.w. in digestive tract, 75.234 µg/g f.w. in gill, 119.819 µg/g f.w. in gonad and 45.839 µg/g f.w. in muscle. Gonad is the main target of DDT and its metabolites. The highest amount of DDTs were found in gonad of both fishes. No residual DDT and its metabolites found in liver. That means DDTs do not bind with the lipid of liver.

Samples collected from Ananda bazaar fish market of Brahmanbaria for Taki fish and Modhukhalibazaar fish market of Faridpur for Gulsha. Large scale study is needed for getting significant results of all the above analyzed fish samples.

Although, organochlorine pesticides were progressively banned in Bangladesh more than a decade ago and the DDT factory was closed in 1993(), the present study showed that organochlorine compounds have persisted in the environment. In the present study DDTs were found in experimental fish samples of Takiand Gulsha fish. Findings of DDT and its metabolites in fish samples indicated that it can be found in other Fresh water fishes also. However, none of the samples was found to contain residual level exceeding the value (5.0 mg/kg for total DDT in fish) of Maximum Residue Limit (MRL) suggested by FAO/WHO (Codex, 1993). We expect that, after few decades DDTs will not be found in the

environment.Source of fresh DDT might be either illegal use in Bangladesh or might be transported from neighboring countries.

Fishermen should be motivated not to use toxic insecticide and it is also the duty of the Government of Bangladesh to give alternative methods for preservation of fresh fish for shorter or longer period (6 months). The present study was area-specific and does not give a holistic picture of Bangladesh. Extensive work is required to determine the overall status of persistent organochlorine pesticide residues before taking any action to the business associated with fishermen.

5. SUMMARY

A pesticide is any substance or mixture of substances intended to control, prevent, destroy, repel or attract or mitigate any pest in order to minimize their detrimental effects. Chemicals are being used for controlling insects from time immemorial but only in the last 50 years, chemical control has been widely used.

Many of the early pesticides such as DDT and other organochlorine compounds were often highly toxic, very persistent or posing a threat to the environment. Though it has been banned in Bangladesh since 1993 due to its adverse effect on human health and environment.The major exposure of the persistent organochlorine compounds to human is via contaminated food, drinking water, inhalation and dermal uptake. Aquatic ecosystems i.e. rivers, canals and ponds in Bangladesh. They are very much susceptible for being contaminated with pesticides and other pollutants as they are often located nearby the rice and vegetables cultivated areas.Pesticides enter into aquatic systems either by direct discharge or transported by evaporation and/or run-off processes.

Modern pesticides are sophisticated compounds, which are very carefully researched to ensure they are effective against target organisms, easily degradable, safe to the environment and can be used without hazards to the operators or consumers.

Cypermethrin, synthesized in 1974 and first marketed in 1977 is a pyrethroid compound used as a modern pesticide which acts as a fast-acting neurotoxin in insects. It can be effective for weeks against a wide range of pests and it is easily degraded on soil and plants. Cypermethrin is used in a wide range of crops. In general, the maximum residue limits are low, ranging from 0.5 to 2.0 mg/kg in the different food commodities.

Cypermethrin undergoes photo, microbial and biological degradation. The *trans*- isomer is more degradable than the *cis*-isomer. The most widely adopted procedures for the determination of cypermethrin residues are based on extraction of the residue with organic solvent, clean-up of the extract, as necessary, by means of solvent-solvent partition and adsorption column chromatography, followed by determination of the residue using gas chromatography with electron capture detector (GC/ECD). The identity of residues can be confirmed by GC with mass selective detection (GC-MSD) or by thin-layer chromatography (TLC) followed by GC/ECD. Alternative procedures, based on HPLC with UV detection and

TLC with a colorimetric end point, have been described, but have not been widely adopted, because of the simplicity and sensitivity of the GC/ECD methods.

The behavior of pesticide is of great importance in agricultural production. Persistence or partial transformation of such a compound determines its usefulness or its partial effects to our environment. Currently organophosphates (OP), carbamates and pyrethroids are mostly used while organochlorine (OC) insecticides have been banned because of their toxicity, persistence and bioaccumulation in the environment.

Even though currently used modern pesticides are of limited persistence, knowledge of withholding period becomes important, specifically in fruits and vegetables since these crops are harvested shortly after pesticide application. Pesticide residues above the Maximum Residue Limit (MRL) in the crop at harvest are a cause of great concern globally and nationally. These residues make food commodities hazardous for human consumption and export and they also pollute the environment.

The vegetables are low in fat, high in dietary fibers; contain water, minerals and vitamins, possessing a very high nutritional density. So it is advised peoples to consume more vegetables in meals. But in our country farmers have been using pesticides frequently in vegetables especially on Bean (*Phaseolus vulgaris)*, Eggplant (*Solanummelongena)*, Cauliflower (*Brassica oleracea)*, and Tomato (*Solanumlycopersicum)* to get higher yields. Bean (*Phaseolus vulgaris* L.) and cauliflower (*Brassica oleracea var. botrytis*) are important winter vegetables in Bangladesh. On the other hand, bean and cauliflowerare susceptible to pest attack throughout the season. So, pesticides are extensively used in this culture at various stages of cultivation to control pests and diseases that may cause yield reduction.

Cypermethrin is one of the most extensively used pesticides in bean, cauliflower and other vegetables in Bangladesh. There is a lack of published data in Bangladesh for the fate of this insecticide on these vegetables. Therefore, the present study was designed to study the dissipation pattern of cypermethrin in bean and cauliflower day by day. The samples were collected according to pre harvest intervals following the World Health Organization (WHO) guideline.

In the present study, for extraction of cypermethrin from bean and cauliflower samples, the QuEChERS methodwas followed(Anastassiades et al., 2003). A florisil – alumina clean up method was adapted in the analysis being necessary to modify the preconditioning steps. The

detection and quantification of cypermethrin in bean and cauliflowerwere done using GC-2010 with an ECD. The purity and recovery of commercial cypermethrin was examined, and the dissipation pattern of cypermethrin in bean and cauliflower were established by this work.

Sampling: bean and cauliflowerwere grown in the experimental field in Bangladesh Agricultural Research Institute (BARI). Commercial cypermethrin, "Ripcord10-EC"(100 g/L) was sprayed with the recommended dose (1 mL/L in water) in two different beds of vegetables. Before spraying, blank/control samples were collected. Then two replicate samples from each bed were collected at the Pre-Harvest Intervals (PHI)of 0 (after 2 hours of spraying), 1, 3, 5, 7, 10 and 15 days after spraying according to WHO guideline. Each sample packet contained 150-250g of bean and cauliflower. They were kept in a freezer by wrapping with clean airtight polythene bag (jeeper lock) at temperature below -50^0C. Each bag was labelled according to sample name, sample ID and date of collection with permanent marker.

Extraction:20 mL ethyl acetate was added to 10 g homogenized sample in 50 mL Teflon tube and shaken for 1 minute in hand & vortex for 1 min. 6 g anhydrous $MgSO_4$& 1.5 g NaCl were added and vortexed for 1 min and then centrifuged for 5 minutes at 4000 rpm. 10 mL supernatant solution was taken in 100 mL RB flask, evaporated in rotary evaporator and then reconstituted in n-hexane (2 mL). This extract was kept in an airtight container and put in a freezer at temperature below -15^0C before clean up.

Clean up: All extracts of the bean and cauliflower samples were cleaned up using florisil-alumina column to remove unwanted compounds. Short stopper columns with an inner diameter of 3cm were used for cleanup. The column was packed with different layers. Very first a cotton bed was placed at the neck of the stopper to ensure that no packing material passed through. Then a layer of 5g florisil was followed by a layer of 5g alumina and 0.5g activated charcoal. Finally a 5g anhydrous sodium sulphate layer was added at the top. Then the column was pre eluted by 60 mL of n-hexane and the liquid was discarded.

The stored extract was kept in room temperature for few minutes and made up to the 1 mL mark. It was then applied to the column and eluted with 100 mL dichloromethane. The eluted solution was collected in a round bottom flask drop wise at a rate of 1mL min^{-1}. Due to the temperature difference between the outer and inner region of the column, there was a possibility of column break during elution. Air bubbles could be stored inside the column bed and hamper the elution process. Hence the column was wrapped with tissue paper and

acetone was applied drop wise on the tissue to maintain the temperature similar in the inner and outer part of the column.

The cleaned extract was then evaporated to dryness, redissolved in n-hexane and again evaporated to about 1 mL using a rotary vacuum pump. After completing all the processes, the cleaned extract was transferred into GC vial for GC run and stored in freezer.

Identificationand Quantification of Cypermethrin by GC-2010

The four peaks of cypermethrin were obtained at a retention timeof around 23 minute. For identification of cypermethrin present in the bean and cauliflower samples, reference standard sample was used. By comparing the retention time of the peak present in the sample chromatogram with the chromatogram of standard solution, cypermethrin was identified.

Cypermethrin shows a group of four close peaks in the chromatogram because of having different stereoisomers. Solutions of different concentrations were made from the certified standard and commercial cypermethrin. These solutions were analyzed and using sigma plot software, calibration curves of cypermethrin were made. From the calibration curve, the amount of cypermethrin present in the sample was calculated. The sum of areas for all four peaks was considered to determine the concentration in the standards and samples.

Calibration curves of cypermethrin

Four different level concentrations of certified standard cypermethrin were prepared to obtain the standard calibration curve. Similarly, four same different level concentrations of cleaned up commercial cypermethrin were also prepared to obtain the calibration curve for commercial cypermethrin.

The calibration curves are shown in figure 21.

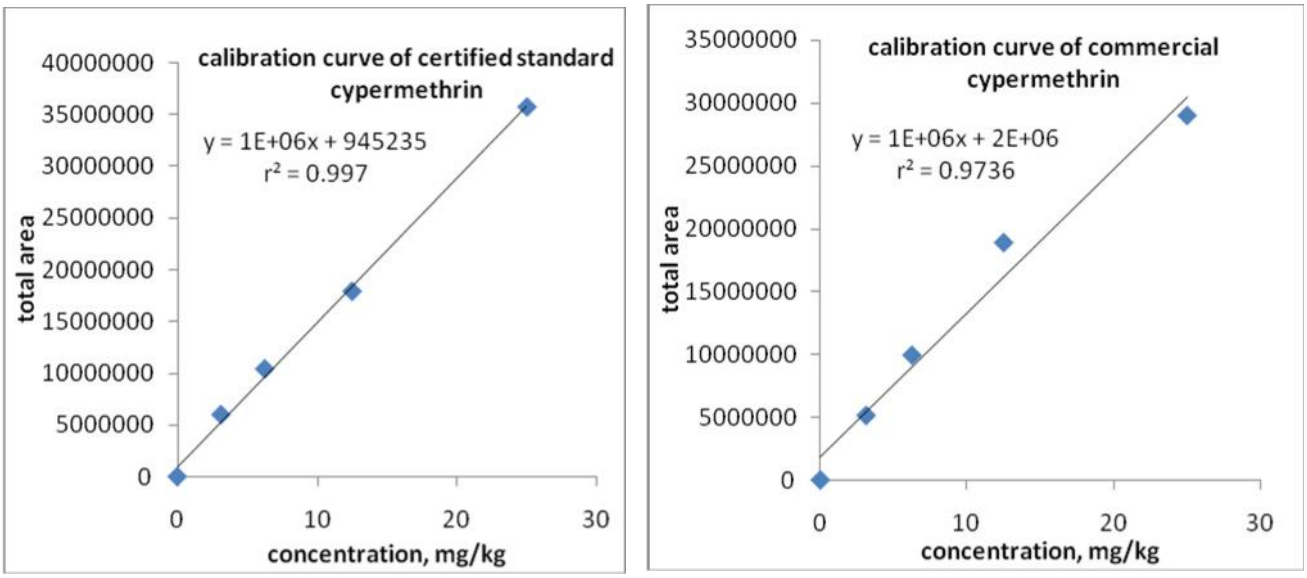


Figure21: calibration curves of cypermethrin.

Calculation of purity of commercial cypermethrin

To determine the purity of commercial cypermethrin, areas for an arbitrary concentration, 15 mg/kg were calculated from the calibration curves of commercial and certified standard cypermethrin. and the purity of commercial cypermethrin was found as 87.01%

Recovery calculation

Recovery

The extraction efficiency was assessed by doing recovery experiment. The recovery experiments were done with control samples collected from field before pesticide spraying which were initially confirmed that there were no pesticides. The recovery experiments were performed in three replicates at two fortified concentrations. The recoveries were 105.95, 106.89% for cypermethrin in bean and75.71, 82.54% for cypermethrin in cauliflower with precision below than 13.32% (Table-13).

Determination of concentrations of cypermethrin in bean and cauliflower samples

Using the value of slope (m) and intercept (c) of the calibration curve concentration of cypermethrin in mg/kg was calculated in different samples from the values of observed area in the chromatogram.

The time of collection, date of collection, sample ID, date of extraction, date of clean up, date of analysis, calculated concentrations and mean concentrations in mg of cypermethrin per kg

bean and cauliflower with respect to commercial cypermethrin in different samples are listed in Table 23 and Table 24.

Table 23: sample collection data and amount of cypermethrin for samples sprayed with recommended dose in bean.

Time of collection	Date of collection	Sample ID	Date of extraction	Date of clean up	Date of analysis	Concentration, mg/kg	Mean concentration, mg/kg
After two hours	18-12-2012	bean(s) 0 DAA-1	08.01.2013	17.01.2013	12.02.2013	3.27	3.77
		bean(s) 0 DAA-2	08.01.2013	17.01.2013	12.02.2013	4.33	
		bean(s) 0 DAA-3	08.01.2013	17.01.2013	12.02.2013	3.70	
After 1 day	19-12-2012	bean(s) 1 DAA-1	08.01.2013	17.01.2013	12.02.2013	2.77	2.69
		bean(s) 1 DAA-2	08.01.2013	18.01.2013	12.02.2013	2.41	
		bean(s) 1 DAA-3	08.01.2013	18.01.2013	12.02.2013	2.69	
After 3 days	21-12-2012	bean(s) 3 DAA-1	08.01.2013	18.01.2013	13.02.2013	1.22	0.21
		bean(s) 3 DAA-2	08.01.2013	18.01.2013	13.02.2013	1.1	
		bean(s) 3 DAA-3	08.01.2013	19.01.2013	13.02.2013	1.07	
After 5 days	23-12-2012	bean(s) 5 DAA-1	09.01.2013	19.01.2013	13.02.2013	0.99	0.94
		bean(s) 5 DAA-2	09.01.2013	19.01.2013	13.02.2013	1.00	
		bean(s) 5 DAA-3	09.01.2013	19.01.2013	13.02.2013	0.81	
After 7 days	25-12-2012	bean(s) 7 DAA-1	09.01.2013	20.01.2013	13.02.2013	0.89	0.89
		bean(s) 7 DAA-2	09.01.2013	20.01.2013	13.02.2013	0.96	
		bean(s) 7 DAA-3	09.01.2013	20.01.2013	13.02.2013	0.83	

After 10 days	28-12-2012	bean(s) 10 DAA-1	10.01.2013	20.01.2013	20.02.2013	0.56	0.45
		bean(s) 10 DAA-2	10.01.2013	21.01.2013	20.02.2013	0.33	
		bean(s) 10 DAA-3	10.01.2013	21.01.2013	20.02.2013	0.44	
After 15 days	02-01-2013	bean(s) 15 DAA-1	10.01.2013	21.01.2013	20.02.2013	0.33	0.32
		bean(s) 15 DAA-2	10.01.2013	21.01.2013	20.02.2013	0.33	
		bean(s) 15 DAA-3	10.01.2013	22.01.2013	20.02.2013	0.31	

Table 24: sample collection data and amount of cypermethrin in for samples sprayed with recommended dose in cauliflower.

Time of collection	Date of collection	Sample ID	Date of extraction	Date of clean up	Date of analysis	Concentration, mg/kg	Mean concentration, mg/kg
After two hours	02-12-2012	CF(s) 0DAA-1	13.01.2013	19.01.2013	14.02.2013	8.83	6.68
		CF(s) 0DAA-2	13.01.2013	20.01.2013	14.02.2013	5.57	
		CF(s) 0DAA-3	13.01.2013	20.01.2013	14.02.2013	5.65	
After 1 day	03-12-2012	CF(s) 1DAA-1	13.01.2013	20.01.2013	14.02.2013	3.03	2.94
		CF(s) 1DAA-2	13.01.2013	20.01.2013	14.02.2013	2.75	
		CF(s) 1DAA-3	13.01.2013	21.01.2013	14.02.2013	3.04	
After 3 days	05-12-2012	CF(s) 3DAA-1	14.01.2013	21.01.2013	15.02.2013	1.66	1.72
		CF(s) 3DAA-2	14.01.2013	21.01.2013	15.02.2013	1.74	
		CF(s) 3DAA-3	14.01.2013	21.01.2013	15.02.2013	1.75	
		CF(s)	14.01.2013	22.01.2013	15.02.2013	1.06	

After 5 days	07-12-2012	5DAA-1					1.12
		CF(s) 5DAA-2	14.01.2013	22.01.2013	15.02.2013	1.15	
		CF(s) 5DAA-3	14.01.2013	22.01.2013	15.02.2013	1.13	
After 7 days	09-12-2012	CF(s) 7DAA-1	14.01.2013	22.01.2013	15.02.2013	0.65	0.68
		CF(s) 7DAA-2	14.01.2013	23.01.2013	15.02.2013	0.74	
		CF(s) 7DAA-3	14.01.2013	23.01.2013	15.02.2013	0.64	
After 10 days	12-12-2012	CF(s) 10DAA-1	15.01.2013	23.01.2013	25.02.2013	0.19	0.17
		CF(s) 10DAA-2	15.01.2013	23.01.2013	25.02.2013	0.13	
		CF(s) 10DAA-3	15.01.2013	24.01.2013	25.02.2013	0.18	
After 15 days	17-12-2012	CF(s) 15DAA-1	15.01.2013	24.01.2013	25.02.2013	0.14	0.13
		CF(s) 15DAA-2	15.01.2013	24.01.2013	25.02.2013	0.12	
		CF(s) 15DAA-3	15.01.2013	24.01.2013	25.02.2013	0.13	

The dissipation curves

Mean concentrations of each sample (threereplicates) were obtained and plotted VS day of collection after spraying for both bean and cauliflower separately to prepare dissipation curves. Figure 4 shows the dissipation curves.

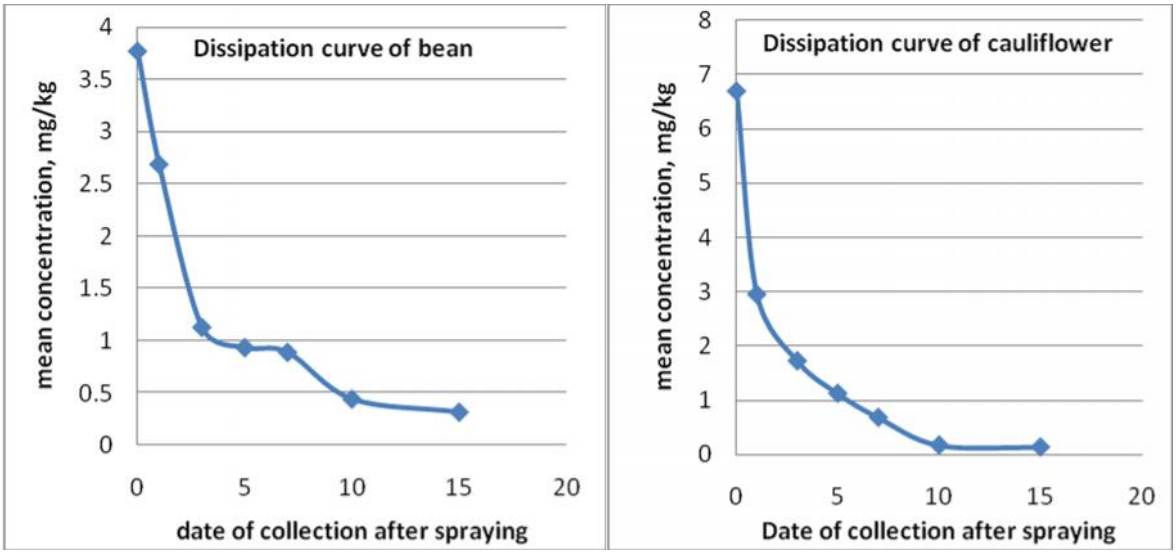


Figure 22: Dissipation pattern (concentration VS day of sampling).

Cypermethrin is a modern pesticide that undergoes degradation quickly. From Table 2 and Table 3 it is found that the residue limit of cypermethrin in bean is 6.68 mg/kg for the sample sprayed according to recommended dose and 0.54 mg/kg for sample sprayed with same dose after two hours of spraying in cauliflower. It degrades rapidly and comes to almost zero at 15th day. The dissipation curves in figure 2 show the rate of dissipation. Both curves show a gradual declining trend. The Maximum Residue Limit (MRL) for cypermethrin in bean and cauliflower are 0.5 mg/kg according to Codex Alimentarius Commission and the Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight. From the study it was found that cypermethrin residue in the bean and cauliflower samples are below the MRL value if it is sprayed according to the recommended dose. It takes 7 days to reach the residue limit below MRL value for bean as it is found that the residue limit is 0.45 at 10th day but higher values than MRL were found for day 7 (0.89 mg/kg) and day 5 (0.94 mg/kg). In cauliflower, it takes 7 days to reach the residue limit below MRL value. It is found that the residue limit is 0.17 at 10th day but higher values than MRL were found for day 7 (0.68 mg/kg) and day 5 (1.12 mg/kg). Cypermethrin is used widely for bean, cauliflower and other vegetables in Bangladesh. Residues present in foods can cause harm to our health. So, farmer should use cypermethrin as recommended and should not intake before 10 day after spraying.

DDT and its metabolites are very less soluble in water but can be present as suspended materials associated with the phytoplankton, algae or through adsorption on soil or sediment. Fish and other organism can easily be contaminated by taking these suspended

materials as their food. Being persistent organic pollutants, highly lipid soluble POPs, DDT and its metabolites can accumulate in the fatty tissues of living organism for long time and continuous consumption of contaminated fish by human may result into biomagnifications and cause various health problem.

Two fish species (Gulsha&Taki fish) were collected from modhukhali bazaar of Faridpur and Titas basin of Brahmanbaria. Then collected their vital parts such as gill, digestive tract, gonad, liver and Muscle etc. Collected vital parts were extracted and cleaned up separately following reported method and validated and analyzed by GC ECD. Retention times of DDTs (DDE, DDD, 2,4′ DDT & 4,4′ DDT) were found to be 12.173, 12.813, 12.899 and 13.426 min, respectively. Recoveries of DDTs (DDE, DDD, 2,4′ DDT & 4,4′ DDT) were found to be 80.05-79.81% in digestive tract, 92.54-105.43% in gill, 88.28-109.35 in gonad&87.31-73.88% in muscle for fish samples, respectively.LOD & LOQ were found to be 0.005 &0.0165 ppm of fish respectively. Our findings revealed that out of two fish samples(gulsha&Taki fish), were found to contain DDTsin digestive tract, gill, gonad and muscle are 64.21, 75.24, 119.82 and 45.83 ppb respectively. No residual DDTs present in liver.

Comparison of DDTs betweengulsha and Taki fish in vital organs are found64.212 & 8.415ppb in digestive tract, 75.234 & 4.037ppb in gill, 119.819 & 56.442ppb in gonad and 45.839 & 23.146ppb respectively. DDTs are higher in gulsha than that of Taki fish.

It might be noted that the amount of 4,4′ DDT is much higher in all four parts compare to its metabolites DDE, DDD and 2,4′ DDT.It is alarming that, Gulsha is susceptible to DDTs. This suggests that the source of fish (different rivers of Bangladesh) contaminated till now. Though, the residual DDTs in all four parts of the fish samples were below maximum residue limit (MRL) of DDTs in fish (5.0 ppm) and ADI/PTDI 0.01 mg/kg body weight(FAO, 2000). DDTs were found another study on Boal fish (*Wallagoattu*). That means DDTs can be bind with the tissues of catfishes (Anonymous, 2008). Continuous consumption Gulsha will accumulate DDTs in our body which may lead to the concentration enough to cause a threat to our health.

Conclusion

Two selected vegetables (Bean & Cauliflower) were collected from experimental field of BARI, Gazipur with the collaboration of University of Dhaka.Cypermethrin is a modern pesticide that undergoes degradation quickly. It degrades rapidly and comes to almost zero at 15^{th} day. The Maximum Residue Limit (MRL) for cypermethrin in bean and cauliflower are 0.5 mg/kg according to Codex Alimentarius Commission and the Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight (Codex, 1993). From the study it was found that cypermethrin residue in the bean and cauliflower samples are below the MRL value if it is sprayed according to the recommended dose. Cypermethrin is used widely for bean, cauliflower and other vegetables in Bangladesh. Residues present in foods can cause harm to our health. So, farmer should use cypermethrin as recommended and should not intake before 10 day after spraying.

Two fish species (Gulsha&Taki fish) were collected from Modhukhalibazaar of Faridpur and Titas basin of Brahmanbaria respectively. The samples were analyzed for the presence of DDTs. The analysis showed that the Modhukhaliand Titas basin is less contaminated with DDTs which has been accumulated by the fishes living and growing in it. The amount of DDTs were found to be very lower than the MRL values, but continuous consumption might cause a threat to human health as a result of biomagnifications. The higher amounts of 4,4′ DDT than that of DDE, DDD and 2,4′ DDT revealed that DDT is still being used although it is banned since 1993.

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