‘Studies of chemical contaminants in different food stuff’

A Dissertation for the Degree

of

Doctor of Philosophy

Submitted by

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Dedicated

To

My Respected Supervisors

&

My Loving Family

DECLARATION

Experimental work described in this thesis has been carried out by the author at the Organic Section, Department of Chemistry, University of Dhaka. The work has not been and will not be presented for any other degree.

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The Author.

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ABBREVIATIONS

<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
°	Degree
µg	Microgram
µL	Micro litre
ADI	Acceptable Daily Intake
ai	Active ingredient
ASE	Accelerated solvent extraction
BDL	Below detection limit
BARI	Bangladesh Agricultural Research Institute
BQL	Below quantification limit
BRRI	Bangladesh Rice Research Institute
C	Celsius
CAS	Chemical Abstracts Service
cm	Centimeter
d.w.	Dry weight
DAS	Days after spraying
DCM	Dichloromethane
EC	Emulsifiable concentrate
ECD	Electron capture detector
EFSA	European Food Safety Authority
EI	Electron ionization
EMAP	Environmental Monitoring Assessment Program
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration

G	Granular
GAP	Good Agricultural Practice
GC	Gas chromatography
GCB	Graphitized carbon black
GC-FPD	Gas chromatography with flame photometric detector
GC-MS	Gas chromatography with mass spectrometer
hr	Hour
ha	Hectre
HR	Higher range
HCl	Hydrogen chloride
i.d.	Internal diameter
i.e.	That is
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
KHIDI	Korea Health Industry Development Institute
L	Litre
LC-MS/MS	Liquid chromatography with mass spectrometer
LOD	Limit of Detection
LOQ	Limit of Quantification
LR	Lower range
MCL	Maximum contaminant level
MeCN	Acetonitrile
mg	Milligram
mL	Milliliter
mm	Millimeter
MRL	Maximum Residue Limit
MRMs	Multi residue methods
MSD	Mass selective detector
MSPD	Matrix solid phase dispersion
n	Number of replicate

ND	Not detected
nm	Nanometer
OCPs	Organochlorine pesticides
OCs	Organochlorine compounds
OHCs	Organohalogen compounds
OHPs	Organohalogen pesticides
OPPs	Organophosphrous pesticides
PCBs	Poly chlorinated biphenyls
PHI	Pre Harvest Interval
POPs	Persistent Organic Pollutants
ppb	Parts per billion
ppm	Parts per million
PRA	Pesticide residue analysis
PRs	Pesticide residues
PSA	Primary secondary amine
PTFE	Poly tetrafluoro ethylene
PULSE	Promoting Understanding and Learning for Society & Environmental Health.
QA	Quality assurance
QC	Quality control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
r^2	Regression factor or correlation coefficient
rpm	Rotations per minute
RSD	Relative standard deviation
RT	Retention time
S/N	Signal to noise ratio
SAX	Strong anion exchange
SD	Standard deviation
SE	Solvent Extraction
SPE	Solid phase extraction
SFE	Supercritical Fluid Extraction
SIM	Selected ion monitoring

SPME	Solid Phase micro extraction
$t_{1/2}$	Half-life
UNEP	United Nations Environment Programme
UPLC	Ultra Performance Liquid Chromatography
US	United State
VOCs	Volatile organic compounds
WHO	World Health Organization

ABSTRACT

Imidacloprid, a neonicotinoid pesticide is allowed to use in food storage. A total of 30 rice samples and 15 wheat flour samples were analyzed to determine the residaul amount of imidacloprid. Among 30 rice samples, 9 (fragrant) packed samples were found to contain imidacloprid residues in the range 1.59 – 4.51 µg g^{-1}which was greater than the MRL value (1.5 µg g^{-1} in rice, EPA 2010) and 9 (fragrant) unpacked samples were found to contain imidaclopridresidues in the range 0.06 – 1.10 µg g^{-1}which was lower than the MRL value. The imidacloprid residue in non-fragrant rice samples were found below detection limit. In cooked rice samples, no trace of imidacloprid was observed. Among 15 wheat flour samples, imidacloprid residues were observed only in 8 samples, ranging from 0.03 – 0.44 µg g^{-1} which were lower than the MRL value (1.5 µg g^{-1} in wheat, EPA 2010). The toxic effect of imidacloprid in rice was evaluated against adults *Sitophylus oryzae*. The average mortality of adult weevils was 57% at 72 hours while spiking the control rice with a concentration level 1.25 µg g^{-1}, therefore, a concentration lower than this value would be enough to control the growth of weevils during storage. Therefore, the results of residual analysis in rice indicate indiscriminate use of imidacloprid in the market samples during storage.

The dissipation pattern of quinalphos was studied in tomato, bean and cauliflower which were grown in the experimental field of Bangladesh Agricultural Research Institute (BARI). Quinalphos treated samples were collected from 0 (2 hours after application) to 15 successive days, transferred to the laboratory rezeerf ni derots dna (ta-C^0 20). sohplaniuq fo tnuoma ehT otamot ni eudiser, fo egnar eht ni eb ot dnuof erew rewolfiluac dna naeb0.05 – 6.31 ,0.05 – 3.100 dna. – 066.5µg g^{-1} and dissipated below MRL (0.20, 0.20 and 0.50 µg g^{-1}in cauliflower, tomato and bean, EPA 2011) value within 6, 4 and 7 days after application, respectively.

The safe period of consumption and the dissipation pattern of five different pesticides; Vitaban 48 EC (chlorpyrifos), Double 50 EC (mix formulation of imidacloprid and cypermethrin), Nitro 505 EC (mix formulation of chlorpyrifos and cypermethrin), Asataf 75 SP (acephate) and Reeva 2.5 EC (lambda-cyhalothrin) in eight different vegetables (cabbage, cucumber, bottle gourd, sweet gourd, sponce gourd, green chili, cauliflower and tomato) were studied. The pesticides

were applied in the agricultural field of Nuritola, Comilla, Bangladesh, collected, brought to the laboratory and analysed for successive 10 days keeping them at ambient temperature.
The residual amount of chlorpyrifos, in tomato, bottle gourd, sweet gourd and green chili, went below the MRL after 5 days and in cabbage, cauliflower, sponce gourd and cucumber, after 8 days of application. The average half life of chlorpyrifos in the vegetables was found to be 1.14 days.

Pesticides of Nitro 505 EC; chlorpyrifos residues, in bottle gourd, tomato and sweet gourd, went below the MRL value after 3 days, in sponce gourd, green chili and cucumber,it was after 5 days and in cabbage and cauliflower, it was after 8 days of application whereas cypermethrin residues, in tomato, cauliflower, sweet gourd, sponce gourd, green chili and cucumber went below MRL value after 1 day, in case of cabbage and sponce gourd, it was after 3days of application. The average half-lives of chlorpyrifos and cyperethrin in Nitro 505 EC were found to be 0.92 and 0.58 days, respectively.

Pesticides of Double 50 EC; imidacloprid residues went below the MRL value after 1day of application for tomato and green chili, after 3 days of application for cabbage, cauliflower, sweet gourd, bottle gourd and cucumber and after 5days of application for sponce gourdwhereas cypermethrin residues went below the MRL values after 1 day of application for tomato, cauliflower, green chili and after 3 day of application for cabbage, bottle gourd, sweet gourd and sponce gourd. The average half-lives were found to be 0.62 and 0.54 day for imidacloprid and cymermethrin, respectively in Double 50 EC.

The initial concentration of lambda-cyhalothrin residue declined very sharply and went below the MRL value after 1 day of application.

The residual amount of acephate went below the MRL value after 5 days of application for cabbage and cauliflower and after 3 days of application for the rest of the samples. The average half life of acephate was 1.1 days.

Storage stability of the five pesticides in freezer (-20^{0}C) were done by fortifying the control vegetable samples with the five pesticides and the samples were then stored in a freezer (-20 ^{0}C) for about 30 days. The average recoveries of the pesticides were found in the range of 84% -

93% which indicates that the pesticides were quiet persistent at freezing condition and not degraded during storage.

QUALITY ASSURANCE (QA)

To maintain the quality of this study, all the steps were performed with maximum efficiency and in an environmentally friendly manner. While collecting of samples, they were transported to the laboratory immediately and stored in a proper way. Samples which were about to stored in freezer, were stored at -20 ^{0}C to reduce degradation and which were needed to be stored at ambient temperature, were stored at fume hood cupboard to allow the samples to be in a contact with air, heat and light.

Certified reference standards were used with highest purity and stored at -20 ^{0}C in freezer. Working standard solutions were prepared in a very sensitive manner and were labeled indicating name, date of preparation and the meniscus layer was marked with permanent ink.

All the glassware were cleaned with detergent, rinsed thoroughly with distilled water and finally rinsed with acetone and the solvent to be used. These were baked at 300 ^{0}C overnight, cooled and wrapped with aluminum foil. Water and the organic solvents were checked for possible interference with other contaminants by the analysis of reagent blank. All the instruments and chromatographic instruments were checked and calibrated regularly.

All the reported methods were validated with the parameters of validations; linearity, specificity, selectivity and by recovery experiments by spiking the control samples. The fortified samples were kept aside to allow perfect adsorption onto the samples. Excellent recoveries (in a range of 70 - 120%) and precisions (less than 20%) were found.

Gas chromatograph coupled with electron capture detector and high performance liquid chromatograph coupled with photo diode-array detector, were used in the analysis for identification and quantification of analytes in the samaples. All the sample extracts fad been cleaned up before injecting into chromatography machines, by respective clean up procedure because the identification is based on retention time of analytes, matched with certified standards and this may cause some problems with co-elutions. Quantification was performed within dynamic range of the respective detector. The control standards were analyzed everyday during analysis to check performance of the analytical systems. To evaluate instrumental limits,

the limit of detection (LOD) and the limit of quantification (LOQ) were measure as 3 and 10 times of the signal to noise ratio (S/N) in the chromatogram, respectively.

Separate laboratories were designated for sample storage, extraction and residual analysis by GC and HPLC. All rooms were neat and clean and separate shoes were available for each room. Separate IPS was used for instruments to avail continuous power supply and freezers and refrigerators were available for sample storage. Sufficient light and ventilation were available in the laboratory.

INTRODUCTION

Food is one of the basic needs of human life which provides energy, nutrition and protects body from diseases. Hunger, poverty, malnutrition and disease are occurred due to improper food which has less nutritional density and are not free from chemical& biological contaminants. Healthy life of human being is more than wealth. Traditionally Bangladesh is an Agricultural country and its economy was mainly dependent on food production and source foreign exchange earning was also on agricultural product. After independence, the country faced food shortage for 70 million people. Government of Bangladesh (GOB) identified agricultural sector is one of the important sectors for alleviation poverty and gave incentive to the farmers by providing pesticides free of cost & fertilizer at cheaper price. Farmers were allowed to use organohalogen (OHP) pesticides; DDT, endrin, endosulfan etc. which were imported to the country and were available for the farmers. Organohalogen pesticides are very effective for the pests; it acts against insects, mites, fungus and other type of pests. As a result, production of agricultural crops; rice, vegetables, fruits etc. had been increased. But due to high toxicity and persistency, twelve hazardous persistent organic pollutants (Aldrien, Chlordane, Dieldrin, DDT, Endrien, Heptachlor, Mirex, Toxaphane, Hexachlorobenzene, PCBs, Dioxins and Furans) were identified and banned, which are commonly known as 'Dirty Dozen', on Stockholm convention, May 23, 2001. Most of these pesticides entered the food chain, accumulated in the fatty tissue of all animals, including humans, and caused cancer and genetic damage. Thus, the aim of the convention is to protect human health and environment by phasing out of these hazardous pollutants from the environment. For this reason, farmers in the recent decades are using organophosphorous, carbamates and pyrethroids types of pesticides that are less persistent and non-bioaccumulative.

Gradually traditional way of cultivation is almost over and food production increased substantially. Today the country is self-sufficient in food having hundred and sixty million people, export food stuff and keeps reserve food to meet the urgent needs like cider, cyclone, unmanageable flood etc. even cultivable agricultural has been decreased due to rapid urbanization. This great achievement became possible by the use of good quality fertilizers, ripening agents, plant growth regulators, cultivating hybrid varieties

crops, and minimizing crop loss by using pesticides. But uncontrolled or indiscriminate use of pesticides decreases food safety. Food Safety and Quality is an emerging issue in the global forum and so also in Bangladesh. Bangladesh Government properly addressed the issue on food safety and quality to provide safe food for all the citizens. Safe Food Law 2013 has been made by the government and to execute the law Bangladesh Food Safety Authorities (BFSA) has been formed. BSFA has taken steps to form a Laboratory Network with different Academic and Research Institution for monitoring and to assess the level of biological and chemical contaminants in agricultural and processed foods, and to upgrade research capacity of the laboratories under the network. Food Safety laboratory of Department of Chemistry, University of Dhaka (DU) is going to be included in the Laboratory Network of BFSA. The Department of Chemistry, DU has been working of chemical contaminants in food stuff; storage (Munia et. al , 2016; Sultana, et.al 2016), agricultural (Nahar et.al 2012; Zamir, et.al 2013; Shoeb, et al.2009), processed food stuff (Mou, 2016), ripening agent (Tamanna, et.al 2007), plant growth regulator (Mandal, et.al 2012) etc.

1.1 Objective of current study

Rice and wheat flour are the staple food in many countries of the world along with Bangladesh. To mitigate the demand of these foods for the huge population of our country and boost up the manufacture of rice and flour, different types of pesticides are applied to field before harvesting, during storage and packaging, but due to over dose and improper use of pesticides; residues of chemical components are often left in these foods and cause toxicity (acute and chronic) to human health. Imidacloprid, one of the most common pesticides used in storage of rice and wheat grains act against rice weevils (pest of rice and wheat flour), acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system of the weevil. In addition to its topical use on pests, it may be applied to structures, crops, soil, and as a seed treatment (Tomlin,2006).The application of any insecticide to food crop draws concerns over food safety and requires a careful monitoring of the residue. In most parts of the world, imidacloprid is used without due regards to dosing and operators are not adequately trained, there is likelihood that the residue of imidacloprid may exceed the threshold limit. Therefore, it is important to

analyze the residue level of imidacloprid in rice and wheat flour from the view point of food safety. Again, to determine the highest toxic level of imidacloprid against rice weevils is also very essential to ensure the proper dose and to minimize the risk of acute toxicity.

Vegetable is a very healthy food which is low in fat, have high dietary fibers; it contains different minerals, vitamin as well as water. Therefore, it has been always suggested to intake vegetables in meals. But in our country, farmers have been using pesticides frequently in vegetables to get higher yields. Using pesticides in vegetables is becoming a food safety concern for consumers and the GOB.The dissipation patterns of quinalphos in three different vegetables were studied in this current analysis where the vegetable samples were collected from the experimental field of BARI, Gazipur, Bangladesh. Quinalphos, is a very toxic chemical and is used for the management of diamond-back moth and tobacco caterpillar on vegetables. It is still not a registered pesticide in Bangladesh. Therefore, to determine the fate of Quinalphos under the agro-climate and soil property of our country, the results will help the government for having a decision about registering quinalphos as a regular insecticide. Therefore, to study dissipation pattern of quinalphos on tomato, bean and cauliflower can play a lead role in identifying the characteristic of quinalphos and to determine the safe harvesting period.

A seminar was organized by BINA (Bangladesh Institute of Nuclear Engineering, Mymensingh) at Nuritola, Comilla, on 9th January, 2016, entitled "Farmer's Seminar on Food Contaminants" as an awareness programme for farmers regarding toxicity of pesticides and their effect on human health and environment. A research group from the Department of Chemistry and Government official (Thana Nirbahi Officer) participated at the seminar. Speeches on "Food Contaminants and Food Safety" were delivered. Main purpose of the seminar was to grow awareness of the farmers about the toxic effect of frequent use of pesticides on their health and the environment. The farmers were addressed by Professor Nilufar Nahar, Professor Mohammad Shoeb, Dr. Jahangir Alam Director, BINA and TNO. During the seminar interview of the farmers were taken regarding their conception of toxicity of pesticides. It is necessary to study residue level of each pesticide on food commodities and the safe period of consumption of pesticide

treated vegetables. Concerning this situation, determination of the dissipation pattern of pesticides and safe level of consumption of crops treated by pesticides is necessary. During the seminar, five different kinds of pesticides; Vitaban 48 EC (chloropyrifos), Double 50 EC (mix formulation of imidacloprid and cypermethrin), Nitro 505 EC (mix formulation of chloropyrifos and cypermethrin), Asataf 75 SP (acefate) and Reeva 2.5 EC (lambda-cyhalothrin)were applied on eight different vegetables (tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber) to the agricultural field of farmers and collected 2 hours after application.

Objectives of the studywere as follows;

- Identification and Quantification of residual Imidacloprid in rice and wheat flour Samples
- Determination of the Toxicity Level of Imidacloprid in Rice
- Analysis of the Dissipation Pattern of Quinalphos in Tomato, Cauliflower and Beans (collected from experimental field, BARI)
- Determination of the dissipation of five pesticides in eight different vegetables(collected from farmer's field, Nuritola, Comilla, Bangladesh)

1.2 Food contaminants

Contaminants are substances added intentionally or unintentionally that makes food unfit for human consumption. These substances may be present in food as a result of the various stages of its production, packaging, transport or holding. They also might result from environmental contamination (Malin, 1995).

1.2.1 Sources of contaminants in food

Contaminants can be present in foods mainly as a result of the use of agrochemicals, such as residues of pesticides, veterinary drugs, contamination from environmental sources (water, air, soil pollution), cross-contamination or formation during processing, migration from food packaging materials, presence or contamination by natural toxins or use of unapproved food additives and adulterants (Vazquez and Pico, 2012).

1.3 Pesticide

Chemical compounds which are used to control pests and diseases of plants, to eradicate weeds, to kill pests and microorganisms that spoil agricultural products, materials and articles and to control parasites and vectors of dangerous diseases of man animals are called pesticides (Gruzdyev et al., 1983).The most common of these are insecticide which account for approximately 80% of all pesticide use ("Pesticides", GRACE Communications, 2017). Most pesticides are intended to serve as plant protection also known as crop protection which protect plants from insects and pest attacks and to control weeds.

According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are organochlorine pesticides (United Nations Environment Programme, April 2005; Gilden, 2010). Therefore, demands of less persistent, easily degradable (into non-toxic products) like synthetic oraganophosphates, pyrethriods and organocarbamatesare increasing day by day.

Pesticides are classified according to the type of pest they control. They can also be considered as either biodegradable pesticides or persistent pesticides, which may take months or years before they are broken down. Another way to think about pesticides is to consider those that are chemical pesticides or are derived from a common source or production method(Giddings, 2014).There are pesticides which are less specific or selective or non-specific. Various types of insects and microorganisms, even animals are killed when non-selective pesticides are being used. Pesticides can also be classified according to the way they work. They may be contact or systemic. A contact pesticide needs to be touched to become effective against the harmful organisms directly. With finer spray mist, the pesticide penetrates the agricultural crop and thus kills the pests by contact. Systemic pesticides attach and penetrate the plant surface and then disperse into the whole plant.

Synthetic or biological pesticides, inorganic and organic compounds are different classes of pesticides according to chemical composition.

Algicides, avicides, bactericides, fungicides, herbicides, insecticides, acaricides, rodenticides are different types of pesticides according to action of pesticides.

According to the mode of action, pesticides can be classified in different groups, such as; antifouling (accumulation of undesired element and inhibition of growth microorganism) agents, antifeedant (inhibition of eating leaves or fruits by insect),attractants, (chemical compoundthat attract insect of opposite sex and killing the pest by making trap), repellants(compounds which repel insect or microgram), plant growth regulators (indirect control of pests), defoliants (chemical compounds cause fall of leaves).

Again, synthetic insecticides are classified into various subgroups;

Neonicotinoid pesticides

Neonicotinoids are neuro-active insecticides chemically similar with nicotine. Imidacloprid is a very widely used insecticide worldwide (FishUpdate.com.2013). In 1990s, neonicotinoids were in a range of studies in order to adverse ecological effects by a reduction in insect populations (Case and Philip, 2013).

Organophosphate pesticides

Organophosphates disrupt acetylcholinesterase activity by affecting the nervous system, the acetylcholinesterase enzyme regulates acetylcholine, a neurotransmitter. Most organophosphates are insecticides. Some are very poisonous. Persistency of this pesticide is very low. Due to the instability, it has to be applied at frequent intervals and therefore, not always economically viable.

Organophosphate pesticides are mutagenic, genotoxic, cytotoxic, teratogenic, carcinogenic and immunotoxic. A few studies reported that exposure of organocarbamate pesticides had affected human health by inhibiting acetyl cholinesterase and modifying cholinergic signaling. Organophosphate pesticides bind to the enzyme acetyl cholinesterase and disrupt nerve functioning which further result in paralysis and death (Dipakshi Sharma et al., 2010).

Carbamate pesticides

Carbamate affect the nervous system by disrupting the enzymatic action of acetylcholine, a neurotransmitter. They are the intermediates in behavior in comparison to organochlorine-hydrocarbons and organophosphates. Carbamates are more toxic than

organophosphate but less persistent than organochlorine pesticides. Examples include carbaryl and methiocarb.

Organochlorine insecticides

Most of the organochlorine insecticides are banned in recent days. These are not very toxic. Structurally these are very stable. It is an advantage as well as disadvantage. On the negative side, these insecticides have the tendency to accumulate in the environment and affect non-target organisms, not only the target pests. Due to their high persistency, they enter into food chain, accumulate in fatty tissue, therefore, severalorganochlorine insecticides including DDT, aldrin, dieldrin have been banned worldwide.

Pyrethroid pesticides

Pyrethrin, is a naturally occurring pesticide and pyrethroid were developed as a synthetic version of pyrethrin. They have been synthesized to increase their stability in the environment. These are neither toxic nor persistent. Therefore, inspite of their high cost, they account for one-third of world insecticide use. For, example pyrethrum, deltamethrin.They are non-systemic contact and stomach chemicals with an additional anti-feeding action. The compound acts on the peripheral and central nervous system of target insects at very low doses (Roberts and Hutson, 1999).

Pyrethroids are lipophilic pesticides which degrade very quickly in the environment. The two main routes of degradation, photodegradation and biodegradation. Greater photo stability is observed in Pyrethroids than the natural pyrethrins. The pathway of pyrethroid metabolism include hydrolysis of the central ester linkage and oxidation of both acid and alcohol moieties.

1.4 Formulation

The physical state of a pesticide can be described by formulation and also it determines the way of application. The effectiveness of a pesticide greatly rely on its form in which the pesticide remains stable. Common formulations are:

a) Emulsifiable concentrate (EC)

It is a solution of pesticide in oil comminuted into drops which is coated with a protective layer of a surfactant.

b) Granules (G)

The active ingredient is made into coarse particles with inert material like fired clay particles.

c) Wettable powders (WP)

The active ingredient is combined with fine powder look like dusts, yields stable suspensions when diluted with water.

d) Soluble powders (SP)

It is obtained by dissolving the active ingredient in water, these are mainly low-concentration solutions, usually applied as a fine spray(Cress, 1990).

1.5 Importance of the use of pesticides

Pesticides have helped to archive higher food production, increased food security by reducing vulnerability of crops to pathogens and lower morbidity and mortality rates for certain vector- borne diseases such as; malaria. 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 (Knutson, R.; 1999). They are used to control harmful organisms. Bees, wasps or ants are also killed by the chemical that can cause allergic reactions.

Pesticides allow consumers to intake high-quality product that is free from insect blemishes and insect contamination. Pest control products protect our families, pets and communities from mosquitoes, ticks, rodents, bedbugs and cockroaches and help prevent health issues associated with these pests. Household pests such as termites, ants, rats, roaches and other pests are controlled by pesticides. To protect and enhance lawns, public parks, playing fields, lakes and ponds, pesticides are also used.

Pesticides are used to kill disease carrying organisms. Many insects spread diseases. Mosquitoes are known to spread over 30 diseases harmful to human. Pesticides are also

used to protect forest produce. For example - Gypsy moth defoliates hemlock trees, spruce budworm destroys balsam trees and these pests can be eliminated by pesticides spraying (Sodhi, 2002).

1.6 Adverse effect of pesticide use

Environmental effects

Pesticide use arises a number of environmental concerns. Over 98% of 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 translocation occurs when pesticides suspended in the air as particles, carried by wind to other areas. Pesticides is one of the main causes of water pollution and some pesticides which are persistent organic pollutants, contribute to soil contamination. Non target organisms, such as predators and parasites of pests, also can be affected by chemical application. The reduction of these beneficial organisms can result in changes in the natural biological balances (FAO, 1998).

Health effects

Pesticides can cause danger to consumers, by standers or workers during manufacture, transportation or during and after use (US EPA, 2007).

The World Health Organization and the UN Environment Programme revealed that each year, about 3 million workers in agriculture experience severe poisoning from pesticides, about 18,000 of die (Miller, 2004). Inadequate safety precautions, 99% of pesticide related deaths occur in developing countries that accounts for only 25% of pesticide usage. Twenty five million workers suffer from mild pesticide poisoning yearly in developing countries (Jeyaratnam,1990). There are other careers aside from agriculture that also put individuals at the risk of health effects including pet groomers, groundskeepers, and fumigators (Pesticide Illness & Injury Surveillance, 2016) A 2014 epidemiological study found link between autism and exposure to certain pesticides, but reported that the evidence was insufficient to conclude that the relationship was causal(Bryden et. al, 2013).

Farmers and workers

There are studies of farmers with the goal of determining the toxicity on human health by pesticide exposure (McCauley et al., 2006). Each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die, estimated by World Health Organization and the UN Environment Programme (Miller, 2004). About 25 million workers in developing countries may suffer mild pesticide poisoning yearly (Jeyaratnam, 1990).

These are associated with acute health problems for workers who handle the chemicals, for example; abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobichon, 1996). Additionally, many studies have indicated, pesticide exposure is associated with long-term health issues 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). Organophosphate exposed workers are mainly the prime victim of having diseases like cancer and neurological disfunctions (Alavanja et al., 2004; Kamel and Hoppin, 2004).

1.7 Toxicity

The toxicity of pesticide is its ability to cause injury or illness and determined by subjecting test animals to varying dosages of the active ingredient and each of its formulated products. Pests are controlled by the active ingredients of a certain compound. Acute toxicity and chronic toxicity are the two type of toxic effects. Acute toxicity of a pesticide refers to chemical's ability to cause illness to a person or animal from a single exposure and is determined by examining the dermal toxicity, inhalation toxicity and oral toxicity of test animals. The toxic effects are mainly expressed by LD_{50} and LC_{50} where these refers to Lethal dose 50 and Lethal concentration 50. Chronic toxicity can be measured by allowing a test organism to be exposed to a pesticide for a long period of time. No particular terms are used to define chronic toxic effects.(Ecobichon, 1996).

1.8 Persistence

Persistence means the active existence of a particular component. Half-life is a term of measure of persistence. The time needed for a substance to degrade to its one half from its initial amount is known as half life. The factors that influence the persistency are the characteristics of the pesticide, including its stability either as parent compound or metabolites, its volatility, solubility, formulation, and the method and site of application. Some environmental factors such as temperature, humidity and air movement, also have important effect on the stability of certain component. The species of a plant, growth rate, general condition, soil characteristic, also influence the persistency of a particular pesticide which is used to control pests in that plant. The most important is that the chemical stability and physical characteristics of the pesticides; its stability exerting the greatest influence. In general, there is a possibility of translocation of a pesticide when it persists for so long (Rahman and Alam, 1997; Aziz, 2005).

1.9 Description of pesticides of the current study

1.9.1 Imidacloprid

Imidacloprid is a systemic insecticide which acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids which act on the central nervous system of insects, with much lower toxicity to mammals. Imidacloprid works by interfering and disrupting the central nervous system. Specifically, it causes a blockage of the nicotinergic neuronal pathway. Imidacloprid blocks the nicotinic acetylcholine receptors of the nervous system and thus transmission of the impulses between nerves is prevented, as a result, the insect becomes paralysed and eventually dead. It is a contact insecticide (Extension Toxicology Network, 2012). It makes a linkage with insects neuron receptors which is stronger than that to a mammal neuron receptor (Gervais et al., 2010).

Imidacloprid has chemical formula of $C_9H_{10}ClN_5O_2$ and the CAS number is 138261-41-3. IUPAC name of imidacloprid is N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide (pesticides manual). It is a colorless crystal with melting point of 136.4 to 143.8 °C (277.5 to 290.8 °F; 409.5 to 416.9 K) and the solubility in water is 0.51 g/L (20 °C) (pesticide manual).

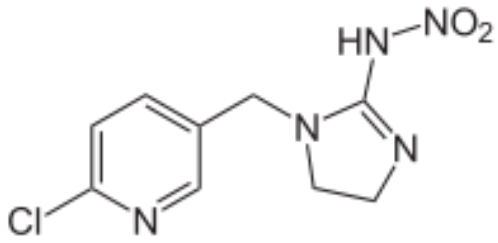


Fig (a): Structure of imidacloprid

Uses of Imidacloprid

Imidacloprid is the most widely used insecticide in the world. Its major uses include; agriculture (Federoff et al.,2008), arboriculture (Preston et al., 2007), home protection (Gervais et al., 2010). It is a systemic insecticide which slowly goes up through plant roots and then upper part of the plant by xylem tissue.

Toxicology

Toxicology Imidacloprid is rated as "moderately toxic" on an acute oral basis to mammals and low toxicity on a dermal basis by the World Health Organization and the United States Environmental Protection Agency . It is rated as an "unlikely" carcinogen and as weakly mutagenic by the U.S. EPA. No effects were observed in rats when exposed to imidacloprid for about two years, at a dose of 100 parts per million (ppm). In rats, imidacloprid effects mainly thyroid. In a one-year feeding study in dogs, no observable effect was seen at 1,250 ppm, while levels up to 2,500 ppm led to hypercholesterolemia and elevated liver cytochrome p-450 measurements (Extension Toxicology Network, 2012), (Canadian Council of Ministers of the Environment, 2007).

Health Effect

Mortality, transient cholinergic effects, growth retardation are the acute oral over-dosed toxic effect of imidacloprid in mammals. Higher dose may also cause cardiovascular and hematological effects. Longer term, low-dose exposure of imidacloprid effects the liver, body weight (reduction), as well as pancreas (USDA, Forest Service , Forest Health Protection, 2005). The effect of imidacloprid may be calculated by the amount of

imidacloprid present and the rate of use (IMIDACLOPRID TECHNICAL FACT SHEET, 2010).

1.9.2 Quinalphos

It is an organothiophosphate. The IUPAC name of quinalphos is *O,O*-Diethyl *O*-2-quinoxalinyl phosphorothioate and chemical formula is $C_{12}H_{15}N_2O_3PS$. At ambient temperature, it is a reddish brown liquid. It is mainly used to control diamond black moth that attacks rice, wheat, maize, cotton, vegetables.

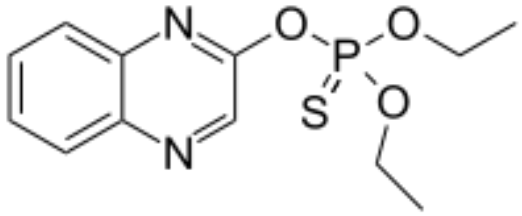


Fig (b):Structure of quinalphos

Toxicity

According to World Health Organization, quinalphos is moderately hazard, but considered as highly toxic in India. Due to its high toxicity, it has been banned in many countries.

1.9.3 Chlorpyrifos

Chlorpyrifos is a crystalline organophosphate insecticide, acaracide and miticide whose IUPAC nameis *O,O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate. The Chemical formula of chlorpyrifos is$C_9H_{11}Cl_3NO_3PS$ and the abstracts Service (CAS) registry number is 2921-88-2. The solubility in water is 2 mg/L (25 °C) with a melting point of 43 °C (109 °F; 316 K).

It was introduced in 1965 by Dow Chemical Company and is known by many trade names, such as; Dursban and Lorsban. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Chlorpyrifos reacts strongly with amines, caustics and strong acids (National Institute for Occupational Safety and Health (NIOSH).

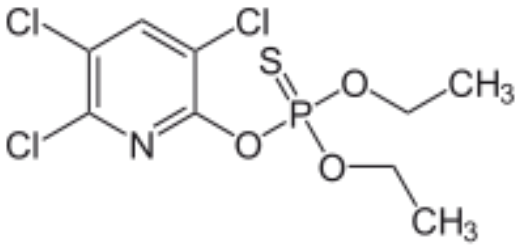


Fig (c): Structure of chlorpyrifos

Chlorpyrifos is moderately toxic to humans, and exposure has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Mentally disordered children were born due to exposure to quinalphos and it was banned in U.S. on 2001 (Scientific American. August 21, 2012). Before being banned (U.S. EPA, 2002), it was one of the most used insecticide in U.S.

Uses of Chlorpyrifos

To control insects in agricultural, commercial settings and residential area, chlorpyrifos is used.

In several countries, it is forbidden to use in residential applications. According to Dow, chlorpyrifos is registered for use in nearly 100 countries and is annually applied to approximately 8.5 million crop acres (The Dow Chemical Company, 2014). It is mainly used in plants like cotton, corn, almonds and fruit ("NASS Agricultural Chemical Database", 2011).

Toxicity

Chlorpyrifos exposure may lead to acute toxicity at higher doses. Long term exposure of chlorpyrifos at low dose may also cause chronic effects.

Mechanism of toxicity

Acetylcholine neurotransmission

Primarily, chlorpyrifos disrupt the function of neurotransmitter acetylcholine (Christensen, 2009). Chlorpyrifos binds with acetylcholinesterase and cause difficulties by deactivating acetylcholine in the synapse (Christensen, 2009).

Non-cholinesterase mechanisms

Experiments on rats suggest that exposure of low doses of chlorpyrifos alter serotonin signaling and increase symptoms of depression; including neuropathy target esterase and several endocannabinoid enzymes; affect components of the cyclic AMP system and influence other chemical pathways (Connors et al.,2008), (Slotkin et al., 2004), (Casidaet al., 2008), (Eaton et al., 2008).

Health effect

Chlorpyrifos exposure during gestation or childhood results lower birth weight with neurological changes (Timofeevaet al., 2010). Chlorpyrifos has been associated with slightly increased risk of wheeze, a whistling sound while breathing due to airway obstruction in the airways (Hoppinet al., 2011). It was associated with higher risks of lung cancer among frequent pesticide applicators than among infrequent users.

Acute health effects

Relatively mild poisoning can result in eye watering, increased saliva and sweating, nausea and headache. Intermediate exposure may lead to muscle spasms or weakness, vomiting or diarrhea and impaired vision. Symptoms of severe poisoning include seizures, unconsciousness, paralysis, and suffocation from lung failure (Christensen et al.,2009).

For acute effects, the World Health Organization classifies chlorpyrifos as Class II: moderately toxic (WHO, 2010). The oral LD_{50} in experimental animals is 32 to 1000 mg/kg. The dermal LD_{50} in rats is greater than 2000 mg/kg and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC_{50} for chlorpyrifos in rats is greater than 200 mg/m^3 (*"Chlorpyrifos"*.Pmep.cce.cornell.edu.).

1.9.4 Cypermethrin

Cypermethrin [(RS)-α-cyano-3-phenoxybenzyl(1RS, 3RS; 1RS, 3RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], Chemical Abstract Name: cyano-(3-phenoxyphenyl)methyl- 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

(CAS number: 52315-07-8) is a digestive and contact insecticide effective against a wide range of pests, particularly leaf- and fruit eating Lepidoptera and Coleoptera in cotton, fruit, vegetables, vines, tobacco and other crops. The chemical formula of cypermethrin is $C_{22}H_{19}Cl_2NO_3$, Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications. It behaves 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. Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN).

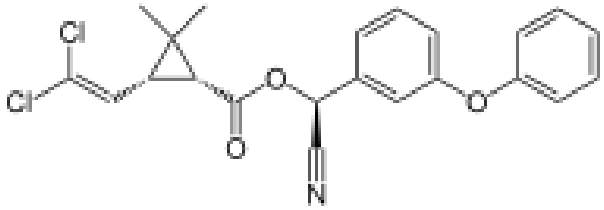


Fig (d): Structure of cypermrthrin

Uses

Cypermethrin is used in agriculture to control ectoparasites which infest cattle, sheep, and poultry (*"Cypermethrin". FAO*). In veterinary medicine, it is effective at controlling ticks on dogs (Somasani, 2014).

Toxicity

Cypermethrin is a moderately toxic through skin contact or ingestion (*"Cypermethrin".* Extension Toxicology Network). Excessive exposure can cause nausea, headache, muscle weakness, salivation, shortness of breath and seizures. In humans, cypermethrin is deactivated by enzymatic hydrolysis to several carboxylic acid metabolites, which are eliminated in the urine (Baseltet al., 2008).

Effect

Cypermethrin is a broad-spectrum insecticide, which means it kills beneficial insects as well as the targeted insects (Pascual, 1992) Fish are particularly susceptible to

cypermethrin but when used according directions, application around residential sites pose little risk to aquatic life (*"Cypermethrin" (PDF). National Pesticide Information Center*).

1.9.5 Lambda- cyhalothrin

It is an insecticide and belongs to synthetic pyrethriod group. It has a very low solubility in waterand is available in the form of powders, pellets, and small capsules. The IUPAC name of lambda-cyhalothrin is 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl cyclopropanecarboxylate and chemical formula is $C_{23}H_{19}ClF_3NO_3$. It is brown or green when solid and colorless when pure.

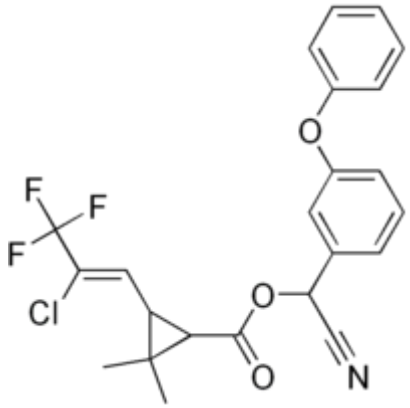


Fig (e): Structure of lambda-cyhalothrin

Uses

It is used to control insects identified as potential disease vectors, such as cockroaches, mosquitoes, ticks, and flies. Aphids, Colorado beetles, and butterfly larvae also cabbe controlled by lambda-cyhalothrin.

Toxicity

By disrupting the action of nervous system, it makes the insect paralyzed and dead. It is highly toxic to fish and bees and moderately toxic to mammals where it is excreted very quickly.

1.9.6 Acephate

The IUPAC name of *N*-(Methoxy-methylsulfanylphosphoryl)acetamide and chemical formula is $C_4H_{10}NO_3PS$. It is sold as a soluble powder, as emulsifiable concentrates, as pressurized aerosol, and in tree injection systems and granular formulations.

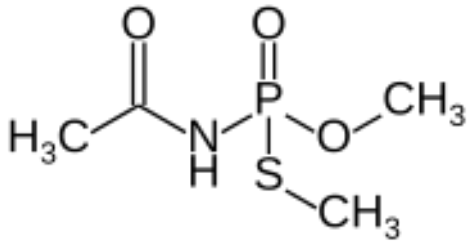


Fig (f): Structure of acephate

Uses

It is used primarily for control of aphids, including resistant species, in vegetables and in horticulture. It also controls leaf miners, caterpillars, sawflies and thrips in the previously stated crops as well as turf, and forestry.

Toxicity

Toxic fumes of various oxides of phosphorus, nitrogen, and sulfur are emitted when acephate is exposed to heat. Eyes and skin irritation may occur when exposed to acephate directly.

1.10 Test insect

Rice weevil, *Sitophylusoryzae*, is one of the most important stored grain pest and was used in the present study during the toxicity test of imidacoprid as test insect.

Common name: Rice weevil
Scientific name: *Sitophylusoryzae*
Order: Coleoptera
Family: Curculionidae

Rice weevil is widely distributed in the tropics and sub- tropics, as a pest of rice, cotton, cereals, beans etc. in storage. It is a serious pest of milled rice and is one of the three primary pests of paddy.

Morphology

The adult has a head produced into a snout like projection. Male and female weevil have different sizes and ranges from 2.0-3.5 mm in length. The colour is generally reddish brown, black (**Fig. 1**). Its antenna has a oval shapped base and apex is wider than base. Rostrum is more coarsely punctured and sculptured in males. Protonum is coarsely and densely pungtured (Booth et al.,1990).

Fig 1: Rice weevil (*Sitophylus oryzae*)

Distribution

It is found all over the world wherever grains are stored. It prefers high temperature and sub-tropical climate. In Bangladesh, India, Pakistan, Srilanka, Bhutan and Nepal, it is a common pests of rice, wheat and cotton.

Food habit

Both husked and nonhusked rice, maize, wheat, cotton, beans are infested by rice weevils. It also causes damage to barley, oats, cotton seeds and cocoa.

Biology

Rice weevil generally breeds from April to October, hibernates in winter as inside cracks and crevices or in godown under grain bags. The female usually bores a small hole in the grain with the help of mandibles before laying the eggs. A single female can lay about 250 eggs at a time. The grub feeds on starchy contents of the grains. A grub is about 3 mm long, it makes a pupal cell and pupates after passing 1 or 2 days as pre-pupa inside the grain (**Fig. 2**). The duration of life of an adult is about one and a half month in Bangladesh (Alam, 1971).

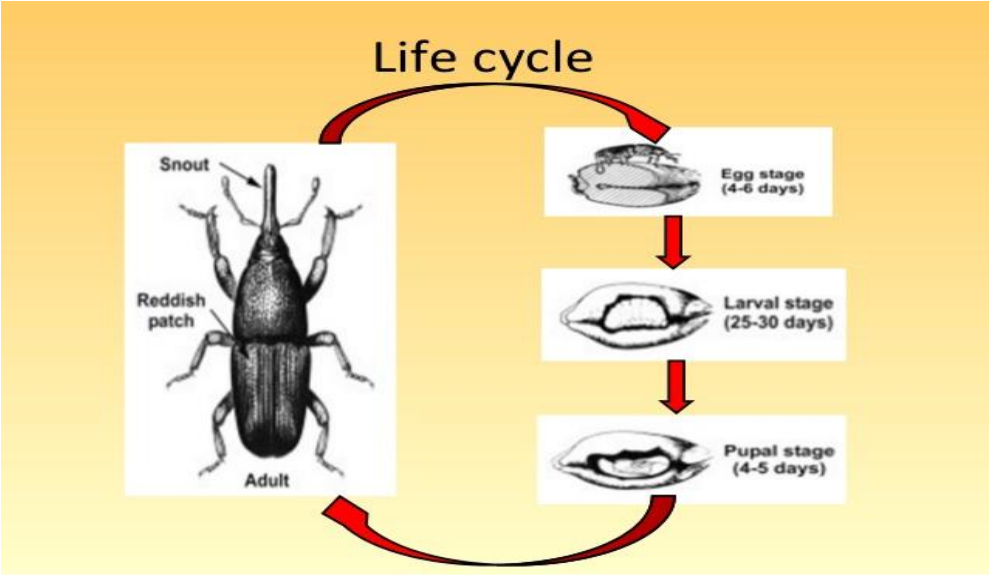


Fig. 2: Life-cycle of rice weevil

1.11The ways of reducing the adverse effect of pesticides

IPM has ability to produce eco-friendly techniques by which healthy crops can be obtained by minimizing the use of pesticide and using pest tolerant crops and thus, increase cost-effectiveness (UNESCAP, 2002). By analyzing suitable methods of

cultivation, biological pest controls (such as pheromones and microbial pesticides), genetic engineering and methods of interfering with insect breeding may reduce the use of pesticides. In addition, EPA is registering reduced-risk carrying pesticides in increasing numbers. Pesticides which are biological such as bacteria, fungi, viruses can cause disease in the pests are frequently used (Pesticide, Wikipedia, 2011). There are insects find their partners by automatic response to various scent cues for mating. Such as male silk moths can smell potential sexual partners and are lured towards them because the female silk moth secrets chemicals called pheromones. Unwanted insects can be trapped by synthetic pheromones instead of spraying a wide area with insecticides.

1.12Degradation of pesticides in environment

Biotic and abiotic transformations are most important mechanisms of degradation of pesticides in soils. Abiotic transformations are involved in the partial degradation of pesticides, resulting in accumulation of metabolites in soils. Complete mineralization to CO_2, H_2O and inorganic ions may results from degradation. Bacteria possess versatile metabolic function on natural and synthetic organic compounds, including pesticides in soils (Chung, 2000).

The microbial metabolism of pesticide is divided into two distinctive processes, mineralization and co-metabolism. Mineralization to inorganic compounds such as CO_2, H_2O and inorganic ions is a normal consequence of microbial activity. Hydrolysis, reduction of nitro group to amino group, oxidation dehalogenation, replacement of sulfur, ring cleavage are various metabolic reactions which helps to degrade synthetic and natural chemicals(Chung, 2000). For the removal of organic and inorganic pollutants from water, photocatalysis has been proved to be an effective tool and also inexpensive and convert pesticides into harmless products (Devipriya et al,.2005).

1.12.1Factors of dissipation of pesticides

Dissipation of pesticides is a behavior that results from the interaction between the chemical and various components of the environment. Pesticides can be dissipated by various routes. The Major routes of pesticide dissipation include adsorption, transfer and degradation.

Adsorption occurs when the pesticide binds to soil particles. Soil sorption is the affinity a chemical has to adhere to soil. The extent to which a pesticide is adsorbed to soil depends on soil type, soil texture, pH of soil, soil moisture and the pesticide itself (Parker and Doxtader, 1983; wild, 1993; Gan et al., 1996).

Transfer of pesticides may occur by-

a) Volatilization to the atmosphere; influenced by the insecticide's volatility or vapor pressure and the temperature and wind movement. As temperature and ambient air movement increases, the potential for pesticide loss through volatilization increases (Yates et al., 2002). Pesticide loss in the air also occur because of spray drift.
b) Washing off by rainfall or overhead irrigation; a function of the chemical's water solubility and the frequency and intensity of waterfall.
c) Runoff of pesticides occurs when pesticides are carried away by surface water. If water addition to a field is faster than it could be absorbed into the soil, runoff occurs (Lecomte et al., 2001).
d) Leaching, downward movement of chemicals in water through soil. Sandy soils are more prone to leach than clay textured soil (Margi and Haith, 2009).
e) Absorption occurs by the movement of chemicals from the surface to the interior part of the plant.
f) Growth dilution; as the apples become larger, the chemical residue concentration will decrease even without physical or chemical dissipation of residue from the fruit.
(g) Metabolism or excretion in the case of animals and some plants. The parent chemical may be converted to one or more degradation products.

Degradation includes photodegradation, chemical degradation, microbial degradation. Microbial degradation is the breakdown of pesticides by micro-organism. The rate of photodegradation is influenced by the intensity, spectrum and duration of exposure of sunlight. Major chemical degradation reactions are hydrolysis, oxidation and reduction (Wheeler, 2002).

Many other studies reported that the dissipation of pesticides depends on several factors, such as the applied dose and formulation, application parameters, the number of application, climatic conditions and the species cultivated (Berger et al., 1999; Cabras et

al., 1999; Garcia-Cazorlaet al., 1998; Herbert et al., 1990; Bowman et al., 1982; Escalada et al., 2008; Rutters et al., 1999).

1.13 Pesticide monitoring by analytical methods

The pesticide residues (PRs) in various fruits, vegetables and environmental samples are analyzed by a number of scientists and the presence of pesticide residues washigher than maximum residue level (MRL) values in many vegetables which were studied in different analysis.The excess amount of pesticides are absorbed by the vegetables and become toxic when consumed by human beings (Kumariet al., 2004). Recently many chromatographic separation or detection procedures coupled with a variety of sample preparation techniques were followed for the multi residue analysis in food and environmental samples.

Many chromatographic separation methods were followed for the multi residue analysis in crops and environmental samples.The first multi residue method (MRM), called the Mills method, was used to determine non-polar organochlorine pesticides in non-fatty foods (Mills et al., 1963) in 1960. This method used acetonitrile to extract samples followed by a dilution of the extract in water and a partitioning of the pesticides into a non-polar solvent. Then, other researchers tried to apply the method to polar pesticides by using different solvents for the initial extraction and adding sodium chloride in the partitioning step (Luke et al.,1975).

The 1990's called for the avoidance of hazardous solvents and the establishment of more environmental and health friendly methods. Solid-phase extraction (SPE) was invented as a cleanup step to avoid using of harmful substances during liquid-liquid partitioning (Hercegova et al.,2007). A number of extraction processes had been evaluated including matrix solid-phase dispersion (MSPD), supercritical fluid extraction (SFE), solid-phase microextraction (SPME), and the quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Anastassiades et al.,2003; Hercegova et al., 2007).These are singular-multiresidue procedure, sufficient cleanup of sample, recoveries approaching 100%, increased concentration of analytes, superior precision, ruggedness, economical,

rapid, easy, environmental-friendly (safe). Modern trends have also added to this list the use of minimal amounts of sample, making methods more safer or "green" (Hercegova et al.,2007).

In many studies, pre-treatment of solid samples were observer (Lehotay et al., 2005b). Fussel (2002),showed that pesticides were lost when samples were pulverized at room temperature. Anastassiades et al. (2003) makes a comparison between shaking and blending in the presence of dry ice as the first step of extraction to ascertain the extent to which pesticide residues could be extracted from produce. Shaking proved best for extraction in their study. Sample extraction efficacy is increased when an adequate homogenization method is used (Lehotay et al., 2005).

In case of developing MRMs, solvent selection is the most important choice. Acetone, ethyl acetate and acetonitrile are the most widely used solvents. Methanol is used more often for the extraction of pesticide residues by high-pressure liquid chromatography with mass spectrometer (HPLC-MS/MS) (Hercegova et al., 2005; Hernandez et al.,2006). There was difficulty in partitioning acetone from aqueous phase since it is wholly water-miscible. Conversely, ethyl acetate is not 100% miscible with water; therefore water can be easily removed by an excess of drying agent, such as anhydrous Na_2SO_4 (van der Hoff and van Zoonen, 1999). Acetonitrile gained popularity after the invention of the QuEChERS method (Anastassiades et al.,2003). Acetonitrile also removes fewer lipophilic compounds from samples compared to acetone and ethyl acetate (Mastovska and Lehotay, 2004). Another benefit of acetonitrile is that it is compatible with reverse phase liquid chromatography (Leandro et al.,2006).

After choosing an appropriate extraction method, the final extracts need to be cleaned by solid phase extraction SPE, dispersive solid-phase extraction (DSPE) or gel permeation chromatography (GPC). Scientists began using different types of carbon sorbents, as graphitized carbon black (GCB) in cleanup step (Schenck et al., 2002). This type of SPE sorbent strongly absorbs planar molecules, such as pigments and isolates them from the sample extract (Hercegova et al., 2007).Other types of SPE columns applied to MRMs of produce have been the reverse phase C18 sorbent and chemical bonded stationary phases,

such as aminopropyl (-NH2), primary-secondary amine (PSA), and strong anion exchange (-SAX) (Leandro et al., 2005). Comparing the cleaning efficiency of these columns on acetone and acetonitrile sample extracts from a variety of produce, Schenck and coworkers determined that -NH2 and PSA normal phase SPE columns were most effective (Schenck et al.,2002). Aminopropyl and PSA columns remove hexadecanoic and octadecanoic acids, fatty acids present in many green vegetables, but C_{18} and SAX can not eliminate the majority of matrix co-eluants in the sample (Schenck et al., 2002) where PSA can also remove sugars and other interferences that are capable of forming hydrogen bonds.

With introduction of QuEChERS by Anastassiades, *et al*., the popularity of using DSPE cleanup techniques increased (Anastassiades et al.,2003). PSA has both a primary and secondary amine, that PSA removed more matrix co-eluants than -NH2 and Alumina which was confirmed by gravimetric analysis. For example, in the QuEChERS method, DSPE give more reproducible recoveries than using regular SPE. Although SPE does provide a better cleanup but higher recoveries make DSPE a better candidate for fatty matrices (Lehotay et al.,2005c).

Sample preparation, extraction, and cleanup should be carefully considered in conjunction with applied chromatographic techniques and detection. The leading approaches in PRA of food – GC and HPLC combined with MS, MS/MS – are used to give the best LOQ and selectivity. In analytical chemistry, GC is the foundation of methodologies for over 60 years (Hercegova et al., 2007). Detectors most widely used in GC, are ECD/MSD. ECD allows to analyze halogenated components at lower detection levels than flame ionization detectors (FID). GC-MS/MS is more accurate than GC-MS because this further reduces matrix effects and lowers LODs (Hercegova et al.,2007). Another detector used with capillary GC is quadrupole MSD. Researchers have used quadrupole MSD in the determination of 19 pesticides at a concentration level of 5.0 ng/g when applied to apple matrices (Hercegova et al.,2005).

HPLC is effective in the partitioning of thermally unstable and non-volatile compounds. Newer classes of pesticides those possess a medium to high polarity and are thermally labile and relatively non-volatile. Past HPLC-based methodologies were applied to UV-Vis and diode array detectors for PRA of food. Both of these detectors are not sufficiently selective or sensitive to deal with complex matrices, such as food samples (Hercegova et al.,2007). For instance, Bicchi, *et al*. determined five pesticide residues at 10 ng g^{-1} using HPLC-UV-Vis but had to pre-concentrate 6.25 fold to obtain data (Bicchiet al.,1996). LC-MS/MS is the most excellent instrument for PRA of all types of matrices because LC-MS/MS has dramatically minimized sample pre-treatment, increased selectivity and sensitivity and increased dependability of quantification and confirmation at ultra-low concentrations. To provide a soft-ionization process that leads to mass spectra with only a few ions, LC-MS/MS interface was designed (Hercegova et al.,2007).

1.14 QuEChERS method

Quick, Easy, Cheap, Effective, Rugged and Safe, the QuEChERS method was developed using an extraction method for determining the residues of pesticides in fruits and vegetables which was followed by a specific clean-up method that removes sugars, organic acids, lipids, pigments and excess water (Schenck and Hobbs, 2004). The method involves two stages; first, the homogenized samples are extracted and partitioned using an organic solvent and salt solution. Then the supernatant is further extracted and cleaned-up by dispersive solid phase extraction (DSPE) technique.

Some modifications to the original QuEChERS method had to be introduced to ensure efficient extraction of pH-dependent compounds (e.g., phenoxyalcanoic acids), to minimize degradation of susceptible compounds (e.g., base and acid labile pesticides) and to expand the spectrum of matrices covered. The analyst homogenizes the sample (fruits, vegetables, tobacco, etc.) in a blender and puts it in a centrifuge tube with a reagent and agitates for 1 minute. The reagents used depend on the type of sample to be analyzed. Following this, the sample is put through a cleanup column prior to analysis by gas-liquid chromatography. Samples prepared using the QuEChERS method can be processed more quickly using a homogenization instrument. Such instruments can homogenize the food sample in a centrifuge tubeand then agitate the sample with the

reagent of choice, before moving the extracted sample for centrifuging. By using such an instrument, the samples can be moved through the QuEChERS method more quickly (A Sample Preparation Approach to Increase the Throughput of Pesticide Analysis by LC–MS-MS, 2009).

1.15 Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

1.16 High Performance Liquid Chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC). The sample is passed by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles. Methods in which the stationary phase is more polar than the mobile phase (e.g., toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (water-methanol mixture as the mobile phase and C_{18} = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC).

Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very different in their

affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength.

In the current study, high performance liquid chromatography was used to analyze imidacloprid residues in rice and wheat flour sample, the model was Shimadzu SCL 10AVP LC (Shimadzu, Kyoto, Japan).

The major components of LC are;

a) *Pumps*

   Two types of pumps are available, low pressure pump and high pressure pump. High pressure pump was used in LC- 10A VP.

b) *Column*

   In the current study, separations were performed by C-18 column (Supelco, end capped 25 cm×4.6 mm *i.d.*, with particle size 5 µm). Column temperature of was maintained at 30^0C.

c) *Injector*

   Manual and auto injectors are available for LC system.In LC-10A VP, analytes were injected through Rheodyne manual injector.

d) *Detector*

   Photo diode array (PDA) detector, refractive index (RI) detector, fluorescence (FLD)

   Detector are the common detectors for HPLC. LC-PDA was used in the current study.

e) *Data monitor*

The minor components are;

Loop(20 µL), mobile phase (any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol), guard column, syringe (with a blunt headed needle) and purging port are the minor components of LC. Before using the mobile phase in the LC system, it was first filtered and then was degassed for about half an hour by mobile phase vacuum filtration apparatus set (1000 mL). Samples were filtered through Millipore filter (0.22 µm pore size) before injecting.

1.17 Diode array detector (DAD, PDA: photodiode Array Detector)

Diode array detectors (also referred to as a DAD detector or more specifically HPLC PDA detector) are used for obtaining spectral profiles from molecular mixtures or chromatographically separated samples. It handles the entire spectrum from the UV at 190nm to the Near IR at 1 micron. These machines provide high sampling speeds of up to 190 Hz and provide thermo electric (Peletier) temperature control of sensors to limit noise. HPLC photo diode array detector detectors offer programmability for processing spectra, utilizing spectral libraries for component identification and multiple spectral displays.

1.18 Gas chromatography

Gas chromatography (GC), also sometimes known as gas-liquid chromatography (GLC), is a separation technique in which the mobile phase is a gas. The first condition for a component to be analyzed in GC is to be volatile. Gas chromatography is based on a partition equilibrium of analyte between a solid or viscous liquid stationary phase (often a liquid silicone-based material) and a carrier gas (most often nitrogen). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat denatures them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields.

Two different models of GC was used in the current study, they were GC-ECD 17A and GC-ECD-2010.

The major components of GC-ECD are;

a) *Column oven*

   The column in a GC is contained in an oven, the temperature of which is precisely controlled electronically. The higher the column temperature, the faster the sample moves through the column. The rate at which a sample passes through the column is directly proportional to the temperature of the column.

b) Column

Gas chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Both types of column are made from non-adsorbent and chemically inert materials. In GC-ECD 17A, ultra performance quartz capillary column (HP 5MS) of 250 µm internal diameter and 30m length having 0.25 µm film thickness was used and in GC-ECD 2010, a non-polar (HP-5 MS) capillary column of dimension, 30 m long x 250 µm i.d.x 0.25 µm film thicknesses from Agilent, USA was used to carry out the separation.

c) Carrier gas

Typical carrier gases include helium, nitrogen, argon, hydrogen and air. In the current study, nitrogen was used as carrier gas.

d) Detectors

The most commonly used detectors are the Electron capture detector (ECD), flame ionization detector (FID), thermal conductivity detector (TCD) and Nitrogen–phosphorus detector (NPD). Both of the GC system used electron capture detector (ECD) in the current study.

e) Data monitor

Again, injector port (manual injector in GC-ECD 17A and auto injector in GC-ECD 2010 were used), syringe (sharp headed needle) are minor but important components of GC.

1.19 Electron Capture Detector (ECD)

The electron capture detector (ECD) is mainly used in environmental testing for detecting PCB's, organohalogen compounds and various halogenated hydrocarbons. It uses a radioactive beta particle (electron) emitter in conjunction with a so-called makeup gas flowing through the detector chamber. The electron emitter typically consists of a metal foil holding 10 millicuries (370 MBq) of the radionuclide ^{63}Ni. Usually, nitrogen is used as makeup gas, because it exhibits a low excitation energy, so it is easy to remove an electron from a nitrogen molecule. The electrons emitted from the electron emitter collide

with the molecules of the makeup gas, resulting in many more free electrons. The electrons are accelerated towards a positively charged anode, generating a current. There is therefore always a background signal present in the chromatogram. As the sample is carried into the detector by the carrier gas, electron-absorbing analyte molecules capture electrons and thereby reduce the current between the collector anode and a cathode. The analyte concentration is thus proportional to the degree of electron capture. ECD detectors are particularly sensitive to halogens, organometallic compounds, nitriles, or nitro compounds.

1.20 Important terms for analytical methods

Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.It is performed in terms of recovery experiment, calibration curve, selectivity, specificity. Results from method validation were used to judge the quality, reliability and consistency of analytical results.

Specificity

It is the ability of a method to quantify accurately and specifically the analyte or analytes in the presence of other compounds present in the matrix It is determined by analyzing standard mixture of pesticides, blank matrices and blank matrices spiked with the mixture of pesticides simultaneously and by checking their retention times.

Sensitivity

It is a response obtained by a given analyte in the machine under used. It is assessed by determining limits of detection (LODs) and limits of quantification (LOQs) for each pesticide in each matrix.

Linearity

It is the ability of the method to produce test results that are proportional to the concentration of in sample within a given range.It is determined by using a minimum of five standards plotting against the corresponding area of those concentrations and should

be established by visual inspection of a plot of analytical response as a function of analyte concentration.

Limit of detection (LOD)

It is the minimum concentration or mass of the analyte that can be detected with acceptable certainty, though not quantifiable with acceptable precision.

Limit of quantification (LOQ)

It is the minimum concentration or mass of the analyte that can be quantified with acceptable accuracy and precision. LOD and LOQ are often the quantity of analyte those generates a response of 3 times and 10 times greater than the noise level of the system.

Accuracy

It is the degree of agreement between an individual test result generated by the method and the true value.

Precision

Itis the agreement among individual test results when the procedure is applied repeatedly to multiple sampling.

Recovery

Studies were undertaken to evaluate the efficiency of the extraction procedure used. The recovery of pesticides was done in replicate and was determined by spiking the previously analysed samples with the pesticide standard at concentrations similar to those expected in the samples. A widely accepted criterion for the acceptability of performance of an analytical method is its capability of providing average recovery within the range of 70%-120% (Codex, 2000). The recovery values expressed in percentages were calculated from the chromatograms.

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

Maximum residue limit

It is defined as "the maximum concentration of a residue that is legally permitted or recognized as acceptable in or on a food or agricultural commodity or animal feedstuff" (FAO code of conduct, 2002). The interval between the last application of pesticide and the safe harvesting of edible crops is called Pre Harvest Interval (PHI) and every pesticide has a Pre Harvest Interval (PHI) for residues to dissipate below the MRL established for that corresponding crop. Food stuff become safe only after pre-harvest interval and it differs from pesticide to pesticide and crop to crop.

1.21 Bangladesh context

In Bangladesh, the use of pesticides started during the middle of the 1950s to promote crop production (Rahman and Alam, 1997). It plays an important role in the lives of the people of Bangladesh. The major crops of the country are rice, wheat, pulses, jute, oilseed, vegetables, potatoes, sugarcane, cotton and tea. The humid climatic conditions of the country increased high yielding varieties of food crops and more use of chemical and natural fertilizers are highly favorable for development of agriculture. The loss in yields was estimated which is due to the pest attack and diseases ranges from 15 to 25 percent annually (Aziz, 2005). Both overuse and misuse may lead to loss of effectiveness of insecticide due to the development of resistance (Forrester, 1990) and thus, cause health hazards and environmental pollution (Maclntyre et al., 1989). Inappropriate use of insecticides and doses, improper spray scheduling and inadequate spray coverage (Phillips et al., 1990) cause failing in controlling insect pests. An increase in the use of pesticides by farmers in combating pests throughout Bangladesh was observed in last few decades. Generally 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(winter rice), potato, bean, eggplant, cabbage, sugarcane and mango growers (Rahman, 2002).

Craig Meisner, (2004) reported that the agricultural output of Bangladesh needs to grow several times during the next several decades, as the population of Bangladesh continues to grow and incomes increase. Bangladesh has to increase yields from the lands which are under cultivation in order to serve this increased demand. Pesticide use warrants a careful assessment of this situation as well as experimentation with feasible alternative production systems such as Integrated Pest Management and organic farming techniques. Like many developing countries, Bangladesh lacks sufficient information on pesticide use, even at the regional level. In 2003, a survey on farm-level pesticidal use was conducted (Development Economics Research Group, Infrastructure and Environment Department of the World Bank). The survey sample was distributed among several districts to capture any possible differences in production or pesticide use. Bogra, Rangpur, Chapainawabgunj, Rajshahi, Comilla, Jessore, Chittagong, Munshiganj, Narsingdi and Mymensingh districts were covered by the survey (**Fig. 3**).

Informations on the type and application rate of pesticides used in each crop were collected and thus calculations on pesticide use intensities (measured as kg of pesticide per kg of crop) were done.

Fig. 3: Pesticide use in different districts (measured as kg of pesticide per kg of crop)

It can be assumed that all pesticides are alike (in terms of toxicity) if the results are summed up. From a health-hazard perspective, risk-weighted measures place a greater weight on more toxic substances. For this, the LD_{50} (or lethal dose 50%) was used. In **Fig.4** and **Fig. 5**, it is shown that pesticide use is increasing in Bangladesh. In several districts where the rate of using pesticide was high in 1994-95, had been intensified by 1999-2000 (Craig Meisner, 2004).

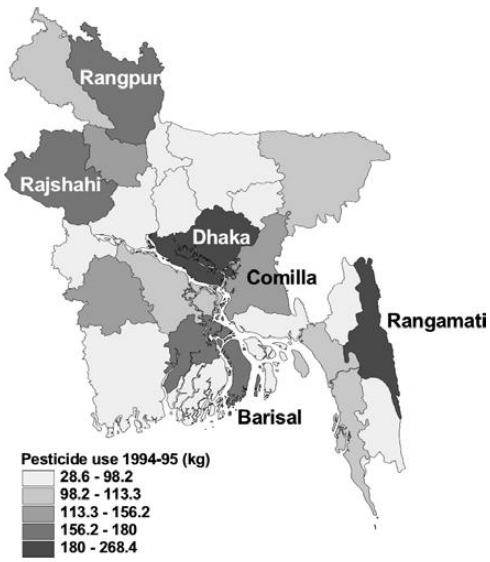


Fig. 4: Pesticide use (un-weighted), 1994- 1995

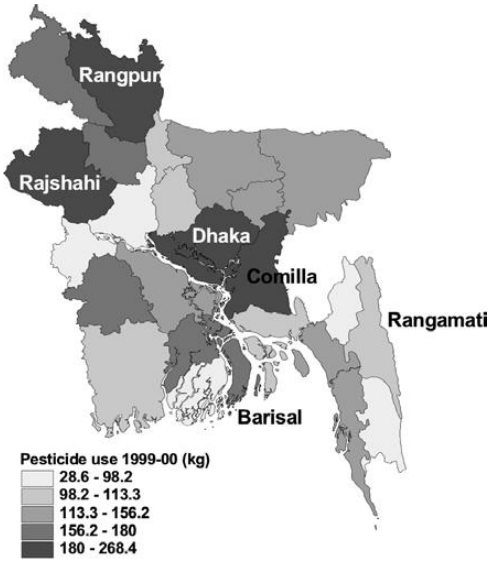


Fig. 5: Pesticide use (un-weighted), 1999-2000

Most of the farmers are not able to take perfect decision on pest management and pesticide application in Bangladesh. Most of the times, they apply pesticides at wrong dose. As a result, beneficial pest management organisms are killed causing greater problems and crop losses. Most of the farmers are hand sprayer and use traditional methods which may cause high risk of toxicity and contaminations (Pingali and Roger, 1995).

Leading to hampering bio-diversity, disrupting natural pest control, causing toxicity in soil, reducing earthworms, developing resistance among target pests and creating hazards to human health are resulted by using broad spectrum pesticides (Islam, 2000; Ibiayo, 2006).

The pollution of environmental resources through the indiscriminate use of pesticides is very common in Bangladesh, although there are Pesticide Acts and Rules (The Environment Court Act, 2000) but some important provisions of the legislation are not strictly followed, which may result in the gradual increase in the risk to humans, animals, fish, birds and the environment. Most of the farmers do not maintain the safe harvesting period due to the lack of knowledge. Even farmers increase the dose to higher levels when the desired effects are not obtained. An interview was taken by our research group on June 22, 2013 in Jhenidah district. It was interviewed about 40 farmers aged about 30-65 years. 98% farmers confessed that they became beneficial by the application of pesticides. About 80% farmers do not know about active ingredients in pesticides. About 60% of them use pesticides every day, 20% in every two days interval, 30% at 4 days interval. About 50% famers harvest crops the next day after application of pesticides which may cause acute health effects.

The interview reflects a general scenario of farming in Bangladesh. Maximum farmers, spray pesticides, especially in vegetables in every day or in every alternate day. Majorities do not care about the side effects of the pesticides and suffer from pesticides related diseases. So it is needed to train the farmers as well as the traders or suppliers about the toxicity of the pesticides. At the same time, awareness of the consumers should be grown about the maximum residue levels of the pesticides in the commodity they consume every day.

EXPERIMENTAL

2.1 General

2.1.1 Materials and methods

2.1.1.1 Chemicals and solvents

Analytical grade anhydrous magnesium sulphate, anhydrous sodium sulphate, sodium chloride, silica gel (0.063-0.200 mm) of Merck, KGaA, Darmstadt Germany, were used for this analysis. Primary secondary amine (PSA) from Supelco USA, was used in cleaninig up steps. Analytical grade acetone, ethyl acetate, dichloromethane, methanol and HPLC grade acetonitrile (ACN) and ultra pure n-hexane were obtained from Merck KGaA, Darmstadt Germany. HPLC grade water (milli-Q water) was used as a constituent of mobile phase, free from cations, anions and hydrocarbons.

2.1.1.2 Certified standards

The standards of imidacloprid (99% purity), chlorpyrifos (99.5% purity), cypermethrin (91% purity), quinalphos (99.2% pure) were purchased from Dr. Ehrenstorfer, Germany. Acephate (84% purity), lamdacyhalothrin (87% purity) were purchased from Jiangsu Hongze Chemicals and Industry Co. Ltd, China and Auto Crop Care Ltd, respectively.

2.1.1.3 Glass and plastic apparatus

Pipettes and volumetric flasks which were used in the current experiment were calibrated by Bangladesh Standard Testing Institute (BSTI). Mobile phase vacuum filtration apparatus set (1000 mL), desiccators, vial, round bottom flask, Teflon tube, graduated test tube, funnel, plastic syringe, micro pipette were also used in different steps of the anlysis. Most of these glass apparatus were brought from Quick fit, England.

2.1.1.4 Minor equipments

Four digit balance and standard weights were used for balancing which were calibrated by BSTI. Rotary vacuum evaporators (Buchi R-210, Switzerland and Heidolph, Germany), vortex machine, water purification system (Boeco, Germany), furnace (GSM 11/8 Hope Valley, S336RB, England), ultrasonic bath, oven (Salvis G- 1020) were used

during the experimental as a part of the work. Two centrifuge machines were used for centrifugation (SIGMA 2-16, Bench top, 10,000 rpm and Cowbell).

2.1.1.5 Major equipments

Gas chromatograph with electron capture detector was used to analyze halogenated pesticides. For particular analysis, a GC-ECD Shimadzu-2010 (**Fig 6**), with auto injector was used. Another gas chromatograph, GC-ECD Shimadzu 17-A with manual injector was also used in the current study.

For particular samples, High Performance Liquid chromatograph, Shimadzu SCL 10A VP system (Shimadzu, Kyoto, Japan), consisted of dual pumps, a manual injector and a SPD-M10A VP photodiode-array detector in the range of 200-400 nm (wavelength). The LC system was fitted with a Supelco-C_{18} (25 cm × 4.6 mm i.d., particle size: 5 μm) column (**Fig. 7**).

Liquid chromatography- tandem mass spectrometry analyses were carried out using Shimadzu LCMS-8050 with electrospray ionization (ESI), a triple quadrupole LC-MS/MS. Shimadzu Prominence Ultra Fast Liquid Chromatograph (Communications Bus Module CBM-20A; Degassing Unit DGU-$20A_{3R}$; Column Oven CTO-10AC ; Solvent Delivery Unit LC-20AD; Auto Sampler SIL-$20AC_{HT}$) was used in the studies (**Fig. 7**). Nebulizing and collision gas was N_2. Separations were performed on a Shim-pack GISS C_{18} column (250 x 4.6 mm i.d.; particle size 5 µm). The carrier gas pipe was 5 m.

Fig. 6: Shimadzu GC-ECD 2010

(a)

(b)

Fig. 7: Shimadzu LC-10A VP (a) and Shimadzu LCMS-8050 (b)

2.1.1.6 Methods

2.1.1.6.1 Cleaning of glass apparatus

All required glass apparatus were cleaned with water using detergent, rinsed with distilled water and finally with acetone. All glassware were baked at 300 ^{0}C overnight, cooled and stored by covering with aluminum foil. Before using, all the glass apparatus were rinsed with the corresponding solvent.

2.1.1.6.2 Activation of anhydrous sodium sulphate and magnesium sulphate

Sodium sulfate and magnesium sulfate was activated by heating at 300 ^{0}C for 8 hrs in a furnace and then these anhydrous materials were kept in a desiccators until use.

2.1.1.6.3 Evaporation

Under reduced pressure, all the evaporations were carried out using rotary vacuum evaporator at water bath at a temperature not exceeding 40 °C. Organic solvents of small volume were evaporated through nitrogen gas while keeping the test tube in block heater.

2.1.2 Preparation of primary standard solutions

Certified pesticide standard (10 mg) was dissolved in 100 mL of ACN or n- hexane to prepare 100 µg mL^{-1} of stock solution. All the prepared solutions were labeled indicating name of each of the standard, concentration and the date of preparation. The meniscuses of the solutions were marked with permanent black ink and stored in the freezer (-20 ^{0}C) away from the sample storing area until further use.

2.1.3 Preparation of middle and working standard solutions

The primary standard solutions were taken from the freezer to reach room temperature and checked the meniscus of the layer. Then 1.0 mL of the primary stock solution was diluted with 9.0 mL of corresponding solvent in a 10 mL volumetric flask to prepare 20 µg mL^{-1} secondary standard solution. These solutions were labeled indicating name of the standard, concentration and date of preparation. The meniscuses of the solutions were marked with permanent ink and also stored in a freezer (-20 ^{0}C) away from the pesticide residue laboratory. The middle standard solution was then serially diluted with the same solvent to obtain the working standard solutions (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019, 0.009 and 0.004 µg mL^{-1}).

2.1.4 Calibration curve

The GC and the HPLC were conditioned until a smooth baseline was obtained. The GC was conditioned at its maximum operating temperature and the HPLC system was conditioned by passing mobile phase (acetonitrile: water) in different proportions until a

smooth baseline is obtained. The instruments were conditioned at specific parameters of different methods to equilibrate the column, for about half an hour. Calibration curve of the pesticides were prepared by serially diluted standard solutions of 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.03, 0.019, 0.009 and 0.004 µg mL^{-1} from the primary standard solution. Then serially diluted standards from lower to higher concentrations were injected gradually into either GC-ECD or LC-PDA to get the area. Then the areas of the standards were plotted against the concentrations (µg mL^{-1}) of the standards for obtaining the calibration curve.

2.1.5 Identification and quantification by GC and HPLC

Under identical analytical conditions, a specific compound in a certain sample and it's reference standard solution, both give peaks at similar retention time in GC and HPLC.

The certified standard solutions were injected into GC or HPLC instrument and under the same conditions of the parameters, cleaned extract of samples were also injected. By matching the retention times of the different peaks of the sample with the retention times of the certified standard compounds, residue present in the samples were identified.

For quantification, concentration of the corresponding analyte was found out from standard calibration curve taking into consideration that the peak area was in the midpoint of the curve. Amount of unknown analyte were found out by using the linear regression formula of calibration curve;

$$y = mx + c$$

Here,

y = Peak area

x = Concentration

m = Slope of the calibration curve

c = Intercept

$$\text{Concentration} = \frac{(\text{Peak area} - \text{intercept}) \times \text{Dilution factor}}{\text{Slope} \times \text{Sample weight}}$$

2.2 Analysis of imidacloprid in rice and wheat flour

Imidacloprid is used in different food stuff, such as, rice, wheat, cotton, maize etc; during storage and packaging, to protect them from pest attacks. Market samples of rice and wheat flour were collected to analyze the residual amount of imidacloprid, from the food safety point of view.

2.2.1 LC conditions

Separations were carried out by maintaing the column temperature at 30 °C. The sample extracts were injected manually by using a 20 μL sample loop and injection volume was 60 μL. Mobile phase was an isocratic elution of acetonitrile: water (70:30). The flow rate was set at 0.5 mL min^{-1} using a UV wavelength of 280 nm. Retention time (t_R) of imidacloprid under these conditions was 6.1 mins.

2.2.2 LC-MS/MS instrumentation

Liquid chromatography-mass spectrometry analyses were carried out using Shimadzu LCMS-8050, a triple quadrupole LC-MS/MS. The analysis mode was positive and ionization process was electronspray ionization (ESI).

2.2.3 Filtration and degassing

Acetonitrile and HPLC grade water (Milli-Q water), both were filtered in a Sartorius vacuum pump device (mobile phase vacuum filtration apparatus set (1000 mL) and degassed for about half an hour in a ultrasonicator before using in HPLC analysis.

2.2.4 Preparation of standard and working solution of imidacloprid

Certified standard of imidacloprid (10 mg) was dissolved in 100 mL of acetonitrile to prepare 100 µg mL^{-1} of stock solution. Then the primary standard solution was serially diluted with acetonitrile to prepare working standard solutions (20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.0195, 0.009, 0.005 µg mL^{-1}). These standard solutions were labeled indicating name of the standard, solvent, concentration, date of preparation and signature and stored in the freezer (at -20 ^{0}C) until use.

2.2.5 Analysis of imidacloprid in rice

2.2.5.1 Purchase and storage of rice sample

In total, thirty one samples were collected to analyze the experiment. Among them, fifteen samples were unpacked, bought from various open markets in Dhaka and sixteen were packed and branded rice samples (**Table 1**). In these samples both fragrant, such as kaligira, chinigura and nonfragrant, such as minikate, najirshail were present (**Fig. 8**). All the collected rice samples were stored in a freezer at -20 $^\circ$C until analysis.

2.2.5.2 Preperation of control sample of rice

Parboiled minikate rice (weighed about 100g) was boiled with 1 L of water for about 30 minutes. The rice water was then discarded and only the boiled rice was taken in a bowl (with holes at the bottom) following by washing thoroughly with boiled luke warm water.The rice sample was then dried in sunlight for 24 hours, collected in a zip-lock bag and stored in a freezer at -20 $^\circ$C until analysis.

Table 1: Rice samples

Rice samples	
Packed	**Unpacked**
Pran Kalijira rice	Chinigura rice, Noyabazar
Pran Chinigura rice	Najirshail rice, Noyabazar
Pran Najirshail rice	Kalijira rice, Polashi
Pran Minikate rice	Minikate, Polashi
Aarong Kalijira rice	Chinigura rice, Moghbazar
Aarong Chinigura rice	Kalijira rice, Moghbazar
Aarong Najirshail rice	Chinigura rice, Farmgate
Aarong Minikate rice	Kalijira rice, Farmgate
Shwapno Kalijira rice	Najirshail rice, Farmgate
ShwapnoNajirshail rice	Kalijira rice, Cantonment
Radhuni Kalijira rice	Minikate rice, Cantonment
Radhuni Chinigura rice	Chinigura rice, Gulshan-1
Radhuni Najirshail rice	Kalijira rice, Gulshan-1
Chashi Kalijira rice	Kalijira rice, Gabtoli
Chashi chinigura rice	Najirshail rice, Gabtoli
Chashi Najirshail rice	

2.2.5.3 Analysis of cooked fragrant rice samples

To analyze whether imidacloprid residue persists or do not persist in rice after cooking, five fragrant rice samples (**Table 2**) were boiled for about 30 minutes, cooled and then extracted according to **Scheme 1**.

Table 2: Cooked rice samples

Samples	Condition
Pran chinigura	Cooked
Radhuni kalijira	
Aarong kalijira	
Chashi kalijira	
Chinigura, Gulshan	

2.2.6 Analysis of Imidacloprid in wheat flour

2.2.6.1 Purchase and storage of wheat flour sample

In total, 16 wheat flour samples (**Fig. 9**) were collected to analyze the experiment. Among them, one is control, ten were branded packed samples and five were unpacked samples brought from various markets in Dhaka (**Table 3**). All the collected samples were stored in a freezer at -20 $^{\circ}$C until analysis.

Fig. 9: Different wheat flour samples

2.2.6.2 Preperation of control sample of wheat flour

Raw wheat (200 g) was bought from Raishabazar, Dhaka. Then 100 g of the sample was boiled with 1.5 L of water for about 1 hour. The water was then discarded and only the

boiled wheat beads were taken in a bowl (with holes at the bottom) followed by washing thoroughly with boiled luke warm water. The sample was then dried in sunlight for 24 hours, ground, collected in a zip-lock bag and stored in a freezer at -20 $^{\circ}$C until analysis.

Table 3: Wheat flour samples

Samples	Condition
Teer atta	Packed
Pusti atta	
Fresh atta	
Fauji atta	
ACI atta	
Ifad atta	
Bashundhara atta	
Diamond atta	
Yusuf atta	
Saad atta	
Wheat flour (Noyabazar)	Unpacked
Wheat flour (Moghbazar)	
Wheat flour (Farmgate)	
Wheat flour (Cantonment)	
Wheat flour (Uttara)	

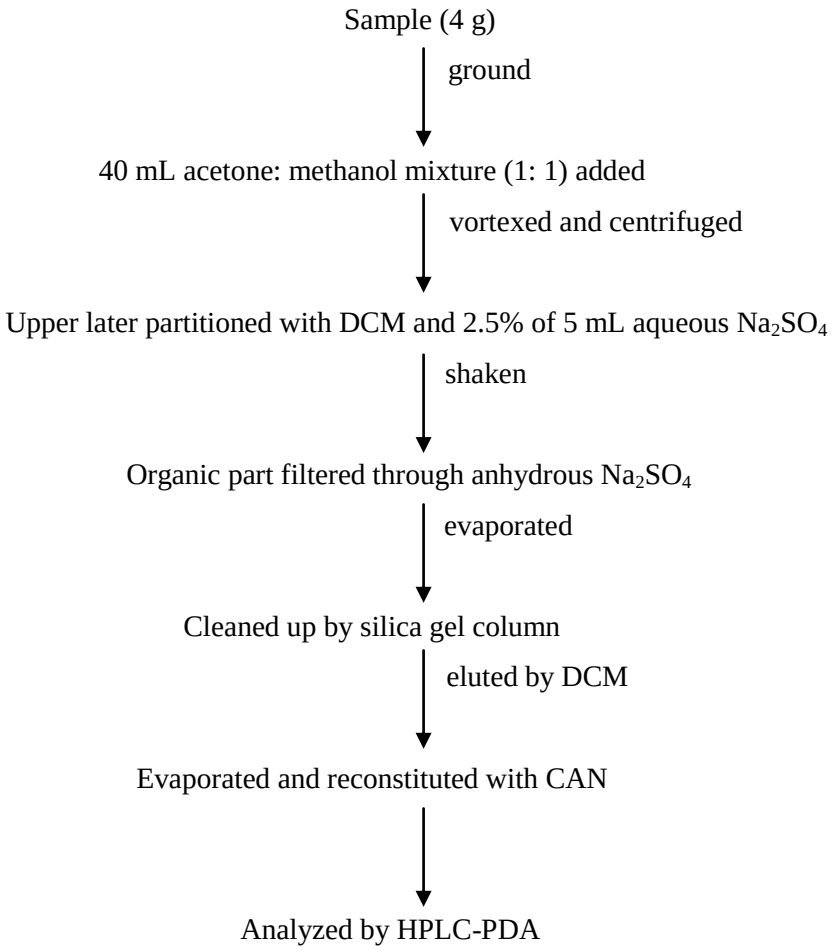
2.2.7 Extraction process for determining imidacloprid residue in rice and wheat flour

The extraction and cleanup were undertaken as method described by Uddin et al., (2011) (**Scheme 1**). The sample (4 g) was homogenously ground by a blender and transferred to a 45 mL Teflon tube. A volume of 40 mL of acetone: methanol (1: 1) mixture was added

to the ground sample. The tube was vortexd for 1 minute, ensuring that the solvents interacted well with the entire sample. The solution was then centrifuged for about 5 minutes. The extract was added to a volume of 25 mL dichloromethane and 200 mL of 2.5% sodium sulfate solution in a 250 mL separatory funnel. The solution was shaken by hand for about 3 min and vented regularly. After shaking, the solution (lower layer) was filtered into the round bottom flask of 500 mL capacity through Whatman filter paper No.1 bearing a bed of anhydrous sodium sulfate. The residual cake was re-extracted with addition a volume of 30 mL acetone: methanol (1:1) and added to 200 mL of 2.5% (w/v) sodium sulphate solution. A volume of 25 mL of dichloromethane was added to the solution. The DCM extract was collected and concentrated to a smaller volume using vacuum evaporator at $\leq 40^{\circ}$C.

2.2.8 Cleanup

The concentrated extract was subjected to further clean up by column chromatography. A glass column packed with silica gel as adsorbent was employed. At the top of silica gel, a 2 cm layer of anhydrous sodium sulfate was added. The column was preconditioned with DCM and concentrated extract was loaded onto top of the column and eluted with 25 mL DCM at 2 mL/min. The elute was collected in a round bottomed flask using Whatman No.1 filter paper. Each elution was concentrated to dryness using rotary vacuum evaporator at $\leq 40^{\circ}$C and dissolved in acetonitrile to make up to 1 mL, finally transferred to a vial using syringe filter. The cleaned-up extract was then analyzed by LC-PDA.



Scheme 1: Extraction and clean-up process for identification of imidacloprid

2.3 Toxicity test of imidacloprid in rice samples

According to World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA), imidacloprid is rated as "moderately toxic" on an acute oral basis to mammals and low toxicity on a dermal basis (Imidacloprid- wikipedia). The current study presents an improved method for analyzing the toxicity of imidacloprid in rice samples which we consume every day, which was performed against rice weevil, *Sitophylus oryzae,* that followed a standard protocol (Abbott et al., 1987) and was evaluated with the collaboration of Bangladesh Institute of Nuclear Agriculture, Mymensingh.

2.3.1 Collection and rearing of rice weevil

The rice weevil was collected from the stock culture of the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh.

The rice weevil was reared in round plastic jars (**Fig. 10**) with rice grains (13-14% moisture) in growth chambers at 30 °C temperature.

The rice grains were sterilized at 60 °C for 30 minutes and then grains were used as food for them. Each jar was set up with one hundred and fifty pairs of adult insects. The mouth of the jar was covered with cheese cloth fastened with rubber bands to prevent contamination and insect escape. The insects were allowed 7 days for mating and oviposition and then they were removed from the jar. One to two weeks old weevils were used for the experiment.

Fig. 10: Rearing of rice weevils

2.3.2 Extraction process for evaluating toxicity test

Toxic effect of imidaclprid was evaluated against adult rice weevils, *S. oryzae*, following a standard protocol. Rice sample (parboiled) was ground into powder and a mould was made from the powder by adding water. Six disks (approximately 0.3 g per disk) were made from the mould. One disk was used as control, one was blank and the rests (n=4) were spiked with four different concentrations of imidacloprid by adding 150 uL per disk. Each of the disk were taken in separate petri dish. The solvent was allowed to evaporate for 24 hrs. Adult rice weevils (n=10), were then placed per petri dish to find out the effect of the pesticide on the insects.Each treatment was replicated thrice. Weevils were

examined daily. Mortality of the insect was counted after 24, 48 and 72 hours after treatment. The percentage of insect mortality at 24, 48 and 72 hours was counted and the toxicity was determined.

2.3.3 Data analysis

The observed mortality was corrected by Abbott's formula (1987).

Corrected mortality (%) = (Observed mortality–Control mortality/100 - Control mortality) x 100.

The data were analysed by analysis of variance (ANOVA) and significant mean values were compared with Duncan's multiple range test (DMRT).The per cent mortality data were transformed into arcsine values before ANOVA. The mortality data were used to determine LD_{50} (Lethal Dose, 50%). Then it was analysed by probit analysis using MSTAT-C Statistical Software in a computer which is based on the method of Finney (1971)/. The probit regression equations and lines were calculated for the relationship between probit mortality of insects and log doses of different plant extracts.

2.4 Dissipation of Quinalphos in tomato, cauliflower and beans

A field experiment was conducted at Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, to study the dissipation of Quinalphos in dna rewolfiluac ,otamot ,selbategev hcae fo doirep gnitsevrah efas eht dna selpmas naebwhen quinalphos was sprayed at recommended dose.

Fig. 11: Vegetable samples collected from BARI

2.4.1 Land preparation

The cultivation land was prepared by harrowing followed by ploughing, cross ploughing and leveling. Fertilizers were applied as recommended dose for 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 was applied as basal dose during land preparation. The entire dose of Urea and the rest of MP were applied as top dressing.

2.4.2 Raising of seedling and transplanting

Seeds were sown in the nursery bed at research field of Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. Light irrigation was done regularly for ensuring proper growth of the seedlings. Seedlings of forty days were transplanted in the well prepared experimental plot. The standard tomato, bean and cauliflower cultivation protocol were followed.

2.4.3 Cultural operation

After transplanting, pit having seedlings were irrigated lightly. At an interval of 2-3 days, supplementary irrigations were applied. Weeding was carried out when necessary.

2.4.4 Sample collection

Quinalphos, 0.03 percent aqueous emulsion prepared from Convoy 25 EC was sprayed at a recommended dose of (1 mL $L^{-1}$of water/ ha), in experimental field of bean, tomato and cauliflower on 24 December, 2014 (**Table 4**). Three replicate samples from each bed of three vegetables (**Fig. 12**) were collected from 0 day (after 2 hours of spraying) and gradually each day upto 15 days after spraying (**Table 5**). Before spraying, control samples (untreated) were harvested. All the samples were taken in jeep-locked bags and immediately brought to the laboratory and kept in a freezer below -20 °C. The collected samples of each day were cut into small pieces and then homogenized by kitchen blender; required amount of homogenized samples was transferred into Teflon tube and then preserved in the freezer until analysis was carried out.

Fig 12: Experimental field of cauliflower (BARI)

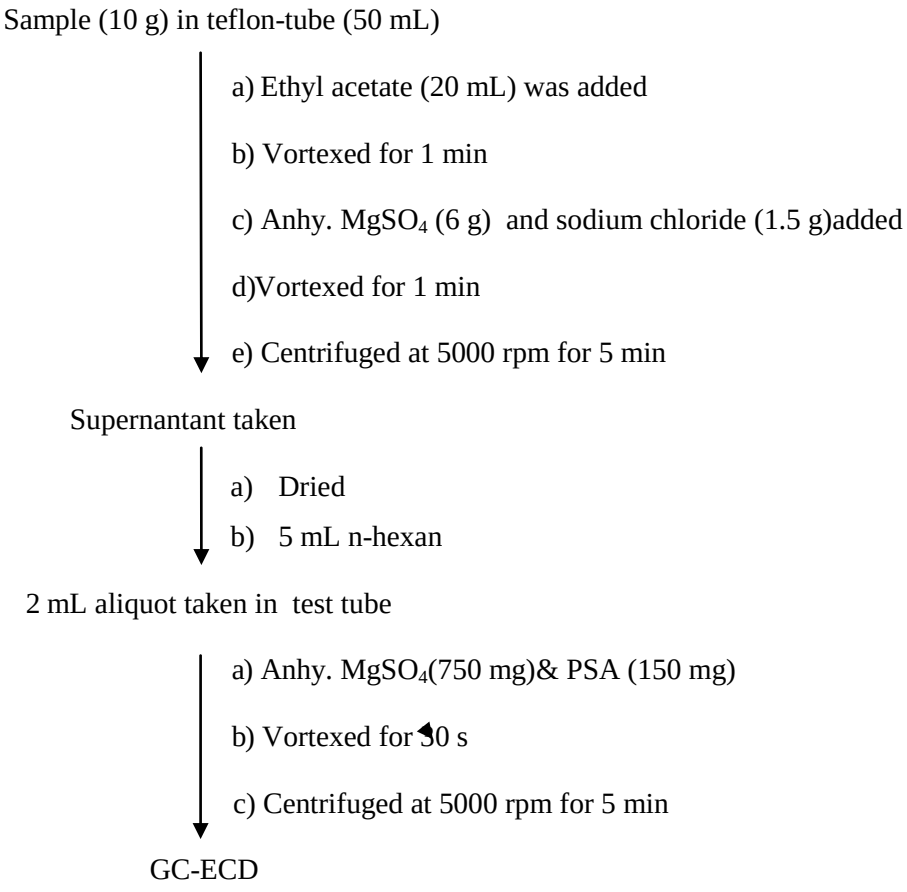
Fig 13: Experimental field of bean (BARI)

Table 4: List of selected vegetable samples

Local Name	English name	Scientific name
Sim	Bean	*Phaseolus vulgaris*
Phulkopi	Cauliflower	*Brassica oleracea var. . botrytis*
Tomato	Tomato	*Lycopersicon lycopersicum*

2.4.5 Extraction

The extraction process followed the QuEChERS method (**Scheme 2**). 10g homogenized sample was taken in a Teflon tube and 20 mL of ethyl acetate was added to it. The tube was then vortexed for about 1 minute. Then 6 g of MgSO4 and 1.5 g of NaCl were added and again vortexed for 1 minute. The mixture was then centrifuged for 5 minutes at 5000 rpm maintaining temperature 20^0C. 10 mL supernatant was taken in a round bottom flask and dried to dryness. 5 mL n- hexane was added to the dried mass and from there; 2 mL solution was taken in a test tube.



Scheme 2. Extraction and clean-up process for identification of quinalphos in beans, cauliflower and tomato

2.4.6 Clean up of sample

For clean-up, 150 mg PSA and 750 mg MgSO4 were added and vortexed for about 1 minute. It was then centrifuged for another 5 minutes and then passed through cotton filter and the sample became ready to analyze by GC-ECD.

2.4.7 GC-ECD analysis

Quinalphos residue was identified and quantified using Gas chromatograph (Shimadzu 17A) equipped with electron capture detector (ECD). Ultra performance quartz capillary column (HP 5MS) of 250 µm internal diameter and 30m long having 0.25 µm film thickness was used. The injector and detector temperatures was set at 280^0C and 300^0, respectively, and the column temperature was programmed at165 ^{0}C as initial temperature, held for 2 minutes, raised @ 15^0C to 255^0C and then held for 4.0 minutes. Nitrogen gas was used as carrier and make up gas and the flow rate was 1 mL min^{-1}. Samples were injected manually in splitless/split mode. Under this condition, the retention time of quinalphos was found to be 9.2 min.

Table 5. Sampling date of bean, tomato and cauliflower of experimental field

Name of vegetables	Date of sampling	Days after spraying	Sample code for Bean	Sample code for cauliflower	Sample code for tomato
Bean, Cauliflower and Tomato	24.12.2014	0	Bn-0	CF-0	Tom-0
	25.12.2014	1	Bn-1	CF-1	Tom-1
	26.12.2014	2	Bn-2	CF-2	Tom-2
	27.12.2014	3	Bn-3	CF-3	Tom-3
	28.12.2014	4	Bn-4	CF-4	Tom-4
	29.12.2014	5	Bn-5	CF-5	Tom-5
	30.12.2014	6	Bn-6	CF-6	Tom-6
	31.12.2014	7	Bn-7	CF-7	Tom-7
	01.01.2015	8	Bn-8	CF-8	Tom-8
	02.01.2015	9	Bn-9	CF-9	Tom-9
	03.01.2014	10	Bn-10	CF-10	Tom-10
	04.01.2014	11	Bn-11	CF-11	Tom-11
	05.01.2014	12	Bn-12	CF-12	Tom-12
	06.01.2014	13	Bn-13	CF-13	Tom-13
	07.01.2014	14	Bn-14	CF-14	Tom-14
	08.01.2014	15	Bn-15	CF-15	Tom-15

2.5 Determination of the dissipation of five pesticides in eight different vegetables

On 9th January, 2016, eight types of vegetables (cabbage, cucumber, bottle gourd, sweet gourd, sponce gourd, green chili, cauliflower and tomato); treated with five different kinds of pesticides; Vitaban 48 EC (chloropyrifos), Double 50 EC (mix formulation of imidacloprid and cypermethrin), Nitro 505 EC (mix formulation of chloropyrifos and

cypermethrin), Asataf 75 SP (acefate) and Reeva 2.5 EC (lambda-cyhalothrin) were harvested from farmer's field (a total of 40 samples) to analyze the dissipation pattern and the safe level of consumption from Nuritola, Comilla, Bangladesh.

2.5.1 Collection of samples

All the pesticides; were sprayed on the vegetables on the same day. But before spraying, fresh and untreated (with pesticides) vegetables of all eight types (weighed about 1 kg) were first collected in zip-locked bag, brought to the laboratory, wiped thoroughly and gently and kept in freezer at -20 °C until analysis.

2.5.2 Pesticide application

Nitro was sprayed at a dose of 1 mL L^{-1} of water/ ha; Vitaban 48 EC was sprayed at a dose of 3 mL L^{-1} of water/ ha, Double 50 EC was sprayed at a dose of 800 mL/ ha, Reeva 2.5 EC was sprayed at a dose of 0.5 mL L^{-1} of water/ ha; Asataf 75 SP was used at a dose of 1gm L^{-1} of water/ ha.

2.5.3 Sample collection

After one hour of pesticides application, all the eight vegetable samples (weighed about 5 kg each) were collected and then transferred to the laboratory as soon as possible. Control samples were kept in freezer at -20 ^{0}C and rest of the samples were kept at ambient temperature, so that they could be in a contact with air, wind, light and heat. From these samples, working samples were chopped, homogenized by kitchen blender and then extracted and cleaned-up on consecutive 1^{st}, 3^{rd}, 5^{th}, 8^{th} and 10^{th} day of application.

Fig 13a: Different vegetables collected from agricultural field

Table 6: Scientific name of collected vegetable samples

Local name	English name	Scientific name
Tomato	Tomato	*Lycopersicon lycopersicum*
Badhakopi	Cabbage	*Brasicca oleracea*
Phulkopi	Cauliflower	*Brassica oleracea* var. *botrytis*
Laao	Bottle gourd	*Lagenaria siceraria*
Mishti kumra	Sweet gourd	*Cucurbita pepo*
Dhundol	Sponce gourd	*Luffa aegyptiaca*
Kacha morich	Green chili	Capsicum spp. (annuum)
Shosha	Cucumber	Cucumis sativus

Table 7: Sampling date of vegetables from farmer's field, Nuritola, Comilla

Pesticide	DAS	Date of pesticide application	Vegetables
Nitro 505 EC	1	10. 01. 2016	tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili, cucumber
Double 50 EC	3	12. 01. 2016	
Asataf 75 SP	5	14. 01. 2016	
Reeva 2.5 EC	8	17. 01. 2016	
Vitaban 48 EC	10	19. 01. 2016	

DAS= Days after spraying

2.5.4 Extraction

The extraction process followed the QuEChERS method (**Scheme 3**). Homogenized vegetable sample, weighed of about 10g was taken in a teflon tube and 20 mL of ethyl acetate was added to it. The tube was then vortexed for about 1 minute. Then 6 g of MgSO4 and 1.5 g of NaCl were added and again vortexed for 1 minute. The mixture was then centrifuged for 5 minutes at 5,000 rpm maintaining temperature 20 ^{0}C. About 10 mL of supernatant was taken in a round bottom flask and dried to dryness. 5 mL n-hexane was added to the dried mass and from there; 2 mL solution was taken in a test tube.

2.5.5 Clean up of sample

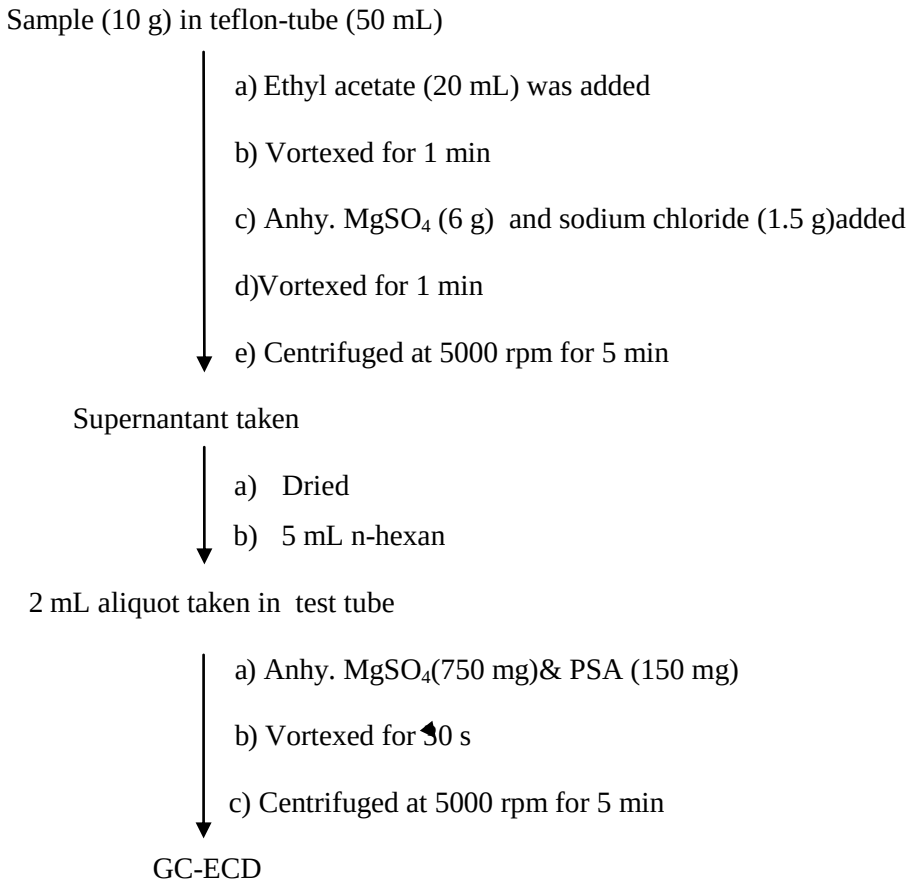
For cleaning up, 150 mg PSA and 750 mg magnesium sulphate were added and vortexed for about 1 minute. It was then centrifuged for another 5 minutes, then passed through cotton filter and the sample became ready to analyze by GC-ECD (**Scheme 3**).

2.5.6 GC-ECD analysis

The quantitative analysis of pesticide residues for this part was conducted by gas chromatograph (GC-2010 Shimadzu) equipped with ^{63}Ni electron capture detector. A non-polar (HP-5 MS) capillary column of dimension, 30 m long x 250 µm i.d.x 0.25 µm film thicknesses from Agilent, USA was used to carry out the separation. Nitrogen was used as both carrier (column flow 1.92 mL/min.) and make up gas. All injections were made in splitless-split mode. Injection volume was 1 µL and column flow rate was 1 mL min^{-1}. The injector and detector temperatures were 250°C and 260°C, respectively.

For determination of chlorpyrifos, lambda-cyhalothrin and acefate, the oven temperature was programmed as: initial temperatureof 140°C hold for 1 minute; increased at 15°C min^{-1} to 260 °C; hold for 2 minutes.

For determination of cypermethrin and imidacloprid, the oven temperature was programmed as: initial temperature of 120 °C held for 1 minute; increased at 14°C min^{-1} to 260 °C; held for 3 minutes.



Scheme 3: Extraction and clean-up process of pesticides in vegetables samples collected from farmers` field

2.5.7 Storage stability of the five pesticides

The storage stability was determined by spiking the control vegetable samples with the five pesticides and then storing them in a freezer at -20 ^{0}C. The control vegetables were spiked with chlorpyrifos, cypermethrin and imidacloprid at a concentration level of 1.0 µg mL^{-1} and with lambda-cyhalothrin and acefate at a concentration level of 0.5 and 2.0 µg mL^{-1}, respectively. Then these spiked samples were stored in freezer for 35 days and extracted, cleaned-up and analyzed following the same procedure (**Scheme 3**) to find out the stability of the pesticides at freezing condition during storage.

RESULTS AND DISCUSSION

3.1Analysis of Imidacloprid in Rice and Wheat Flour

3.1.1Determination of residual amount of imidacloprid in rice

In total, thirty one rice samples were collectedfrom various parts of Dhaka city to analyze the presence of imidacloprid. Among them nineteen samples were fragrant rice and twelve of them were non fragrant rice samples.To analyze the presence of imidacloprid after cooking, five fragrant rice samples were boiled and then analyzed. The extraction process was carried out according to a method described by Uddin et al. (2011). Imidacloprid residue was identified and quantified by LC-PDA (Shimadzu SCL 10A VP system) system which was consisted of LC 10AT VP dual pumps, a manual injector and a SPD-M10A VP photodiode-array detector in the range of 200-400 nm (wavelength). Among all the wavelengths, 280 nm was found to be the best for determination of imidacloprid in terms of peak area (**Fig 15**). During the study, different parameters of the LC-PDA were taken into consideration for best performance of the method (temperature of column, different wavelengths, ratios and flow rate of mobile phase).

The method validation for the analysis of imidacloprid was done in terms of recovery experiments, linearity, selectivity and sensitivity. The residual amount of imidacloprid in the spiked and collected samples were determined through calibration curve. The calibration curve showed peak area with a very good linear correlation (r^2= 0.996). LOD was found to be 0.009 µg $mL^{-1}$and LOQ was found to be 0.027 µg mL^{-1}.

Table 8: Percent recovery and percent RSD values of the spiked rice samples

Conc (µg mL^{-1})	Recovery % (n=5)					Average %	± SD	%RSD
	1	2	3	4	5			
0.625	73	75	70	71	74	72.6	2.07	2.85
1.25	80	82	76	75	78	79.33	2.49	3.14
2.5	78	83	80	81	79	80.33	2.52	3.13
5.0	81	84	78	79	80	81.0	3.0	3.7
10	75	80	84	90	91	84.2	5.24	6.22

The recovery experiments of imidacloprid residue (**Table 8**) in spiked rice samples were carried out in five different concentrations, 0.625, 1.25, 2.5, 5 and 10 µg g^{-1}, with five replications each. The recoveries of imidacloprid in rice samples were 72.6 ± 2.07, 79.33± 2.49, 80.33± 2.52, 81± 3.0 and 84.2± 5.24% with RSD values of 2.85, 3.14, 3.13, 3.7 and 6.22, respectively.

Table 9: Amount of Imidacloprid (µg g^{-1}) in rice samples

Rice samples				
Packed	**Amount of imidacloprid ± SD (µg g^{-1})**		**Unpacked**	**Amount of imidacloprid ± SD (µg g^{-1})**
Pran Kalijira rice	2.55± 0.13		Chinigura rice, Noyabazar	0.13±0.04
Pran Chinigura rice	1.97± 0.02		Najirshail rice, Noyabazar	bdl
Pran Najirshail rice	bdl		Kalijira rice, Polashi	1.18±0.04
Pran Minikate rice	bdl		Minikate, Polashi	bdl
Aarong Kalijira rice	1.77± 0.06		Chinigura rice, Moghbazar	0.12±0.07
Aarong Chinigura rice	1.86±0.03		Kalijira rice, Moghbazar	0.88±0.15
Aarong Najirshail rice	bdl		Chinigura rice, Farmgate	1.01±0.09
Aarong Minikate rice	bdl		Kalijira rice, Farmgate	0.08±0.03
Shwapno Kalijira rice	3.14±0.03		Najirshail rice, Farmgate	bdl
ShwapnoNajirshail rice	bdl		Kalijira rice, Cantonment	0.27±0.02
Radhuni Kalijira rice	1.59±0.07		Minikate rice, Cantonment	bdl
Radhuni Chinigura rice	2.72±0.09		Chinigura rice, Gulshan-1	0.06±0.01
Radhuni Najirshail rice	bdl		Kalijira rice, Gulshan-1	0.09±0.04
Chashi Kalijira rice	4.51±0.10		Kalijira rice, Gabtoli	0.63±0.02
Chashi chinigura rice	2.90±0.06		Najirshail rice, Gabtoli	bdl
Chashi Najirshail rice	bdl		control	bdl

bdl= below detection limit

Non- fragrant rice samples did not show any traces of imidacloprid (**Table 9)**. Only fragrant rice samples were observed to contain residues of imidacloprid. Packed and unpacked fragrant samples had higher and lower values than the MRL of imidacloprid in rice.

The amounts of imidacloprid in boiled rice samples (**Table 10**) are shown below, the results revealed that boiled rice samples did not contain any residue of imidacloprid.

Table 10: Amount of imidacloprid in boiled rice samples

Samples name	**Amount of imidacloprid ± SD(µg g^{-1})**
Pranchinigura	bdl
Radhunikalijira	bdl
Aarongkalijira	bdl
Chashikalijira	bdl
Chinigura, gulshan	bdl

bdl= below detection limit

3.1.2 Determination of the residual amount of imidacloprid in wheat flour

Sixteen wheat flour samples were collected from different areas of Dhaka to analyze the presence of imidacloprid residue. Among them, one is control, ten were branded packed samples and five were unpacked samples brought from various markets. The extraction process was carried out according to a method described by Uddinet al., (2011).

Imidacloprid residue in wheat flour was detected by LC-PDA.In spiked wheat samples, recovery experiments (**Table 11**)were carried out in three concentration levels with five replications; 1.25, 2.5 and 5.0 µg mL^{-1}. The recoveries of imidaclopridin wheat were 83± 2.54, 90.8± 2.3 and 86.6± 2.7 with RSD value of 3.06, 2.53 and 3.11, respectively.

Table 11: Percent recovery and percent RSD values of the spiked wheat samples

Conc ($\mu g\ mL^{-1}$)	Recovery %(n=5)					Average%	±SD	%RSD
	1	2	3	4	5			
1.25	85	83	80	86	81	83.0	2.54	3.06
2.5	90	95	88	90	91	90.8	2.30	2.53
5.0	87	88	82	89	87	86.6	2.70	3.11

Table 12: Amount of Imidacloprid ($\mu g\ g^{-1}$) in wheat flour samples

Samples	Condition	Amount Of imidacloprid ± SD ($\mu g\ g^{-1}$)
Teer atta	Packed	0.44±0.10
Pusti atta		0.05±0.08
Fresh atta		bdl
Fauji atta		0.28±0.04
ACI atta		bdl
Ifad atta		bdl
Bashundhara atta		0.03±0.09
Diamond atta		0.11±0.03
Yusuf atta		0.38±0.07
Saad atta		bdl
Wheat flour (Noyabazar)	Unpacked	0.29±0.06
Wheat flour (Moghbazar)		bdl
Wheat flour (Farmgate)		bdl
Wheat flour (Cantonment)		bdl
Wheat flour (Uttara)		0.08±0.09
control		bdl

bdl= below detection limit

Among the 10 packed wheat flour samples, only 6 samples were detectable and the amount of imidacloprid residues ranged from 0.03 ±0.08 µg $g^{-1}$to 0.44 ±0.10 µg g^{-1} and out of five unpacked samples, only 2 samples showed traces of imidacloprid, valued 0.08 ±0.09 µg g^{-1} and 0.29 ±0.06 µg g^{-1}(**Table 12**). Control wheat sample did not show any residues of imidaccloprid.

3.1.3 Discussion

Validation of the method

Extraction efficiency was evaluated through recovery experiments. The recovery experiments of imidacloprid residue in spiked rice samples were carried out in five different concentration levels with five replications; 0.625, 1.25, 2.5, 5 and 10 µg mL^{-1}. The recoveries of imidacloprid in rice samples were 72.6 ± 2.07, 79.33± 2.49, 80.33± 2.52, 81± 3.0 and 84.2± 5.24% with RSD values of 2.85, 3.14, 3.13, 3.7 and 6.22, respectively. Therefore, the recovery was at the range of 72 - 84% which are acceptable (Codex Alimentarius, 1993). Recovery experiments for wheat samples were carried out in three concentration levels with five replications; 1.25, 2.5 and 5 µg mL^{-1}. The recoveries ofimidacloprid in wheat were 83± 2.54, 90.8± 2.3 and 86.6± 2.7% with RSD value of 3.06, 2.53 and 3.11, respectively. Also these recoveries were at the range of 83 - 90% which are acceptable (Codex Alimentarius, 2010). A widely accepted criterion for the acceptability of performance of an analytical method is its capability of providing average recovery within the range of 70-120% (Uddinet al., 2011). From the results obtained in this study, the results of recovery experiments are therefore within the range.

Linearity of the LC-PDA was evaluated by calibration curves. The residual amount of imidacloprid in samples,spiked samples and control were determined via calibration curve (**Fig. 14**). The calibration curve showed an excellent linear coefficient of r^2=0.996 and were constructed as peak area *vs* concentration (µg g^{-1}).

Selectivity was evaluated by analyzing standard pesticides mixture, blank matrices and blank matrices spiked with pesticides mixture simultaneously and monitoring retention times. Unwanted components interfering with analytes (if any) were analyzed by comparing the chromatograms of the standard, blank sample and fortified sample. There was no interference peak at the retention time of imidacloprid under similar LC-condition. Overlaying chromatograms of two different standard solutions (0.009 and 0.019 µg mL^{-1}) and one fortified rice sample of imidacloprid (fortification level 0.039 µg mL^{-1}) is shown in **Fig 15**.

Sensitivity of the LC system was examined by determining LOD and LOQ. The LOD was determined using a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample, whereas the LOQ were determined with a signal-to-noise ratio of 10. The LOD was found to be 0.009 µg $g^{-1}$and LOQ was found to be 0.027 µg g^{-1} for imidacloprid.

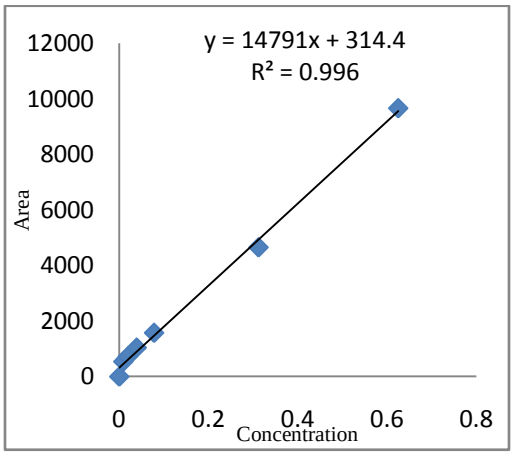


Fig. 14: Calibration curve of different certified imidacloprid standards

3.1.3.1 Rice samples

In **Table 9**, it is observed that non- fragrant (najishail and minikate) rice samples of different brands (a total of 12 samples) were analyzed and no trace of imidacloprid was observed in these samples. Among the 18 fragrant rice samples, 9 (packed) samples were found to contain imidacloprid residues in the range 1.59±0.07–4.51±0.10 µg g^{-1} which was greater than the MRL value (1.5 µg g^{-1} in rice, EPA 2010) and 9 (unpacked) samples were found to contain imidacloprid residues in the range 0.06±0.01–1.01±0.09 µg g^{-1} which was lower than the MRL value. The most interesting part of the analysis was that there was no trace of imidacloprid in rice samples when the sample was boiled. As we consume rice after cooking, so it is safe to intake it without any risk of health hazard.

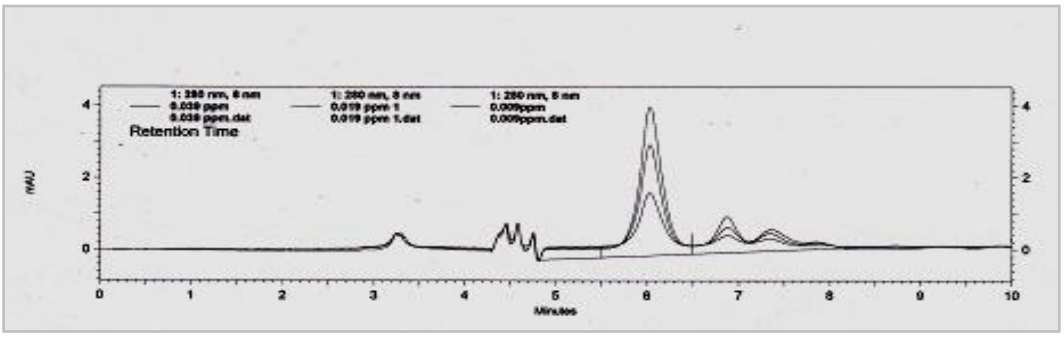


Fig 15: **Overlaying chromatograms of two different standard solutions (0.009 and 0.019 µg mL^{-1}) and one fortified rice sample of imidacloprid (fortification level 0.039 µg mL^{-1})**

3.1.3.2 Wheat flour samples

Among the sixteen wheat flour samples, only few showed traces of imidacloprid above the detection limit and all the samples showed a minute amount of imidacloprid in the samples which were below the MRL value (for wheat flour sample, MRL value is 1.5 µg g^{-1} , according to European Food Safety Authority, 2000), ranged from 0.03–0.44 µg g^{-1}. Although, low level does not absolutely mean safer wheat flour regarding to its

high consumption but as it is consumed after cooking, therefore, the residues of imidacloprid actually went below the ADI value.

Nobuko et al., (2003) reported determination of imidacloprid in different agricultural products using HPLC. Similarly, a quantitative analysis of imidacloprid in wheat-seed and soil has been reported in Guiyinget al., (2006). Samnani et al. (2011) also had reported the quantitative determination of imidacloprid residues in soil and water samples.

3.1.4. Confirmation by LC-MS

Two product ion, 175 and 209, were obtained from precursor ion 256 which confirms the residual presence of imidacloprid (**Fig 16**) in the sample by LC-MS/MS, ionization technique was electron spray ionization and mode was positive, method was MRM.

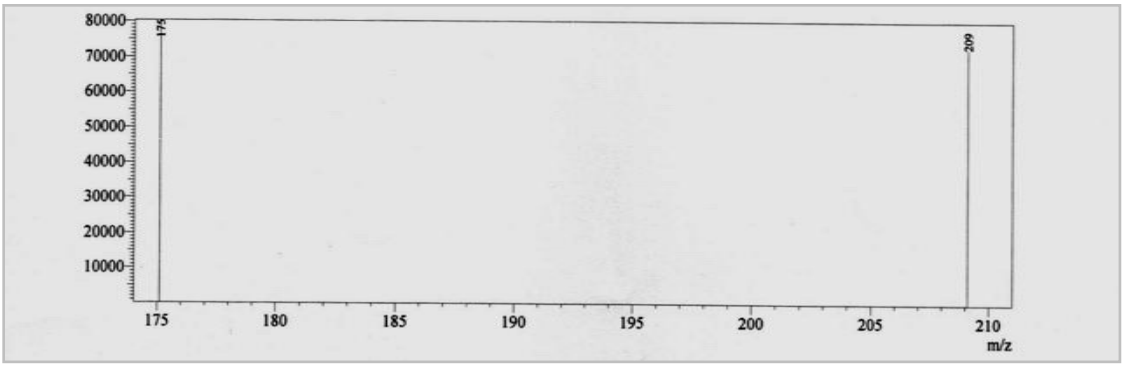


Fig16: LC-MS/MS chromatogram of imidaclopridа in a sample

3.2 Toxicity test of imidacloprid against rice weevils

Disk flour bioassay was carried out to evaluate toxic action by ingestion.The percentage of insect mortality at 24, 48 and 72 hours after treatment indicated that the imidacloprid possessed the highest toxic (74.44 %) effect at the highest concentration (10.0 µg g^{-1}) level (**Table 13**). Mortality percentage was found directly proportional to the level of concentrations and time after treatment.

Table 13: Toxic effect of imidacloprid at different concentration on rice weevil, *Sitophylus oryzae* at different HAT (Interaction of concentration and time)

Name of the insecticide	Conc. (µg g^{-1})	Mortality percentage			
		24 HAT	48 HAT	72 HAT	Average
Imidacloprid	control	0	0	0	0
	1.25	0.004 (0.371)	33.33 (35.20)	56.67 (48.91)	30.00 c (28.16)
	2.50	23.33 (28.27)	60.00 (50.83)	80.00 (63.90)	54.44 b (47.67)
	5.00	26.67 (30.77)	70.00 (56.77)	86.67 (68.82)	61.11 b (52.12)
	10.00	43.33 (41.13)	86.66 (72.13)	93.33 (77.55)	74.44 a (63.61)
$S_{\bar{X}}$		4.338			2.505
Probability level		NS			0.01

HAT= Hour after treatment.

NS= Not significant.

Original data corrected by Abbot's formula and then transformed into arcsin √percentage values before ANOVA and DMRT test.

3.2.1 Probit Analysis for Toxicity

The result of the Probit analysis for the estimation of LC_{50} values and their 95% fiducial limits at 24, 48 and 72 HAT for the mortality of rice weevil is presented in **Table 14**. The LC_{50} values of imidacloprid (1.204, 0.221 and 0.0724µg g^{-1}) at 24, 48 and 72 HAT indicated that highest toxicity possessed the lowest LC_{50} values in all weevils. From the above Probit results, it is clear that imidacloprid will be more effective for controlling the rice weevil.

Table 14: Probit analysis for ingestion toxicity of imidacloprid to rice weevil, *S. oryzae* (total no of weevils used n=120) at 24, 48 and 72 HAT

Hours after treatment(HAT)	LC_{50} value ($\mu g\ g^{-1}$)	95% fiducial limits	$\chi 2$ value	Slope ± SE
24	1.204	0.737-1.958	1.73	2.01±0.05
48	0.221	0.136-0.359	1.19	1.33±0.02
72	0.0724	0.029-0.199	0.23	1.20±0.03

HAT= Hour after treatment.

SE= Standard error.

Concentrations were transformed by the log transformation before analysis.

Values were based on four concentrations, three replications of 10 insects each.

χ^2= Goodness of fit. The tabulated value of χ^2 is 5.99 (d .f = 2 at 5 % level)

3.2.2 Probit Regression Lines

The Probit regression lines for the effect of imidacloprid are presented in **Fig. 17**. The insect mortality rate showed positive correlation with concentrations in all cases. The Probit regression lines for the effects of imidacloprid on rice weevil showed a clear linear relationship between Probit mortality and their log concentrations. The Probit regression lines became steeper as concentrations increased, because the adult insects were treated with more toxins for the same period at higher concentrations.

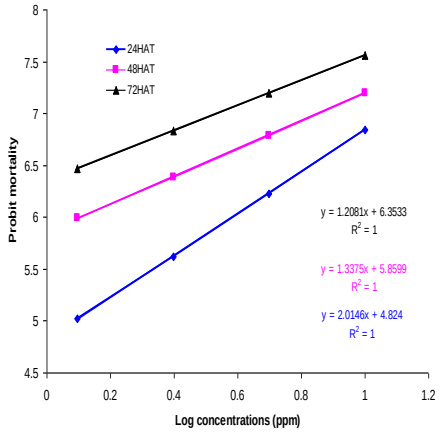


Fig 17: Relationship between Probit mortality and log concentrations for imidacloprid against *S. oryzae* at 24, 48 and 72 HAT

The toxic effect of imidacloprid in rice was evaluated against adults *Sitophylus oryzae*. In **Table 13**, it is observed that the average mortality of adult weevils was 30, 55, 61 and 74% while spiking the control rice with a concentration level of 1.25, 2.5, 5.0 and 10 µg g^{-1}. The mortality of weevils was 57% at 72 hours while spiking the control rice with a concentration level of 1.25 µg g^{-1} which is lower than the MRL value of imidacloprid in rice. This experiment was performed on adult rice weevils whereas imidacloprid is mainly used in food storage to inhibit the breeding process or the growth of weevils. A concentration lower than 1.25 µg g^{-1} would be enough to control the growth of weevils during storage. The residual analysis of imidacloprid (**Table 9**) showed that among 30 rice samples, 8 samples were found to contain imidacloprid residue higher than 1.25 µg g^{-1} which indicate indiscriminate use of imidacloprid in the market samples during storage.

3.2 Dissipation of Quinalphos in bean, tomato and cauliflower

Quinalphos, *O,O*-diethyl-*O*-quinoxalin-2-yl-phosphorothioate is an insecticide and is a solid at low temperature having melting point of 31.5 °C and is very toxic to mammals (LD_{50} 71 mg kg^{-1}) (Anonymous, 2011). It is mainly used to control diamond-back moth and tobacco caterpillar on vegetables (Anonymous, 2009). Accumulation of quinalphos residues in vegetables may pose human health hazards as quinalphos is very toxic chemical (Banerjee et al.,2006).

From different studies, it has been revealed that about 50-70% of vegetables are contaminated with insecticides (Karanth, 2000), due to harvesting the crops before the safe waiting period. A waiting period between pesticide treatment and harvesting of crops minimize the risk of hazardous effects of insecticides for consumer (Kumari, 2004). Quinalphos is still not a registered pesticide in Bangladesh, therefore, the result of the present study is very important to determine the dissipation of quinalphos under the weather condition and soil property of our country and will help the government for having a decision about registering quinalphos as a regular insecticide.

The samples were extracted by QuEChERS method (Anastassiades et al., 2003) and clean-up was performed with primary secondary amine (PSA) and then the samples extracts were run through GC- ECD. The retention time of quinalphos was at 9.2 minute under certain parameters of the GC-ECD system (**section 2.4.8**).

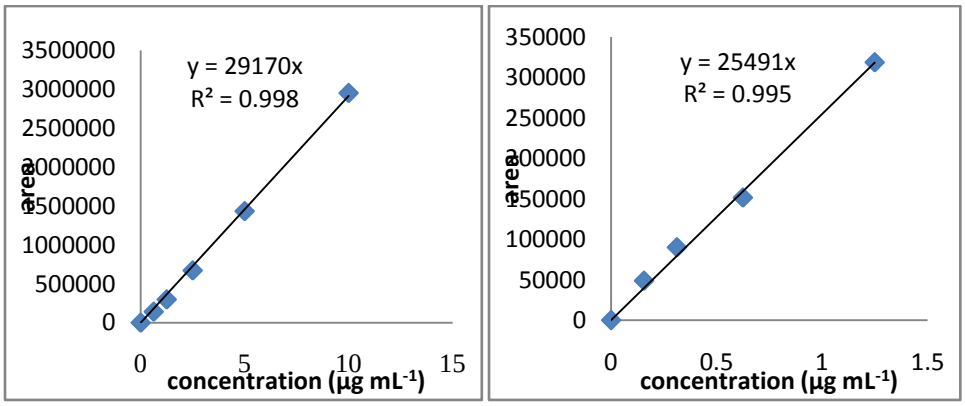
Due to the vast variation in the residual amount in the field samples, three calibration curves were prepared (higher, middle and lower levels) in order to cover the range.High, middle and lower concentration calibration curves were obtained with linear correlation coefficient (r^2) 0.998, 0.995and 0.993, respectively (**Fig. 18**), the linearity range for high, middle and lower concentration calibration curves were 2.5 - 6.5, 1.0 - 2.5 and 0.06 - 0.13 µg mL^{-1}, respectively (**Table 15**).

3.2.1 Limit of detection and Limit of quantification

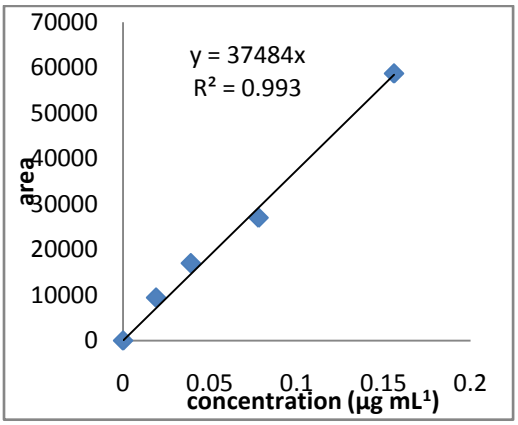
The LOD and the LOQ were found to be 0.009 µg g^{-1} and 0.027 µg g^{-1}, respectively (**Table 15**).

Table 15: Name, linearity range, correlation coefficients (r^2), LOD and LOQ of quinalphos

Pesticide	Calibration curve	Linearity range (µg mL^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Quinalphos	higher conc.	2.5 - 6.5	0.998	0.009	0.027
	middle conc.	1.0 - 2.5	0.995		
	lower conc.	0.06 – 0.13	0.993		



(a) (b)



(c)

Fig. 18: Calibration curve of higher, medium and lower concentration (a, b, c) of quinalphos standards

3.2.2 Recovery experiment

The percentages of the recovery of quinalphosresidues in spiked vegetable samples at three different concentration levels (0.625, 2.5 and 5.0 µg mL^{-1}) were in the range of 74-86%. The percent recoveries of the method were 85, 74 and 86% with RSD values of 6.2, 4.2 and 5.5, respectively (**Table 16**).

Table 16: Percent recovery, ±SD and percent RSD values of quinalphos

Conc. (µg mL^{1})	**Recovery % (n=5)**					**Average%**	**±SD**	**RSD%**
0.625	89	80	85	92	80	85	5.31	6.2
2.5	71	76	73	73	79	74	3.13	4.2
5.0	92	84	90	83	81	86	4.74	5.5

The concentration of quinalphos gradually dissipated from 0 day to 15 day and went to bdl. The amount of quinalphos residue in tomato, bean and cauliflower were in the range of 6.31 ± 0.35 – 0.05 ± 0.09 (**Table 17**) , 3.10 ± 0.18 – 0.05±0.07 (**Table 18**)and 6.5± 1.06 – 0.06 ± 0.03 µg g^{-1}(**Table 19**) and went bdl after 11, 10 and 11 days, respectively.

Table 17: Amount of quinalphos (Av. ± SD, µg g^{-1}) in tomato

Day	Sample code	Tomato	
		Amount(µg g^{-1})	% of Dissipation
0	QT 0	6.31 ± 0.15	
1	QT 1	4.48 ± 0.19	29
2	QT 2	2.71 ± 0.25	57
3	QT 3	2.02 ± 0.25	67
4	QT 4	1.51 ± 0.20	76
5	QT 5	1.12 ± 0.17	82
6	QT 6	0.19 ± 0.18	94
7	QT 7	0.10± 0.06	98
9	QT 9	0.05 ± 0.09	99
10	QT 10	bdl	
11	QT 11	bdl	
12	QT 12	bdl	
13	QT 13	bdl	
14	QT 14	bdl	
15	QT 15	bdl	

bdl = below detection limit

Table 18: Amount of quinalphos (Av. ± SD, µg g^{-1}) in bean

Day	Sample code	Bean	
		Amount(µg g^{-1})	% of Dissipation
0	QB 0	3.10 ± 0.18	
1	QB 1	1.99 ± 0.16	35
2	QB 2	1.12 ± 0.03	63
3	QB 3	0.88 ± 0.08	71
4	QB 4	0.59 ± 0.05	80
5	QB 5	0.42 ± 0.02	86
6	QB 6	0.28 ± 0.02	90
7	QB 7	0.21± 0.02	93

9	QB 9	0.12 ± 0.18	96
10	QB 10	0.05 ± 0.07	98
11	QB 11	bdl	
12	QB 12	bdl	
13	QB 13	bdl	
14	QB 14	bdl	
15	QB 15	bdl	

bdl = below detection limit

Table 19: Amount of quinalphos (Av. ± SD, µg g^{-1}) in cauliflower

Day	**Sample code**	**Cauliflower**	
		Amount (µg g^{-1})	**% of Dissipation**
0	QB 0	6.51 ± 1.06	
1	QB 1	4.87 ± 0.26	25
2	QB 2	4.81 ± 0.18	27
3	QB 3	3.43 ± 0.11	47
4	QB 4	2.68 ± 0.16	58
5	QB 5	1.81 ± 0.03	72
6	QB 6	1.06 ± 0.03	83
7	QB 7	0.81 ± 0.19	87
9	QB 9	0.11 ± 0.23	98
10	QB 10	0.06 ± 0.03	99
11	QB 11	bdl	
12	QB 12	bdl	
13	QB 13	bdl	
14	QB 14	bdl	

bdl= below detection limit

3.2.3 Discussionof Quinalphos treated tomato, bean and cauliflower

3.2.3.1 Validation of the method

The recovery experiments evaluate the efficiency of the method for analyzing quinalphos in vegetable samples. The recovery experiments of quinalphos were carried out only against spiked tomato samples and were done in three different concentration levels with five replications; 0.625, 2.5 and 5 µg mL^{-1}. The percent recoveries of the method were 85±5.31%, 74± 3.13% and 86± 4.74 with RSD values of 6.2, 4.2 and 5.5, respectively. Therefore, the recovery was at the range of 72% - 84% which are acceptable (Codex Alimentarius, 2010). A widely accepted criterion for the acceptability of performance of an analytical method is its capability of providing average recovery within the range of 70%-120%. From the results obtained in this study, the results of recovery experiments are therefore within the range.

The linearity was evaluated by calibration curves plotted with concentration of standard quinalphos solution against area at those respective concentrations. The calibration curve showed an excellent linear coefficient of r^2=0.998, 0.995 and 0.993 for, higher, medium and lower concentration calibration curves, respectively.

Sensitivity of the LC system was examined by determining LOD and LOQ. The LOD was found to be 0.009 µg mL^{-1} and the LOQ was found to be 0.027 µg mL^{-1} for quinalphos.

Quinalphos was detected at 9.2 min (retention time) in GC-ECD. Specificity was confirmed by injecting control vegetable extract and no matrix peaks was found to interfere with the retention time of quinalphos. **Fig. 19** and **Fig. 20** show chromatograms of quinalphos in tomato sample and certified quinalphos standard.

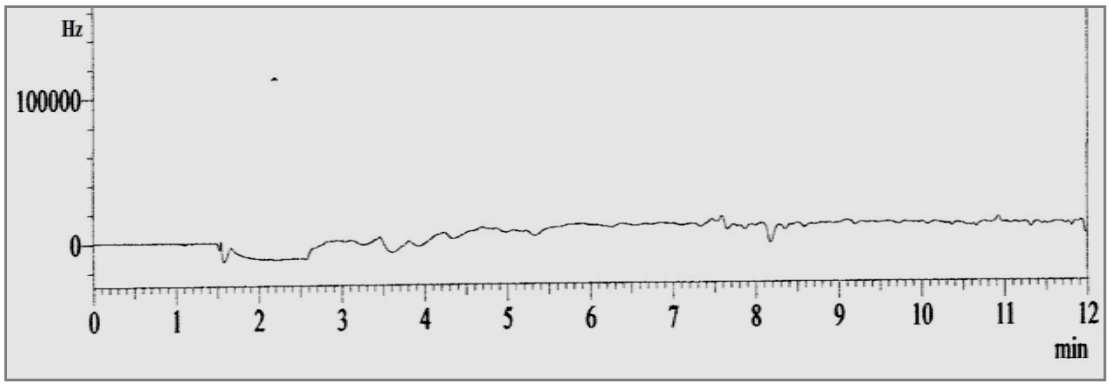


Fig 18: GC-ECD Chromatograph of blank sample

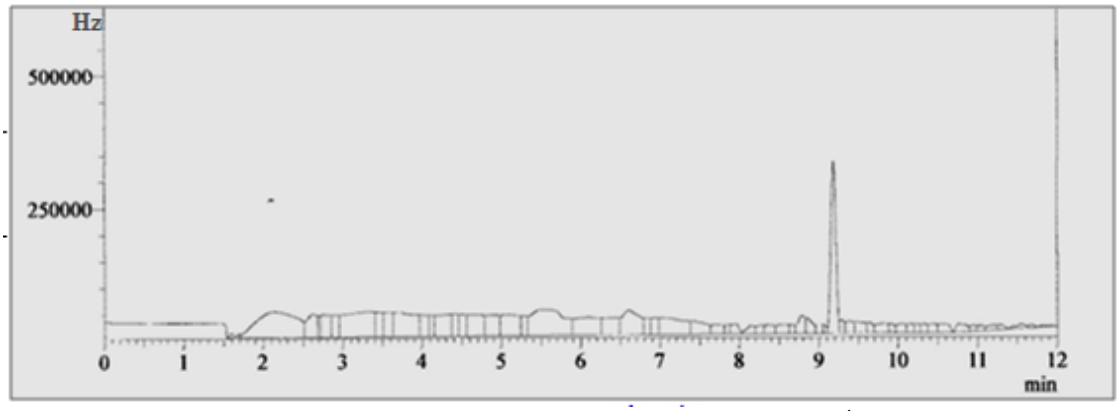


Fig 19: GC-ECD Chromatograph of tomato sample (1st day of pesticide application) treated with quinalphos

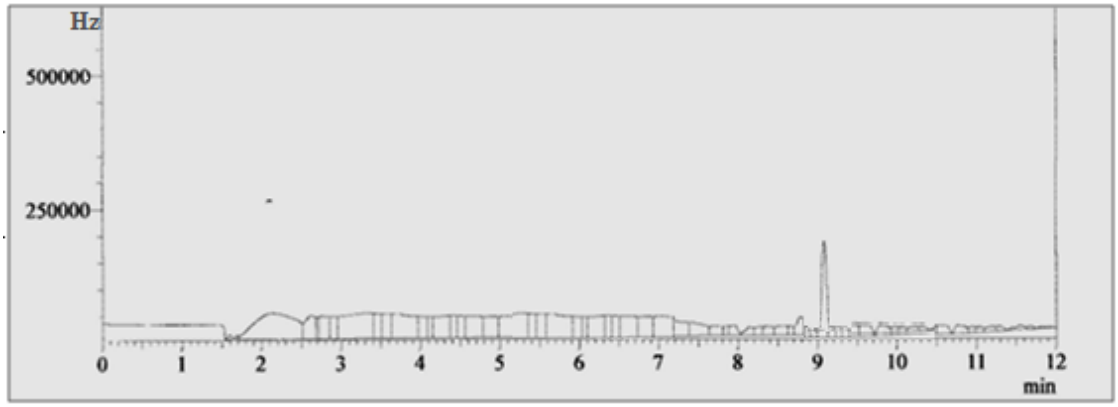


Fig 20: GC-ECD Chromatograph of certified quinalphos standard (0.312 µg g^{-1})

3.2.4 Dissipation pattern of quinalphos in tomato, bean and cauliflower

The QuEChERS method gave an excellent recovery within the limit of 70% - 120%. From the results obtained in this study, it can be said that the method was perfect for determining quinalphos in vegetables.The dissipation percentage of pesticide residues in/on vegetables depends on the climatic conditions, type of application, plant species, dosage, the interval between application and harvest. Tomato, bean and cauliflower are winter vegetables of Bangladesh. Among the vegetables grown in Bangladesh, tomato, cauliflower and bean are popular and highly valued. It has been published that these vegetable are attacked by pests and farmers use pesticides quite often even every day (Miahet al., 2000). Therefore, the dissipation pattern of quinalphos was studied to analyze the persistency of this pesticide in these three vegetables. From this study, it was analyzed that residue of quinalphos in tomato, bean and cauliflower could be detected up to 9, 10 and 10 days after spraying, respectively.

From **Fig. 21**below, we see that residue of quinalphos was found to be the higher in cauliflower, then in tomato and then comes bean in 0 day, 3rd day and 7th days after spraying, respectively. On 0 day (2 hrs after spraying), the residual amounts of quinalphos were as; Cauliflower (6.31 ± 0.15 µg g^{-1}) > Tomato (3.10 ± 0.18 µg g^{-1}) > Bean (6.51 ± 1.06 µg g^{-1}). On 3rd day, the residual amounts of quinalphos were as; Cauliflower (2.02 ± 0.25 µg g^{-1}) > Tomato (0.88 ± 0.08 µg g^{-1}) > Bean (3.43 ± 0.11 µg g^{-1}) and on 7th day, the residual amounts were as; Cauliflower (0.10 ± 0.06 µg g^{-1}) > Tomato (0.21 ± 0.02 µg g^{-1}) > Bean (0.81 ± 0.19 µg g^{-1}). A comparison between the residual value of quinalphos in tomato, bean and cauliflower is shown below in fig. .

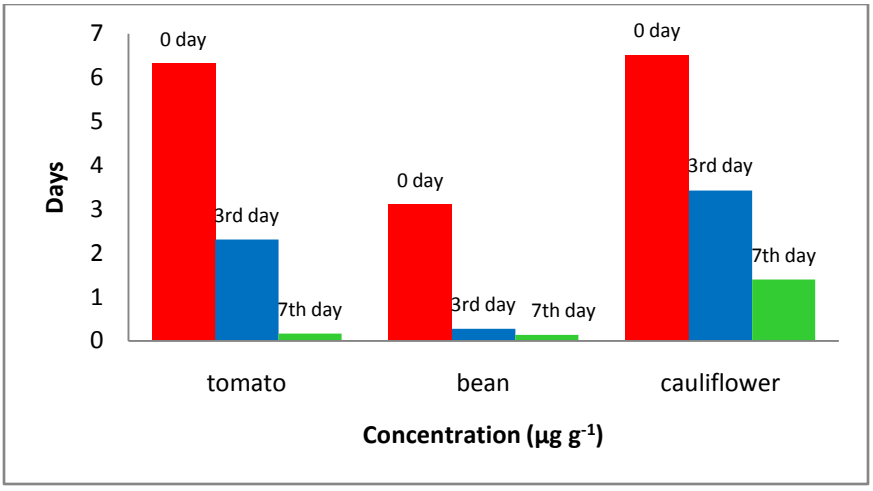


Fig 21: Amount of quinalphos in tomato, bean and cauliflower in 0 day (2 hrs after spraying), 3rd day and 7th day after spraying

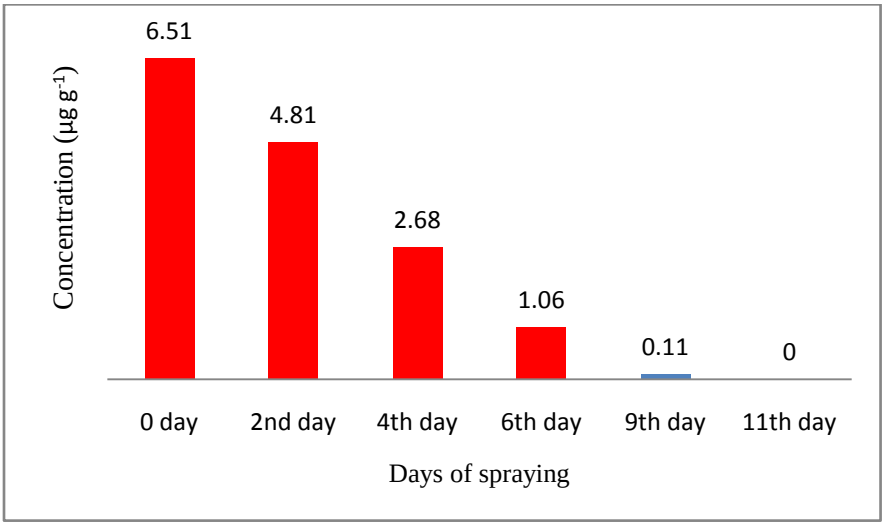


Fig 22: Dissipation at six alternate days of spraying of quinalphos in cauliflower

In case of cauliflower, the pesticide reaches to the target point properly. Cauliflower grows in upper direction and its surface is large and non-homogeneous. So, the pesticide has much space to accommodate easily. Therefore, higher persistency of quinalphos is observed. But the skin of tomato is smooth compare to cauliflower. Tomato grows in down direction. Thus the residue was lower in tomato than

cauliflower. In bean, the pesticide reaches to the target point less properly compare tomato and cauliflower. So, rapid dissipation was observed.

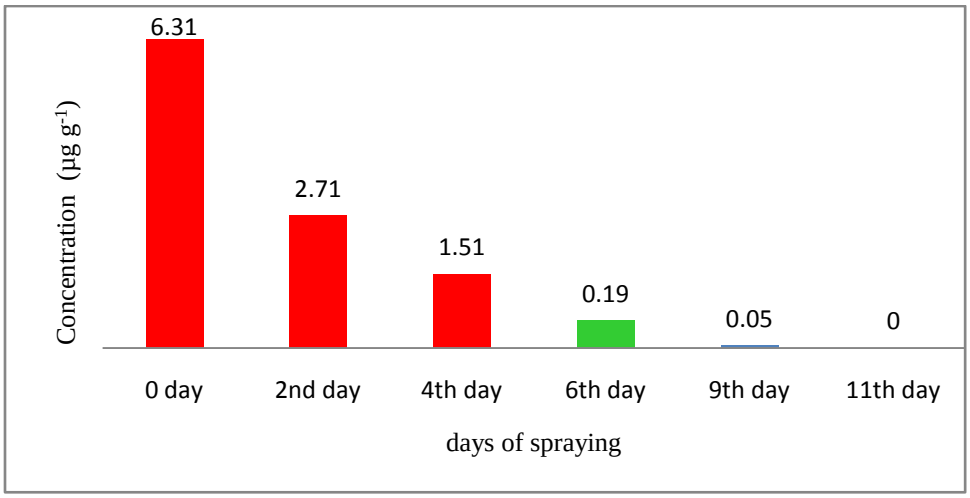


Fig 23: Dissipation of six alternate days of spraying of quinalphos in tomato

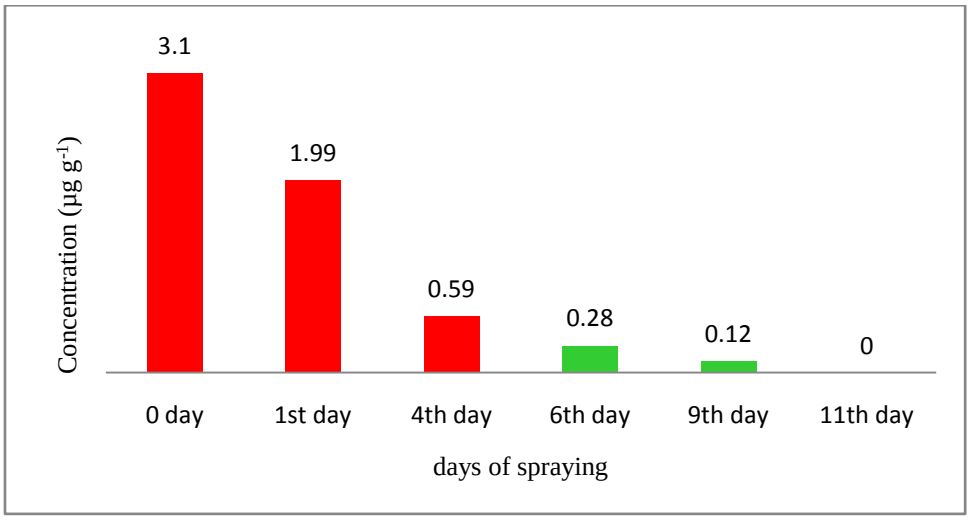


Fig 24: Dissipation of six alternate days of spraying of quinalphos in bean

In cauliflower (**Fig. 22**), quinalphos was in an amont of 6.31± 0.15 µg g^{-1} on 0 day (2 hours of application), then it decreases to 4.87 ± 0.26 µg g^{-1} on next day. The amount decreases to 4.81 ± 0.18 µg g^{-1} on 2^{nd} days after spraying, then became 3.43± 0.11 ,

2.68± 0.16 , 1.81± 0.03 , 1.06± 0.03 , 0.81 ± 0.19 , 0.11 ±0.23 and 0.06± 0.03 µg g^{-1}on 3^{rd},4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th} and 10^{th} day of spraying, respectively and went below detection limit on 11^{th} day of spraying (**Table 19**).The percentages of dissipation were 25, 27, 47, 58, 72, 83, 87, 98 and 99% on 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th} and 10^{th} days of spraying respectively. The maximum residual limit of quinalophosfor cauliflower is 0.20 µg g^{-1} (EPA guidelines, 2011). The residual value dissipated to a value less than the MRL value after 7 days of spraying (0.11± 0.23 µg g^{-1}), Therefore, according to my analysis, it is suggested that quinalphos should be applied on cauliflower at every 7 days of final spraying, not before that.

In tomato (**Fig. 23**), the initial deposition was found to be 6.31± 0.15 µg g^{-1}on 0 day (2 hours of application) which gradually decreased to 4.48 ± 0.19 , 2.71± 0.25 , 2.02 ± 0.25 , 1.51 ± 0.20 , 1.12 ± 0.17 , 0.19± 0.18 , 0.10 ± 0.06 and 0.05 ± 0.09 µg g^{-1} on 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th} day of spraying, respectively. The dissipation percent were 29, 57, 67, 76, 82, 91, 98 and 99% on 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 9^{th} days after spraying, respectively (**Table 17**).The maximum residual limit of quinalophos for tomato is 0.20 µg g^{-1} (EPA guidelines, 2011). The residual value dissipated to a value less than the MRL value on 6 days (0.10±0.06 µg g^{-1}). Therefore, quinalphos should be applied on tomato at every 6 days of final application.

In bean (**Fig. 24**), the residual amount of quinalphos degraded sharply from 1^{st} day to 10^{th} days and then went below detection limit. The concentrations were 3.1 ± 0.18 , 1.99 ± 0.16 , 1.12 ± 0.03 , 0.88 ± 0.08 , 0.50 ± 0.05 , 0.42 ± 0.02 , 0.28 ± 0.02 , 0.21 ± 0.02 , 0.21± 0.02 , 0.12 ± 0.18 , 0.05 ± 0.07 µg g^{-1} for 0 day (2 hours of application), 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th} and 10^{th} days after spraying, respectively (**Table 18**). And the percentages of dissipation were 35, 63, 71, 80, 86, 90, 93, 96 and 98% for 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th} and 10^{th} days after spraying, respectively The amount of quinalphos went below detection limit on 11^{th} day after spraying. The maximum residual limit of quinalophos for bean is 0.50 µg g^{-1} (EPA guidelines, 2011). So, the safe harvesting period for bean, treated with quinalphos is after 4 days of application. Therefore, it should be applied on bean at every 4 days of final application.

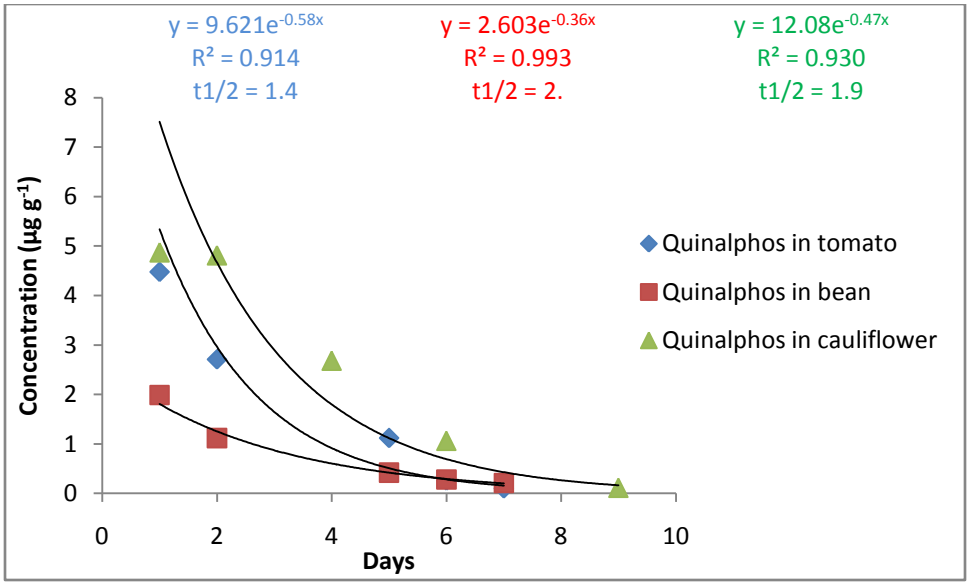


Fig 25: Dissipation curves of quinalphos in tomato, bean and cauliflower

The degradation of a component was described by the first order function ($C_t = C_0$ x e $^{-kt}$). The half lives of the pesticides were obtained by the equation $t_{1/2} = \ln 2/k$, where C_t is the concentration (µg L^{-1}) at time t (days) after application, C_0 is the initial concentration (µg L^{-1}) and k is the first order rate constant (day $^{-1}$) (Zhang et al. 2012). The dissipation curves of quinalphos for tomato, bean and cauliflower were made. The half-life of quinalphosfor tomato, bean and cauliflower were 1.4, 2 and 1.9 days, respectively (**Fig. 25**).

Gurminder S. (2011) analyzed degradation dynamics of quinalphos on cabbage under subtropical conditions of Ludhiana, India. He collected the samples of 0 (1 hr of application), 1, 3, 5, 7, 10 and 15 days of application. The initial deposition was 0.41± 0.01 µg g^{-1} and the amount went below detection limit after 7th days.

AR Pathan et al.,2012, reported the degradation pattern of quinalphos 25 EC in/on brinjal and soil. The initial deposition was 0.0866 mg kg^{-1}. The half life was 2 days and safe waiting period was 6 days. No residual traces of quinalphos was detected in soil.

R.P. Chawla, (1979) analyzed the dissipation percentage of quinalphos in cauliflower. He reported that for cauliflower, the safe waiting period was 8 days.Christensen,

(2004) analyzed that the degradation of pesticides may be due to biological, chemical or physical processes, also due to dilution by growth of the crop.

Besides, criteria of growth of plant is also responsible for decreasing the pesticide residue concentrations due to growth dilution effect (Walgenbah et al., 1991).

The growing pattern of cauliflower, tomato and bean is different, therefore, when the pesticide was sprayed onto the vegetables, different type of accumulation of the pesticide on/in these vegetables occurs.

Again, the rapid dissipation of applied pesticide is dependent on environmental factors; sunlight and temperature (Lichtenstein, 1972). However, high temperature is a major factor in declining the pesticides from plant surfaces (Awad et al., 1967).

Light plays a lead role in the behaviour of pesticide (Zepp and Cline, 1977).

The upper surface area of Cauliflower is more than tomato and bean, so took 11 days to degrade totally under various environmental condition. On the other hand, bean may contain the minimum amount of pesticide but due to its thick skin, the pesticide took longer period (11 days) under the effect of light and heat of sun than that of tomato (went below detection limit on 10^{th} day)

This can be related to the statement of El-Sayed et al. (1976). According to him, the deposition amount depends on rate of application, nature of surface to be treated and its weight.

Sangama et al., *(1989)* reported that the safe waiting period for quinalphos was 9.03 days when treated on brinjal, but in this current study it was found that the safe waiting period for cauliflower, tomato and bean were 7, 6 and 4 days, respectively. Therefore, weather condition took the lead role here to differentiate between these values.

As the quinalphos treatment on these three vegetables took place on the same day and in same climate area, therefore it can be said that the variation of the dissipation rate depends on accumulation criteria on these three vegetables and also on the biological, chemical or physical differences of these respective vegetables.

The current analysis reveals that quinalphos application on vegetables under open field requires longer pre-harvest interval for allowing the residues to dissipate to the safe level. For minimizing the long pre-harvest interval, the application dose could be

reduced, so that, quinalphos could be used as a useful protective pesticide in vegetables without any health risk of toxicity to consumers if the safe waiting period is maintained.

According to the current study, quinalphos dissipates completely and went below detection level after certain days in the vegetable samples, for cauliflower, bean and tomato, these were 11, 10 and 11 after spraying. The residue dissipated over MRL value within7^{th}, 6^{th} and 4^{th}days after spraying, for cauliflower, tomato and bean, respectively. Therefore, it is suggested not to spray quinalphos twice with an interval of at least 11 days after final spraying.

3.3 Results of the dissipation of five pesticides in eight different vegetables

Pesticides are used to manage pests and insects in agricultural sector, but misuse and overdoses of pesticide may lead to public concerns on food safety, secured environment and human health (Wentzell, 2014; Rauhet al., 2012; Janssens, 2013), environmental contamination (Watts, 2012; Popp, 2013), insect resistance (Zhang et al., 2012;Ouyang, 2010), etc. In Bangladesh, using agrochemicals is a essential part to provide food supplies for its increasing population with limited arable lands.In order to meet the demand and boost up the cultivation of grains and vegetables, the use of organophosphate, organocarbamate and pyrethroids insecticides are increasing. Farmers of our country use these pesticides in open fields, without any self protection and most of the times, they overdosed the crops. As a result, the residual amount of these pesticides retain above the MRL value. Therefore, determination of safe period of consumption of pesticide treated crops from farmer's field is very important in order to find out the efficient application rate to control pests while leaving minimum residues (MacLachlan, 2010; Malhat et al., 2013).

Vegetables are being sprayed with various types of pesticides; therefore, to analyze the safe period of consumption, eight different vegetables (cabbage, cucumber, bottle gourd, sweet gourd, sponce gourd, green chili, cauliflower and tomato)treated with five different pesticides; (Vitaban 48 EC (chloropyrifos), Double 50 EC (mix formulation of imidacloprid and cypermethrin), Nitro 505 EC (mix formulation of chloropyrifos and cypermethrin), Asataf 75 SP (acefate) and Reeva 2.5 EC (lambda-cyhalothrin))were collected, extracted according to QuEChERS method (Anastassiades et al., 2003) and quantified by GC-ECD. Also the half life of each pesticide in every particular vegetable was estimated.

3.3.1Dissipation ofVitaban 48 EC (chlorpyrifos) in eight vegetables

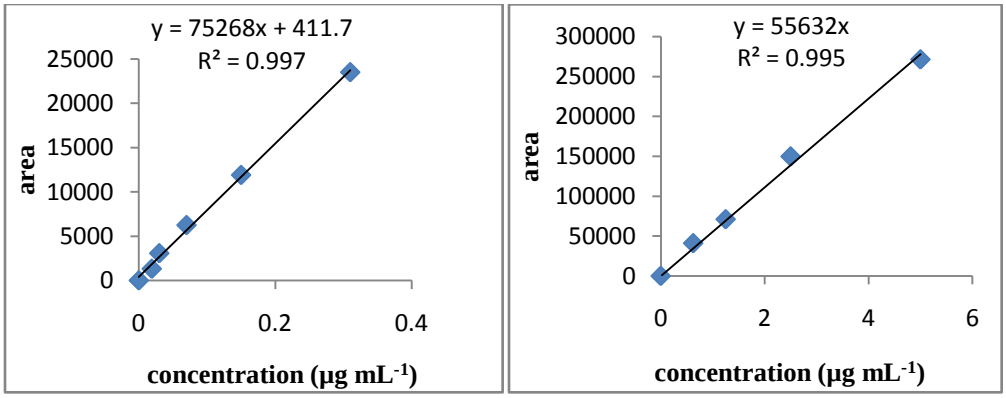
Vitaban 48 EC or Vita was sprayed on the eight kind of vegetables in the farmer's field, Nuritola, Comilla, Bangladesh at a dose of 3mL per Liter of water/ ha. The residue of Vita was analyzed by GC-ECD (Shimadzu, 2010).Under specific condition of the parameters (**section 2.5.6**), chlorpyrifos showed the retention time at 7.2 minutes.

3.3.1.2 Calibration curve of certified chlorpyrifos standards

Higher and lower concentration calibration curves (**Fig. 26**) of certified chlorpyrifos standards showed the linear coefficient (r^2) 0.995 and 0.997 with linearity range of 1.0– 3.2 and 0.10 – 0.30 µg mL^{-1}(**Table 20**)

3.3.2.3 Limit of detection and Limit of quantification

The LOD and the LOQ were found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1}, respectively.



(a) (b)

Fig. 26: Calibration curve of lower and higher concentration (a and b) Chlorpyrifos standards

Table 20: Linearity range, correlation coefficients (r^2), LOD and LOQ

Pesticide	Calibration curve	Linearity range (µg mL^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Chlorpyrifos	higher	1.0- 3.2	0.995	0.019	0.057
	lower	0.10-0.30	0.997		

3.3.2.4 Recovery Experiments

Recovery percentages of chlorpyrifos in spiked vegetable samples were done in two different concentration levels with three replications each (**Table 21**). The recovery percentages were in between the range of 73% - 99% with standard deviations less than and equal to 4.2. The relative standard deviation (RSD) values ranged from 0.50 to 4.24.

Table 20 (a): Recovery percentages of Chlorpyrifos in spiked vegetable samples

Vegetable	Spiking level ($\mu g\ mL^{-1}$)	Recovery %	Mean Recovery % ± SD	%RSD
Tomato	L0.5	82, 83, 86	84 ± 2.0	2.30
	H 0.2	74, 77, 71	74 ± 3.0	4.05
Cauliflower	L 0.5	90, 89, 91	93 ± 1.0	1.10
	H1.0	75, 74, 78	99 ± 2.0	2.60
Cabbage	L 0.5	99, 94, 103	96 ± 4.2	4.24
	H1.0	73, 72, 75	73 ±1.5	2.05
Bottle gourd	L 0.5	92, 91, 96	93 ± 2.6	2.78
	H1.0	82, 85, 88	85 ± 3.0	3.50
Sweet gourd	L0.5	90, 90, 86	89 ± 2.3	2.50
	H 0.2	76, 77, 78	77 ± 1.0	1.20
Sponce gourd	L 0.2	81, 79, 80	80 ± 1.0	1.25
	H 1.0	82, 84, 87	85 ± 2.3	2.70
Green chilli	L 0.5	75, 74, 73	74 ± 1.0	1.35
	H1 .0	88, 90, 86	88 ± 2.0	2.27
Cucumber	L 0.2	83, 86, 80	83 ± 3.0	3.61
	H 1.0	95, 95, 94	95 ± 0.5	0.52

L = Lower, H = Higher

3.3.2.5 Residual amount of chlorpyrifos in vegetables

The vegetable samples were kept at ambient temperature and the residual amount of chlorpyrifos was analyzed from 1st day to 10th day, which are shown below in **Table 22**.

Table 22:Amount of chlorpyrifos (Av. ± SD, µg g^{-1}) in different vegetables

DAS	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chili	Cucumber
Control	bdl							
1	3.12±0.8	4.30±08	2.94±0.18	2.75±1.01	4.23±1.03	4.11±0.33	5.14±1.33	4.21±1.01
3	2.22±0.8	1.97±11	1.02±0.75	1.41±2.33	2.11±0.74	3.31±0.10	3.12±3.31	2.75±1.23
5	1.31±0.4	0.51±2.1	0.31±0.31	0.61±0.77	1.23±0.22	1.95±0.02	1.42±2.11	1.35± 0.09
8	bdl	0.11±11	0.05±0.74	bdl	bdl	0.81±0.08	bdl	0.82± 0.19
10	ND	bdl	ND	ND	ND	bdl	ND	ND

DAS= Days After Spraying
bdl= below detection limit
ND= Not Detected

Table 23: Dissipation percentage of Vita in vegetable on 3rd, 5th, 8th and 10th day of spraying

DAS	Dissipation of chlorpyrifos (%)							
	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chilli	Cucumber
3	29	45	65	49	50	19	39	34
5	58	76	89	77	71	52	72	68
8	91	93	99	100	86	80	100	81
10	ND	100	ND	ND	ND	100	ND	ND

DAS= Days After Spraying
ND= Not Detected

3.3.2.6 Discussion of dissipation of chlorpyrifos treated vegetables

Recovery experiments were done in two different concentration levels (**Table 21**), at a lower and higher spiking level.The recoveries of Chlorpyrifos ranged from 74- 84% for tomato, 93- 99% for cauliflower, 73-96% for cabbage, 85- 93% for bottle gourd, 75- 89% for sweet gourd, 80- 85% for sponce gourd, 83- 88% for green chili and 83-95% for cucumber which is in the range of 70- 120%, acceptable range for a

acceptable recovery experiment, reported by CODEX, (2010). The variations in recovery percentages may be due to many factors. It is not possible to describe accurately the behavior of a pesticide to vegetables combination based on the information with other pesticides (Wheeler et al., 1983).

Higher and lower concentration calibration curves showed the linear coefficient (r^2) 0.995 and 0.997 with linearity range of about 1.0 – 3.2 µg mL^{-1} and 0.10 – 0.30 µg mL^{-1}.

Sensitivity of instrument was evaluated by determining LOD and LOQ. The LOD and LOQ were found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1}, respectively.

No substrate interferences were observed as evidenced by the control sample analysis. These data indicates that the extraction method is satisfactory for the requirement of chlorpyrifos residue analysis.

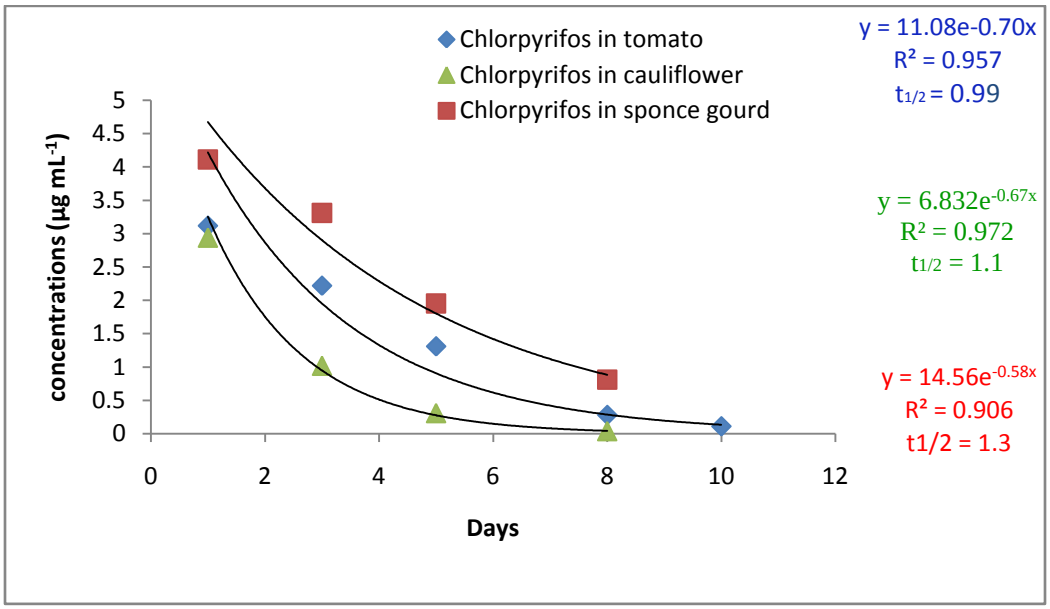


Fig. 27: Dissipation curve of chlorpyrifos in tomato, cauliflower and sponce gourd

No traces of chlorpyrifos were observed in the control samples of vegetables. The initial residual amount of chlorpyrifos on 1st day, in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber were 3.12±0.8, 4.30±08, 2.94±0.18, 2.75±1.01, 4.23±1.03, 4.11±0.33, 5.14 ±1.33 and 4.21±1.01 µg g^{-1}, respectively (**Table 22**).

According to CODEX Alimentaris Commission, April 2000; MRL of Chlorpyrifos;in tomato 0.5 µg g^{-1}, in cabbage, cauliflower, cucumber 0.05 µg g^{-1}, in chili 0.2 µg g^{-1}, in sweet gourd 0.1 µg g^{-1}.

All the eight vegetables showed much higher residual value of Chlorpyrifos than the MRL value on the 1st day after application (**Fig. 28**). Therefore, the vegetables should not be consumed on the 1st day of chlorpyrifos application. Otherwise they can cause harmful impact on human health.Parveen et al. (2005)monitored 24 pesticides residues, belonging to different pesticide classes like organophosphates, organocarbamates and pyrethroids in 206 samples of 27 different vegetables that collected from retail markets of Karachi, Pakistan during 2000-2003. About 63% samples were contaminated with one or another pesticide and 46% of contaminated samples had pesticide residues more than MRL values as given by FAO/WHO.

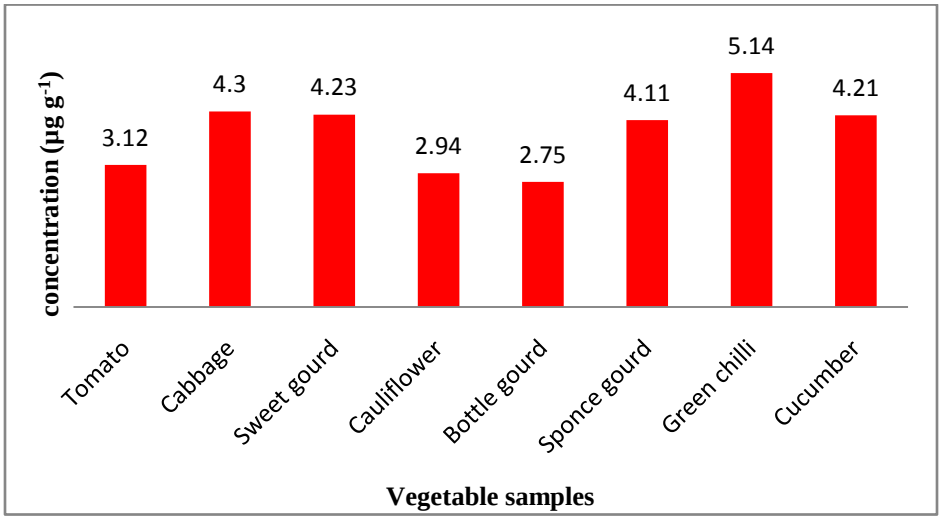


Fig 28: Residual amount of Chlorpyrifos on 1st day in eight different vegetables

The dissipation of chlorpyrifos followed a pseudo first-order kinetics pattern. The biological half-life ($t_{1/2}$) was calculated by the formula $t_{1/2} = \ln 2/k$ (Park 2011), in which the constant *k* is the slope of the linear regression ($A=A_0e^{-kt}$). The half lives of Chlorpyrifos in the vegetables were found to be 0.99, 1.2, 1.1, 1.3, 1.4, 1.3, 0.80 and 0.90 days, respectively for tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber, respectively (**Fig 27)**. That is supported by Zhang et al. (2012) for the dissipation of chlorpyrifos from soil under paddy field conditions. They reported the half life of chlorpyrifos in soil was 1.35 day. In the current study, residues in tomato, bottle gourd, sweet gourd and green chili,treated with Chlorpyrifos went under MRL value after 5 days of application, on the other hand, cabbage, cauliflower, sponce gourd and cucumber, treated with chlorpyrifos went under MRL value after 8 days. From **Table 23** of dissipation percentage of chlorpyrifos in the vegetables, it was observed that dissipation process is slow. From the work of Patel (1999); we see that the persistence of chlorpyrifos was lengthier than other pesticides. In few earlier studies, it was reported that dissipation rate of chlorpyrifos is slower than other modern pesticides. Ravi and Verma (1997) reported that initial residues of chlorpyriphos were 5.41 mg kg^{-1} on chickpea pods that degraded below MRL of 0.2 mg kg^{-1} in 20 days.

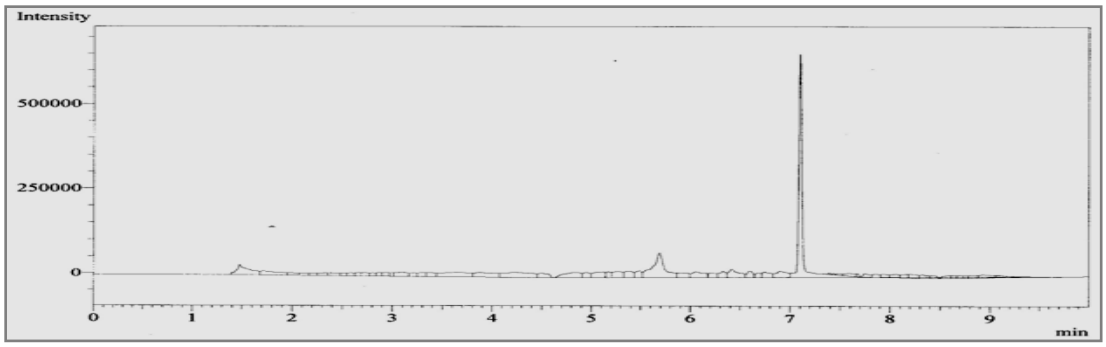


Fig 29: Chromatograph of cabbage sample treated with chlorpyrifos (1st day of pesticide application)

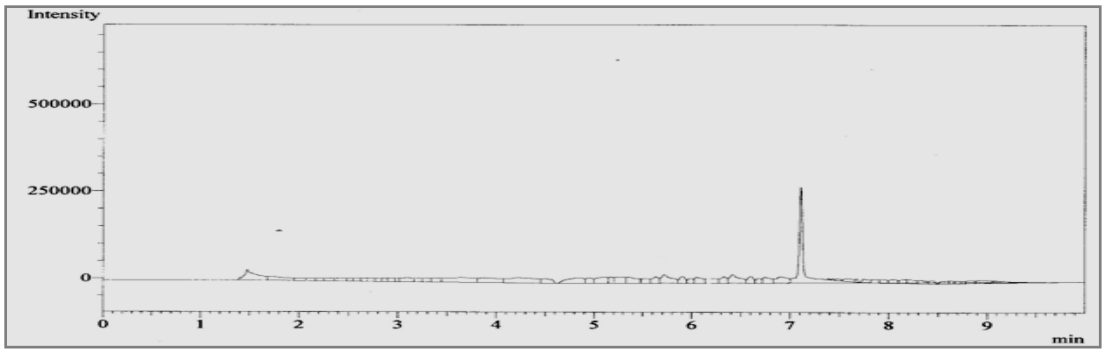


Fig 30: Chromatograph of certified chlorpyrifos standard (1.25 µg g^{-1})

Subhash (2014) reported that Chlorpyrifos dissipates slowly and it went under MRL value in 15 days, in okra.

Thus, the results from the current experiment revealed that the safe period of consumption of chloropyrifos treated tomato, bottle gourd, sweet gourd and green chili is 5 days after application and for cabbage, cauliflower, sponce gourd and cucumber, it is 8 days after application.

3.3.3 Dissipation of Nitro505 EC (mix formulation of chloropyrifos and cypermethrin) in vegetables

Nitro 505 EC or Nitro was sprayed on the eight kind of vegetables at a dose of 1 mL per Liter of water/ ha. After extraction and clean up, Nitro treated vegetables were analyzed by GC-ECD. At specific condition of the parameters (**section 2.5.6**), Nitro showed retention times at 7.5 and 12.4 minutes.

3.3.3.1 Active component of Nitro 505 EC

Nitro 505 EC is a mix formulation of chlorpyrifos (50%) and cypermethrin (5%). Chlopyrifos and cypermethrin showed retention time of 8.4 and 12.3 minutes, respectively in GC-ECD.

3.3.3.2 Calibration curve of certified cypermethrin standards

Calibration curves of higher and lower concentrations of certified chlorpyrifos standards have been shown on section 3.3.1.2.

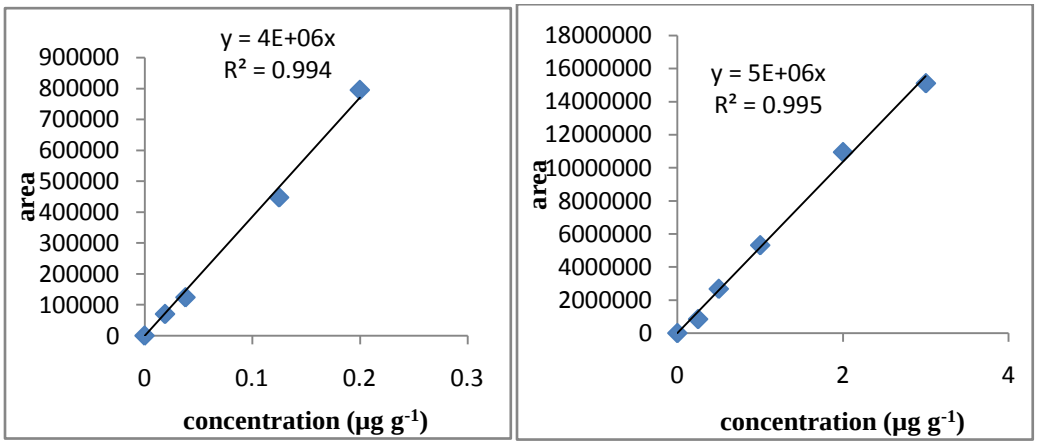
Two individual calibration curves were made to cover the vast range of concentration of cypermethrin in the vegetables from 1st day to 10th day (**Fig. 31**).Higher and lower concentration calibration curves showed the linear coefficient (r^2) 0.995 and 0.994 with linearity range of about 1.5 – 3.5 and 0.05 – 0.20 µg mL^{-1} (**Table 24**).

3.3.3.3 Limit of detection and Limit of quantification

The LOD and the LOQ were found to be 0.019 and 0.057 µg mL^{-1}, respectively (**Table 24**).

Table 24: Linearity range, correlation coefficients (r^2), LOD and LOQ

Pesticide	Calibration curve	Linearity range (µg mL^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Cypermethrin	Higher	1.5- 3.5	0.995	0.019	0.057
	Lower	0.05-0.20	0.994		



(a) (b)

Fig 31: Calibration curves of lower and higher concentrations (a and b) of cypermethrin standards

3.3.3.4 Recovery Experiments

Recovery percentages of cypermethrin in spiked vegetable samples were done in two different concentration levels with three replications each(**Table 25**). The recoveries of cypermethrin ranged from 88- 96% for tomato, 98- 102% for cauliflower, 84-93% for cabbage, 85- 88% for bottle gourd, 90- 92% for sweet gourd, 76- 93% for sponce gourd, 82- 85% for green chili and 77- 98% for cucumber which is in between the range of 70 - 120%, acceptable range for a acceptable recovery experiment, reported by CODEX, (2010).The standard deviations were less than or equal to 3.0. The relative standard deviation (RSD) values ranged from 0.50 to 3.52.

Table 25: Recovery percentages of Cypermethrin in eight spiked vegetable samples

Vegetable	Spiking level ($\mu g\ mL^{-1}$)	Mean Recovery % ± SD	%RSD
Tomato	L 0.05	96± 1.2	1.25
	H 0.2	88 ± 2.3	2.61
Cauliflower	L 0.5	98 ± 1.5	1.97
	H 2.0	102 ± 2.0	2.20
Cabbage	L 0.5	84 ± 2.5	2.97
	H 2.0	93 ±0.5	0.54
Bottle gourd	L 0.5	85 ± 3.0	3.52
	H 2.0	88 ± 2.5	2.84
Sweet gourd	L 0.05	92 ± 1.2	1.30
	H 0.2	90 ± 0.5	0.56
Sponce gourd	L 0.2	93 ± 1.0	1.07
	H 1.0	76 ± 2.5	3.20
Green chilli	L 0.5	82 ± 2.5	3.04
	H 2 .0	85 ± 2.0	2.36
Cucumber	L 0.2	77 ± 1.0	1.29
	H 1.0	98 ± 1.5	1.53

L= Lower, H= Higher

3.3.3.5 Residual amount of Nitro 505 EC in vegetables

The vegetable samples were kept at ambient temperature and the residual amount of Nitro(chlorpyrifos and cypermethrin) were analyzed from 1st day to 10th day, which are shown below in **Table 26 – 32.** .

Table 26:Amount of Nitro (Av. ± SD, µg g^{-1}) in tomato

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			chlorpyrifos	Cypermethrin
1	tomato	TN 1	2.11 ±0.08	1.22 ±0.08
3		TN 3	1.68 ±0.50	0.13 ±0.05
5		TN 5	0.62 ±0.46	0.07 ±0.05
8		TN 8	bdl	bdl
10		TN 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 27:Amount of Nitro (Av. ± SD, µg g^{-1}) in cabbage

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Chlorpyrifos	Cypermethrin
1	cabbage	Cb N 1	1.20 ±0.08	1.27 ±0.15
3		Cb N 3	0.94 ±0.44	1.05 ±0.06
5		Cb N 5	0.13 ±0.36	0.08 ±0.10
8		Cb N 8	0.06 ±0.09	bdl
10		Cb N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 27 (a):Amount of Nitro (Av. ± SD, µg g^{-1}) in cauliflower

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Chlorpyrifos	Cypermethrin
1	cauliflower	Cf N 1	1.35 ±0.35	1.01 ±0.08
3		Cf N 3	1.01 ±0.55	0.63 ±0.04
5		Cf N 5	0.38 ±0.08	0.09 ±0.15
8		Cf N 8	0.06 ±0.05	bdl
10		Cf N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 28:Amount of Nitro (Av. ± SD, µg g^{-1}) in bottle gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Chlorpyrifos	Cypermethrin
1	Bottle Gourd	Bg N 1	1.91 ±0.22	0.85 ±0.03
3		Bg N 3	1.25 ±0.07	0.49 ±0.07
5		Bg N 5	0.13 ±0.65	0.10 ±0.12
8		Bg N 8	bdl	bdl
10		Bg N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 29:Amount of Nitro (Av. ± SD, µg g^{-1}) in sweet gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Chlorpyrifos	Cypermethrin
1	Sweet Gourd	Swt N 1	2.43 ±0.09	1.32 ±0.02
3		Swt N 3	1.98 ±0.06	0.13 ±0.55
5		Swt N 5	0.15 ±0.11	bdl
8		Swt N 8	0.06 ±0.03	ND
10		Swt N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 30:Amount of Nitro (Av. ± SD, µg g^{-1}) in sponce gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			chlorpyrifos	Cypermethrin
1	Sponce Gourd	Sg N 1	3.44 ±0.07	1.87 ±0.06
3		Sg N 3	2.60 ±0.10	1.04 ±0.09
5		Sg N 5	0.92 ±0.05	0.07 ±0.35
8		Sg N 8	0.07 ± 0.09	bdl
10		Sg N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 31:Amount of Nitro (Av. ± SD, µg g^{-1}) in green chili

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			chlorpyrifos	Cypermethrin
1	Green chili	Chl N 1	2.28 ±0.10	1.94 ±0.02
3		Chl N 3	1.40 ±0.15	0.41 ±0.08
5		Chl N 5	bdl	bdl
8		Chl N 8	ND	ND
10		Chl N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 32:Amount of Nitro (Av. ± SD, µg g^{-1}) in cucumber

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			chlorpyrifos	Cypermethrin
1	Cucumber	Cu N 1	2.10 ±0.02	1.02 ±0.20
3		Cu N 3	1.70 ±0.05	0.17 ±0.08
5		Cu N 5	0.08 ± 0.10	bdl
8		Cu N 8	ND	ND
10		Cu N 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 33: Dissipation percentage of chlorpyrifos and cypermethrin in the vegetable samples

Days of spraying	Pesticide	Dissipation (%)							
		Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chili	Cucumber
3	Chlorpyrifos	43	17	25	34	19	44	39	19
	Cypermethrin	64	35	37	42	90	68	76	83
5	Chlorpyrifos	76	89	71	93	93	78	100	99
	Cypermethrin	94	95	91	88	100	100	100	100
8	Chlorpyrifos	100	95	94	100	100	100	-	-
	Cypermethrin	100	100	100	100	-	-	-	-

3.3.3.6 Discussion on Nitro 505 EC

Nitro 505 EC is an insecticide and is a mix formulation of 50% chlorpyrifos and 5% cypermethrin. It was extracted according to QuEChERS method and cleaned up using primary secondary amine. Method validation were performed to provide evidence that the method was fit for the purpose for which it is to be used. Two individual components are present in Nitro 505 EC, chlorpyrifos and cypermethrin. Validation of the extraction method for determining chlorpyrifos residues was mentioned above in section 3.3.2.6.

Recovery experiments for cypermethrin were done in two different concentration levels, at a lower and higher spiking level of 0.05 and 0.2 µg g^{-1} for tomato; 0.5 and 2 µg g^{-1} for cauliflower; 0.5 and 2 µg g^{-1} for cabbage; 0.5 and 2 µg g^{-1} for bottle gourd; 0.05 and 0.2 µg g^{-1} for sweet gourd; 0.2 and 1 µg g^{-1} for sponce gourd; 0.5 and 2 µg g^{-1} for green chilli, 0.2 and 1 µg g^{-1} for cucumber. The recovery for lower and higher spiking levels (**Table 25**) were, for tomato, 96 and 88%; for cauliflower 98 and 102%; for cabbage, 84 and 93%; for bottle gourd, 85 and 88%; for sweet gourd, 92 and 90%; for sponce gourd, 93 and 76%; for green chili, 82 and 85%; for cucumber, 77 and 98%. The average recoveries of cypermethrin ranged from 76 to 98%, with relative standard deviation less than 5, which are in the range of 70 - 120%, acceptable range for a recovery experiment, reported by CODEX (2000).RSD, ranging from 0.54% to

3.5%, indicates the good performance of extraction, clean-up and chromatographic parameters for the analysis. The variations in recovery percentages may be due to many factors. Higher recoveries were found for cypermethrin in cauliflower and chlorpyrifos in cauliflower indicating that matrix may play a role affecting the recovery (Ajay et al., 2004).

Higher and lower concentration calibration curves of certified cypermethrin standards showed the linear coefficient (r^2) 0.998 and 0.998 with linearity range of about 1.5–3.5 µg mL^{-1} and 0.05– 0.20 µg mL^{-1}, respectively (**Table 24**).

Sensitivity of instrument was evaluated by determining LOD and LOQ. The LOD and the LOQ were found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1} for both chlorpyrifos and cypermethrin.

Certified standards of chlorpyrifos and cypermethrin gave sharp peaks at 8.4 and 12.3 minutes respectively in GC-ECD according to specific parameters of the system. Specificity was confirmed by injecting control vegetable extract and no matrix peaks was found to interfere with the retention time of chlorpyrifos and cypermethrin.
These data indicates that the extraction method is satisfactory for the requirement of chlorpyrifos and cypermethrin residue analysis.

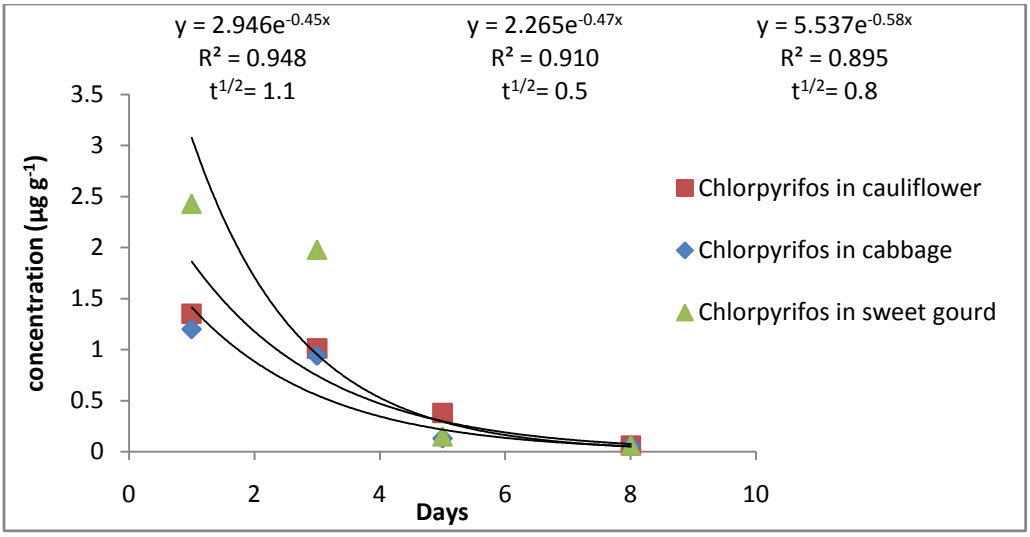


Fig 32: Dissipation curve of chlorpyrifos in cauliflower, cabbage and sweet gourd as the constituent of Nitro 505 EC

Tajeda et al., (1983) reported that the disappearance of residues in and on plants is the effect of the interactions of environmental conditions such as wind, rain, sun, humidity and temperature and chemical and physical factors such as volatilization and growth of the plants.Nitro 505 EC, the mix formulation of 50% chlorpyrifos and 5% cypermethrin, is used mainly as an insecticide and spayed at a dose of 1 mL per Liter of water/ ha. From the composition of Nitro, it can be seen that the concentration of chlorpyrifos is higher than that of cypermethrin. On the other hand, chlorpyrifos has greater persistence than that of cypermethrin. The dissipation percentage of chlorpyrifos is much slower than cypermethrin's dissipation percentage, shown in table 3.27.

Parmar et al., (2012), studied dissipation and decontamination ofcypermethrin and initial deposits were 0.53 µg $g^{-1}$and safe waiting periods were 4.7 days. Nath et al., (2005) described dissipation behaviour of ready mix polytrin C 44EC (profenophos 40% + cypermethrin 4%) applied at 1 L ha^{-1} in okra crop during Kharif in year 2000 was studied at 0, 1, 3, 5 and 7 days after treatment.Half life ($t_{1/2}$) values for profenophos and cypermethrin were 1.35 and 4.11 days, respectively.

In another study, 84 farm gate seasonal vegetable samples, viz., brinjal, okra, cauliflower, cabbage, knolknol, summer squash, smooth gourd, cucumber, pea and potato from Hisar were analyzed for monocrotophos, endosulfan, cypermethrin, malathion, methyl parathion, chloropyrifos, aldicarb and quinalphos by Kumari *et al.* (2004). It was revealed that 26% samples contained residues above MRL values.

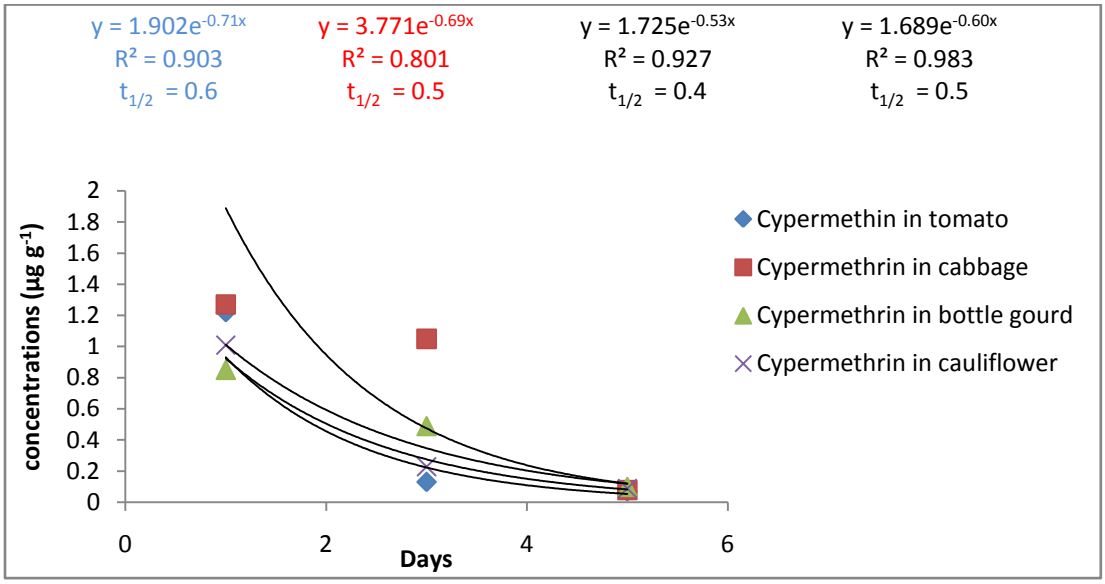


Fig 33: Dissipation curve of cypermethrin in tomato, cabbage, bottle gourd and cauliflower as a constituent of Nitro 505 EC

In the current study, the initial amounts of chlorpyrifos and cypermethrin in all the vegetables on first day were much higher than the MRL values of both pesticides. The MRL value of cypermethrin in tomato, 0.2 mg kg-^{1} (Codex 2013), in cabbage, cauliflower and cucumber, 1.0 mg kg-^{1} (Codex 2013), in green chili, 0.5 mg kg-^{1} (Codex 2013) and in sweet gourd and bottle gourd, 1.0 mg kg-^{1} (Codex 2000).The MRL value of chlorpyrifos in tomato, 1.0 mg kg-^{1} (Codex 2013), in cabbage, cauliflower and cucumber, 0.05 mg kg-^{1} (Codex 2013), in sweet gourd and bottle gourd, 0.5 mg kg-^{1} (Codex 2000).

Islam et al. (2009a) reported on pesticides residues (diazinon, malathion, chlorpyrifos and cypermethrin) in cauliflower. Recommended and double of the recommended doses of pesticides were sprayed and were collected after 6 hours. Residues of diazinon and chlorpyrifos were above the MRL values but malathion and cypermethrin were below the detection limit.

Chowdhury et al. (2013) collected tomato samples from various local market shops of Savar Upazilla and analyzed to identify cypermethrin, chlorpyrifos and diazinon residues. Five samples were contaminated with cypermethrin and one with chlorpyrifos below the respective MRL values.

In fig below, the initial concentrations of chlorpyrifosin all the mentioned vegetable samples on first day are shown; the values are much higher than those of the MRL values of the respective vegetables. Again, the amount of cypermethrin in the samples was higher than the MRL values of the respective value. Therefore, it can be certainly reported that the consumption of Chlorpyrifos and cypermrthrin treated vegetables on the first day is highly risky as it may pose acute toxicity to the health.

From the dissipation curves, the average half-lives of chlorpyrifos and cyperethrin in Nitro were found to be 0.92 and 0.58 days, respectively (**Fig. 32 and 33**). The average half –life value in chlorpyrifos is higher than that of cypermethrin. Similar results were reported by Awasthi (1994) whereas he determined persistence and degradation of different pyrethroids on green chilli fruits. The half-life of permethrin was 0.42–0.68 days as that of cypermethrin in the current study was 0.46 -0.81 days.

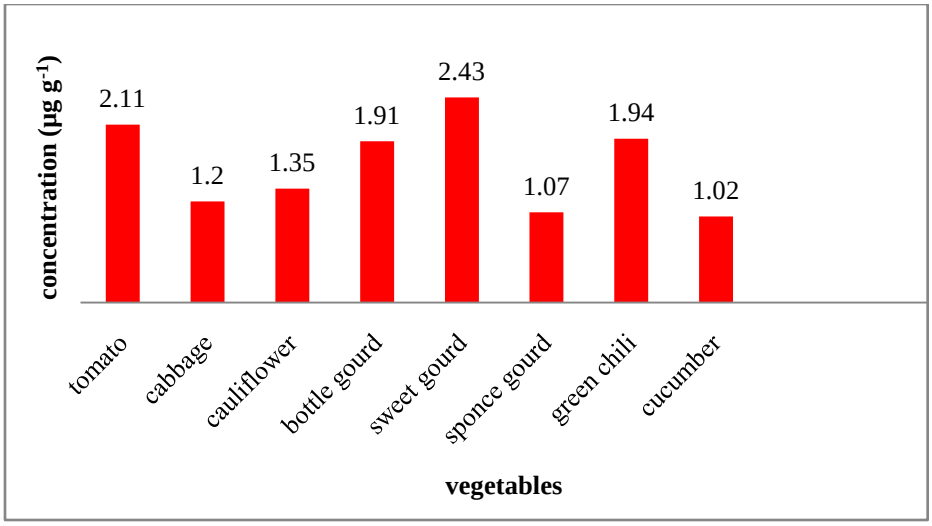


Fig 34: Residual amount of chlorpyrifos (in Nitro) on 1st day in the vegetables

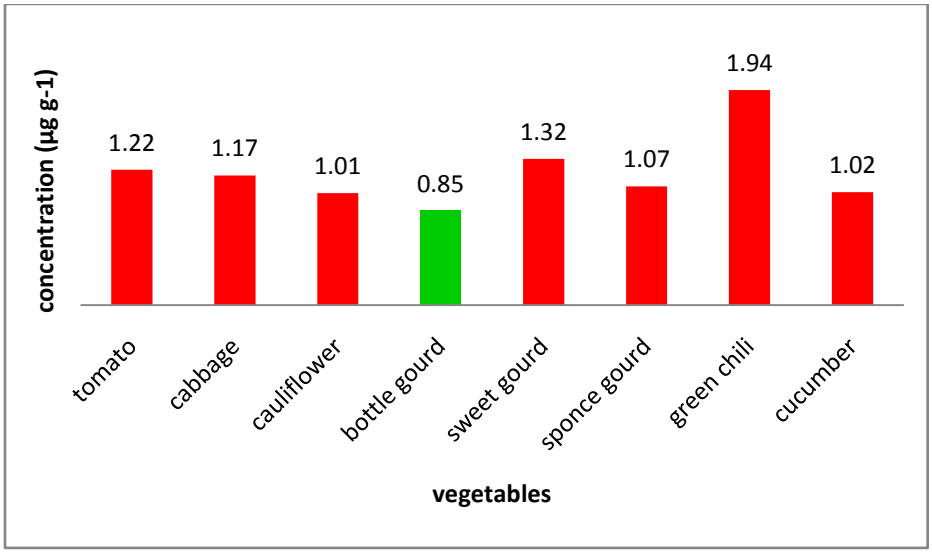


Fig 35: Residual amount of cypermethrin (in Nitro) on 1st day in the vegetables

The initial amount of chlorpyrifos in the Nitro treated vegetable samples were above MRL values of the vegetable samples on the first day (**Fig 34**). The residual concentration of chlorpyrifos in tomato was 2.11 ±0.08µg g^{-1}, in cabbage, cauliflower, bottle gourd, sweetgourd, sponce gourd, green chili and cucumber were 1.20 ± 0.08 ,

1.35 ± 0.35 , 1.91 ± 0.22 , 2.43 ± 0.09 , 3.44 ± 0.07 , 2.28 ± 0.10 and 2.10 ± 0.02 µg g^{-1}, respectively (**Table 26– 32**). From **Table 33,** it was observed that dissipation of 100% of chlorpyrifos occurred on 5^{th} day after spraying for green chili and on 8^{th} day after spraying for rest of the vegetables.For tomato, the initial amount declined by 43% upto 3^{rd} day after application and went below MRL value. For cabbage and cauliflower, the initial amount declined by 95 and 94% upto 8^{th} day after application. For bottle gourd and sweet gourd, the safe level of consumption, MRL, were obtained after 3^{rd} day of application and dissipates by 34% and 19%, respectively. The safe level of consumption of chlorpyrifos treated sponce gourd, green chili and cucumber is after5 days of application, where 78, 100 and 99% dissipation occurs. Therefore, the safe period of consumption of chlorpyrifos treated tomato, bottle gourd and sweet gourd is 3^{rd} day after application; in case of cabbage and cauliflower, it is 8 days after application; sponce gourd green chili and cucumber are safe to consume after 5 days after application at ambient temperature.

The residual concentration of cypermethrinon 1^{st} day (**Fig 35**) in tomato, cabbage, cauliflower, sweetgourd, sponce gourd, green chili and cucumber were 1.22 ±0.08 , 1.17 ±0.15 , 1.01 ±0.08 , 1.32 ±0.02 , 1.07 ± 0.06 , 1.94 ±0.02 and 1.02 ±0.20 µg g^{-1}, respectively (**Table 26- 32**). Cypermethrin in tomato, cauliflower, sweet gourd, sponce gourd, green chili and cucumber went below MRL value after 1^{st} day of application, in case of cabbage and sponce gourd, the initial concentration went below the MRL value after 3^{rd} day of application. Almost 100% dissipation of cypermethrin occurs within 5 days after application. Therefore, it can be suggested from the current study that it is safe to consume cypermethrin treated tomato, cauliflower, sweet gourd, green chili and cucumber atleast after 1 day of application and for cabbage and sponce gourd, the safe level of consumption reaches after 3 days of application at ambient temperature.

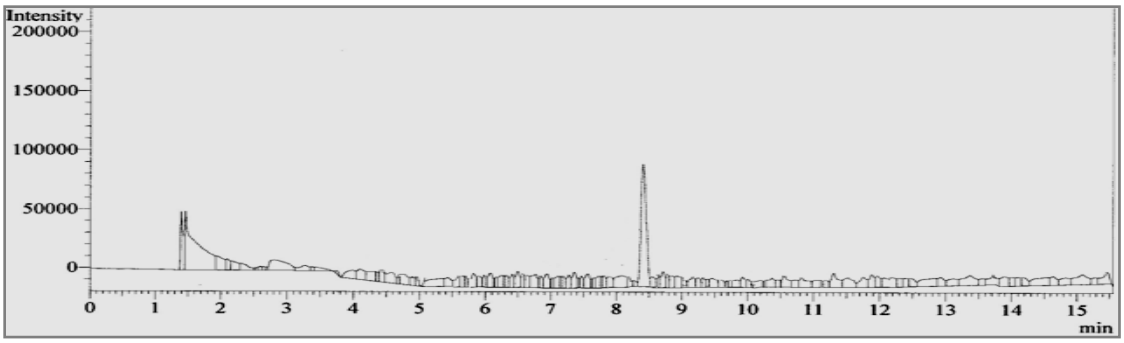


Fig 36: GC–ECD chromatograph of certified chlorpyrifos standard (0.625 µg g^{-1})

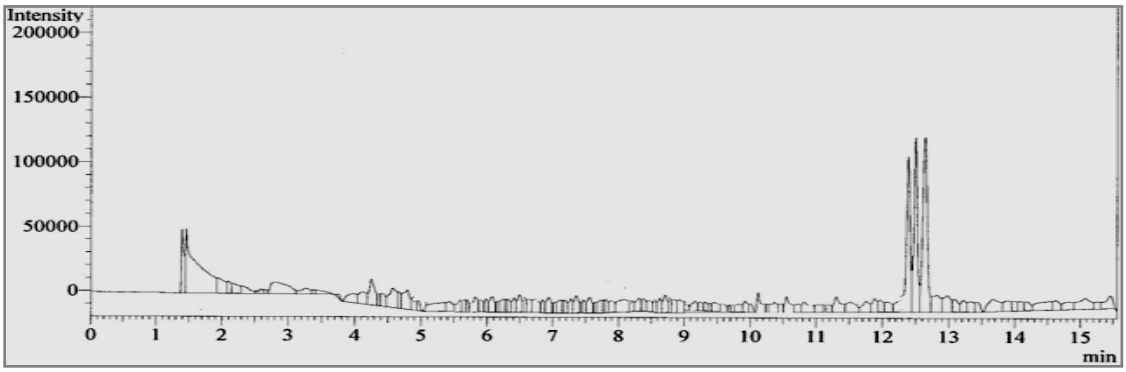


Fig 37: GC–ECD chromatograph of certified cypermethrin standard (1.25 µg g^{-1})

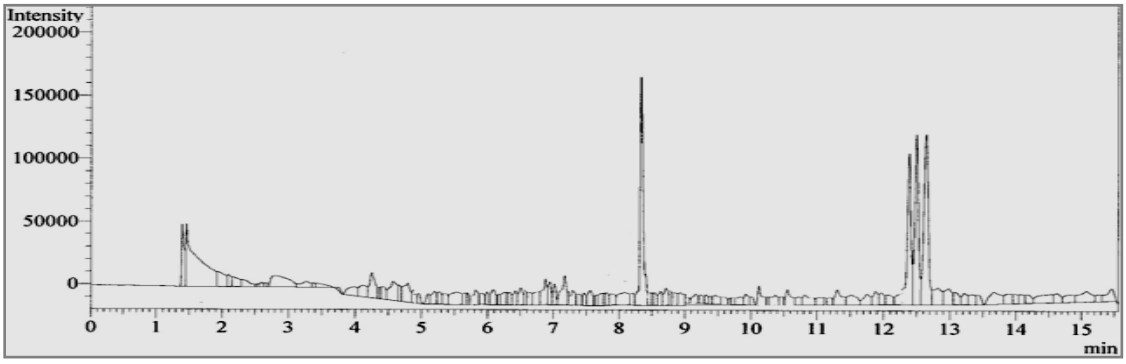


Fig 38: GC–ECD chromatograph of tomato sample treated with Nitro 505 EC (1st day of application)

3.3.4 Dissipation of Double 50 EC (mix formulation of imidacloprid and cypermethrin)

Double 50 EC or Double was sprayed at a dose of 800 mL/ ha. The residue of Double in the vegetables was determined by QuEChERS method and cleaned up with PSA, then the residue was analyzed by GC-ECD (Shimadzu, 2010).

3.3.4.1 Active component Double 50 EC

Double 50EC is the mix formulation of imidacloprid (40%) and cypermethrin (10%). Double showed retention times at 7.5 and 12.3 minutes, respectively for imidacloprid and cypermethrin in GC-ECD at specific condition of the parameter (**mentioned in sec. 2.5.6**).The retention times for the individual pesticide were confirmed by running certified standards of imidacloprid and cypermethrin.

3.3.4.2 Calibration curve of certified imidacloprid standards

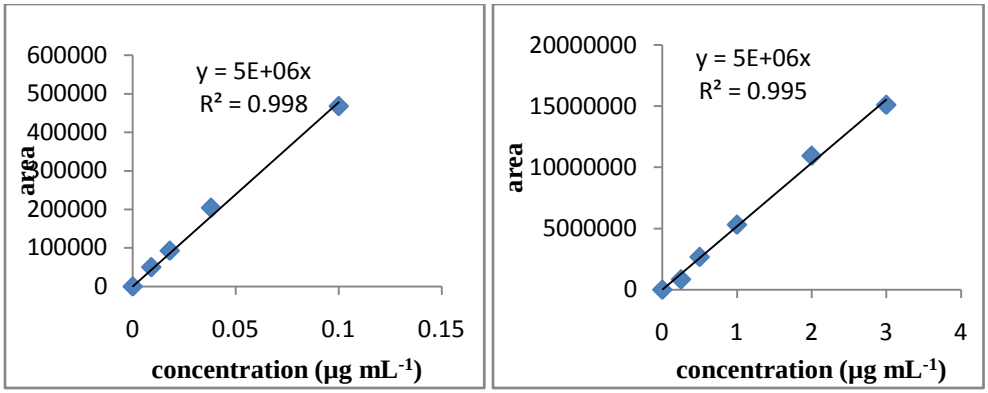
Two individual calibration curves were made to cover the vast range of concentration of imidacloprid in the vegetables from 1st day to 10th day (**Table 34**). Higher and lower concentration calibration curves showed the linear coefficient (r^2) 0.998 and 0.994 with linearity range of about 1.0– 2.0 and 0.03 – 0.08 µg mL^{-1} (**Fig. 39**).

3.3.4.3 Limit of detection and Limit of quantification

The LOD and the LOQ were found to be 0.009 µg mL^{-1} and 0.027 µg mL^{-1}, respectively.

Table 34: Linearity range, correlation coefficients (r^2), LOD and LOQ

Pesticide	Calibration curve	Linearity range (µg mL^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Imidacloprid	higher	1.0 -2.0	0.998	0.009	0.027
	lower	0.03 -0.08	0.994		



(a) **(b)**

Fig 39: Calibration curve of lower and higher concentration (a and b) of imidacloprid standards

3.3.4.4 Recovery Experiments

The method for determining imidacloprid and cypermrthrin were optimized and validated by evaluating recovery experiments with control matrices that were previously confirmed the absence of targeted compound (s) in the samples. Recovery experiments for cypermethrin were described before in section 3.3.3.4 For imidacloprid, the recovery experiments were performed in 3 replicates at 2 fortification levels. The spiked samples were kept asidefor 4 hrs to allow for the adsorption of pesticide onto the samples. Good recoveries were found for spiked samples.

Recovery percentages of imidacloprid in spiked vegetable samples were done in two different concentration levels with three replications each (**Table 35**). The recovery percentages were in the range of 71 -104% with standard deviations less than and equal to 3.5. The relative standard deviation (RSD) values ranged from 0.8 to 4.2. The results confirmed that the extraction efficiency for analyzing imidacloprid is good.

Table 35: Recovery percentages of imidacloprid in eight spiked vegetable samples

Vegetable	Spiking level ($\mu g\ mL^{-1}$)	Mean Recovery % ± SD	%RSD
Cauliflower	L 0.5	96 ± 2.0	2.2
	H 2.0	104 ± 1.0	1.4
Cabbage	L 0.5	95 ± 1.5	1.5
	H 2.0	102 ± 2.0	1.9
Bottle gourd	L 0.5	87 ± 2.5	2.8
	H 2.0	90 ± 1.2	1.1
Sweet gourd	L 0.05	83 ± 3.5	4.2
	H 0.2	82 ± 1.0	1.2
Sponce gourd	L 0.2	88 ± 2.5	2.8
	H 1.0	93 ± 1.5	1.6
Green chilli	L 0.5	76 ± 2.5	3.2
	H 2 .0	80 ± 3.0	3.7
Cucumber	L 0.2	87 ± 1.2	1.3
	H 1.0	79 ± 1.0	1.2
Tomato	L 0.5	71 ± 0.5	0.7
	H 1.0	80 ± 1.5	0.8

L= Lower, H= Higher

3.3.4.5 Residual amount of Double 50 EC in vegetables

The vegetable samples were kept at ambient temperature to allow the pesticide treated vegetables to be effected by the air, heat and light, as well as biological processes. As after harvesting, most of the people buy or brought them from field and then keep vegetables at ambient temperature at home before eating, sometimes eat them raw, as salad ingredients. Therefore, it was required to analyze the time period after which the pesticides declined below MRL value and became safe for consumption without the risk of health hazard. The residual amount of Double 50 EC (chlorpyrifos and cypermethrin) and dissipation percentage, analyzed are shown below in **Table 36**.

Table 36:Amount of Double (Av. ± SD, µg g^{-1}) in tomato

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	tomato	TD 1	1.13± 0.15	0.65± 0.09
3		TD 3	0.47± 0.06	0.17± 0.09
5		TD 5	0.06± 0.15	0.04± 0.10
8		TD 8	bdl	bdl
10		TD 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 37:Amount of Double (Av. ± SD, µg g^{-1}) in cabbage

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	cabbage	Cb D 1	1.44± 0.08	1.61± 0.08
3		Cb D 3	0.60± 0.07	0.45± 0.10
5		Cb D 5	0.05± 0.15	0.06± 0.03
8		CbD 8	bdl	bdl
10		Cb D 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 38:Amount of Double (Av. ± SD, µg g^{-1}) in cauliflower

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	cauliflower	Cf D 1	1.62± 0.06	1.05± 0.08
3		Cf D 3	0.88± 0.10	0.13± 0.05
5		CfD 5	0.04± 0.10	0.06± 0.09
8		Cf D 8	bdl	bdl
10		Cf D 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 39:Amount of Double (Av. ± SD, µg g^{-1}) in bottle gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	bottle gourd	Bg D 1	1.65± 0.10	1.17± 0.15
3		Bg D 3	1.27± 0.09	1.03± 0.10
5		Bg D 5	0.03± 0.15	0.06± 0.04
8		Bg D 8	bdl	bdl
10		Bg D 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 40:Amount of Double (Av. ± SD, µg g^{-1}) in sweet gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	sweet gourd	Swt D 1	1.58 ±0.09	1.25 ±0.06
3		SwtD 3	1.12 ± 0.10	1.00 ±0.06
5		Swt D 5	0.65±0.08	0.35 ±0.05
8		Swt D 8	ND	ND
10		Swt D 10	ND	ND

ND= Not Detected

Table 41:Amount of Double (Av. ± SD, µg g^{-1}) in sponce gourd

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	sponce gourd	Sg D 1	1.98± 0.03	1.60± 0.05
3		Sg D 3	1.52± 0.08	1.02± 0.06
5		Sg D 5	1.01± 0.08	0.30± 0.10
8		Sg D 8	ND	ND
10		Sg D 10	ND	ND

ND= Not Detected

Table 42:Amount of Double (Av. ± SD, µg g^{-1}) in green chili

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	green chili	Chl D 1	1.02± 0.10	0.52± 0.03
3		ChlD 3	0.41± 0.03	0.09± 0.06
5		Chl D 5	0.03± 0.07	bdl
8		Chl D 8	bdl	bdl
10		Chl D 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 43:Amount of Double (Av. ± SD, µg g^{-1}) in cucumber

Days of spraying	Samples code		Residual amount (µg g^{-1}) ± SD	
			Imidacloprid	Cypermethrin
1	cucumber	Cu D 1	2.05±0.02	1.87± 0.04
3		Cu D 3	1.12± 0.01	1.01± 0.04
5		Cu D 5	0.33± 0.04	0.10± 0.06
8		Cu D 8	bdl	bdl
10		Cu D 10	ND	ND

bdl= below detection limit

ND= Not Detected

Table 44: Dissipation percentage of imidacloprid and cypermethrin in the vegetable samples

Days of spraying	Pesticide	Dissipation percentage (%)							
		tomato	cabbage	cauliflower	bottle gourd	sweet gourd	sponce gourd	green chili	Cucumber
3	Imidacloprid	58	60	45	23	34	34	59	45
	Cypermethrin	73	72	56	26	32	36	82	47
5	Imidacloprid	94	97	96	98	58	56	97	83
	Cypermethrin	93	96	94	96	80	82	100	95
8	Imidacloprid	100	100	100	100	-	-	100	100
	Cypermethrin	100	100	100	100	-	-	100	100

3.3.4.6 Discussion on Double 50 EC

Double 50 EC was extracted according to QuEChERS method and cleaned up using PSA. Two individual components are present in Double 50 EC, imidacloprid and cypermethrin. Validation of the extraction method for determining cypermethrin was mentioned above in section 3.3.3.4.

Recovery experiments for imidacloprid (**Table 35**) were done in two different levels, at a lower and higher spiking level. The recovery for lower and higher spiking levels were, for tomato, 71 and 80%; for cauliflower 90 and 71%; for cabbage, 95 and 102%; for bottle gourd, 87 and 104%; for sweet gourd, 83 and 82%; for sponce gourd, 88 and 93%; for green chili, 76 and 80%; for cucumber, 87 and 79%. The average recoveries of imidacloprid ranged from 71 to 104%, with relative standard deviation less than 5, which are in between the range of 70 - 120%, acceptable range for a recovery experiment, reported by Codex. The result indicates the good performance of extraction, clean-up and chromatographic parameters for the analysis. The variations in recovery percentages may be due to many factors. It is not possible to describe accurately the behavior of a pesticide to vegetables combination based on the information with other pesticides (Wheeler et al., 1983).

Higher and lower concentration calibration curves of certified imidacloprid and cypermethrin standards were made. Imidacloprid showed the linear coefficient (r^2) 0.995 and 0.998 for higher and lower concentration calibration curves with linearity range of about 1.0– 2.0 and 0.03– 0.08 µg mL^{-1}, respectively. Again, higher and lower concentration calibration curves of standard cypermethrin showed linear coefficient (r^2) 0.995 and 0.994 with linearity range of about 1.5– 3.5 and 0.05– 0.20 µg mL^{-1}, respectively (**Table 34**).

Sensitivity of instrument was evaluated by determining LOD and LOQ.The LOD and the LOQ were found to be 0.019 and 0.057 µg mL^{-1} for cypermethrin and 0.009 µg mL^{-1} and 0.027 µg mL^{-1} for imidacloprid, respectively.

Certified standards of imidacloprid and cypermethrin gave sharp peaks at 7.5 and 12.3 minutes respectively in GC-ECD according to specific parameters of the system. Specificity was confirmed by injecting control vegetable extract and no matrix peaks was found to interfere with the retention time of imidacloprid and cypermethrin.
These data indicates that the extraction method is satisfactory for the requirement of imidaclopridand cypermethrin residue analysis.

In the current study, half-lives for tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponcegourd,green chili and cucumber were found to be 0.38, 0.85, 0.80, 0.41, 0.40, 0.52, 0.35 and 0.7 day and 0.30, 0.72, 0.52, 0.39, 0.53, 0.60, 0.34 and 0.41 day for imidacloprid and cymermethrin, respectively (**Fig 42 and 44**).

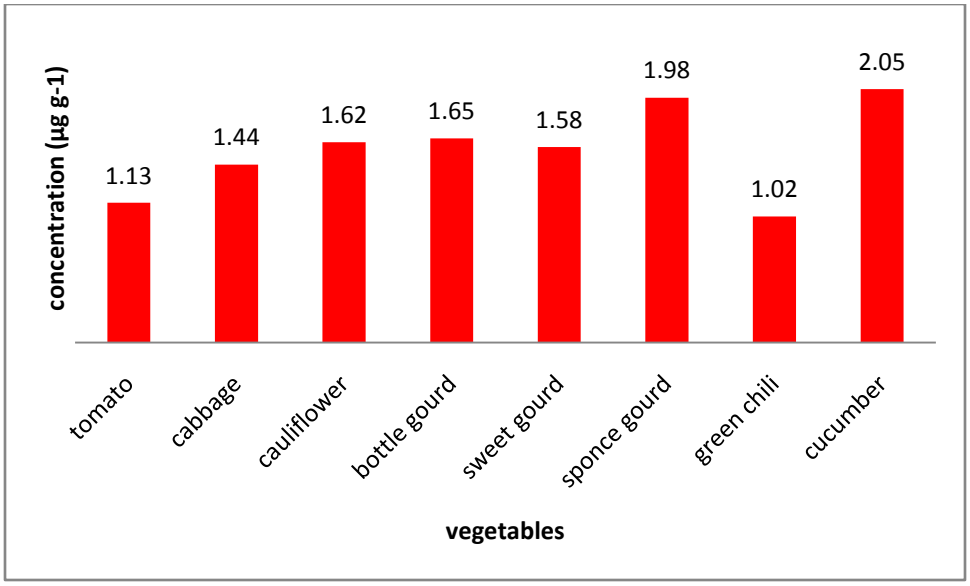


Fig 40: Amount of imidacloprid (in Double 50 EC) on 1st day in eight vegetables

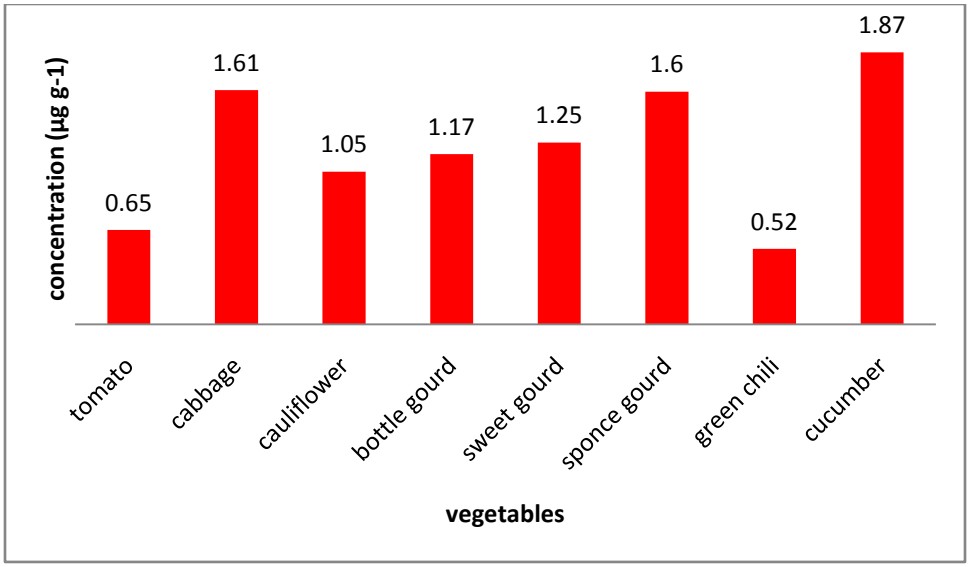


Fig 41: Residual amount of cypermethrin (in Double 50 EC) on 1st day in eight vegetables

For understanding the possible hazardous effect of pesticide residues, dissipation studies are important to analyze the appropriateness of pesticide treatment strategies (Lu et al., 2014). There are earlier studies on vegetables and fruits treated by

imidacloprid and cypermethrin done in many countries of the world, results on the dissipation pattern and residual amount of the pesticides had been published in many journals.

The bar diagram (**Fig 40** and **41**) is obtained by plotting theinitial amount of these two pesticides against the vegetable samples. As we know that the MRL value of Cypermethrin in tomato, 0.2 mg kg^{-1} (Codex 2013), in cabbage, cauliflower and cucumber, 1.0 mg kg^{-1} (Codex 2013), in green chili, 0.5 mg kg^{-1} (Codex 2013) and in sweet gourd and bottle gourd, 1.0 mg kg^{-1} (Codex 2000) and MRL value of imidacloprid in tomato, 0.5 mg kg^{-1}, in cabbage and cauliflower, 0.5 mg kg^{-1} , in cucumber, 1.0 mg kg^{-1}, in green chili, 0.5 mg kg^{-1} and in sweet gourd and bottle gourd, 1.0 mg kg^{-1} (according to EPA, 2000). From the diagram, it was observed that the residual amounts of the pesticides on the first day in all the vegetables are slightly higher than the MRL values. Therefore, it is suggested not to eat these vegetables on the first day.

The initial amounts of imidacloprid in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber were 1.13 ± 0.15 , 1.44 ± 0.08 , 1.62 ± 0.06 , 1.65 ± 0.10 , 1.58 ± 0.09 , 1.98 ± 0.03 , 1.02 ± 0.10 and 2.05 ± 0.02 µg g^{-1}, respectively (**Table 36- 43**). The initial value of imidacloprid went below MRL after 1^{st} day of application for tomato, green chili and after 3^{rd} day of application for cabbage, cauliflower, sweet gourd, bottle gourd and cucumber, after 5^{th} day of application for sponce gourd.

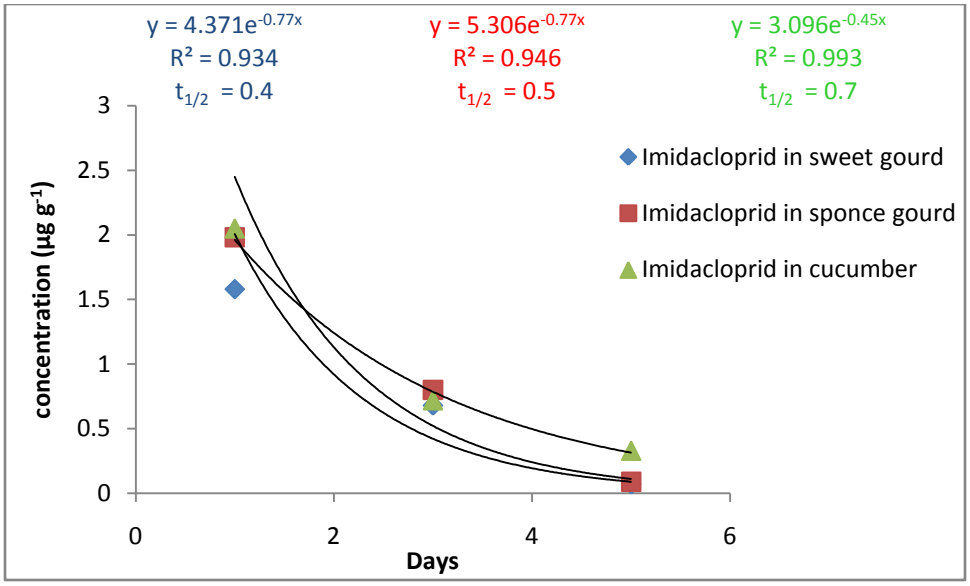


Fig 42: Dissipation curve of imidacloprid in sweet gourd, sponce gourd and cucumber as a constituent of Double 50 EC

The maximum residue limit was exceeded by 58, 97, 96, 98, 98, 99, 59 and 83% dissipation of the initial concentration of tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber, respectively (**Table 44**). The amount of imidacloprid went below detetion limit after 8th day of application for all the vegetables.

T. Banerjee et al.,(2013) studied on the persistence of Imidacloprid and Beta-Cyfluthrin in vegetables. It analyzed the persistence of imidacloprid and beta-cyfluthrin, when applied as a mix formulation, Solomon 300 OD @ 200 and 400 mL ha(-1) in brinjal, tomato and okra. The half life values of imidacloprid in the vegetables varied from 0.98 and 1.30 days. It was also suggested that suggesting that the persistence of beta-cyfluthrin is lower than that of imidacloprid in fruits of these vegetables. Similar result is observed in current study (**Fig 43**), here, it was analyzed that the persisitance of cypermethrin in the vegetable samples is lower than that of imidacloprid and both of beta- cyfluthrin and cypermethrin are in the group of pyrethroids.

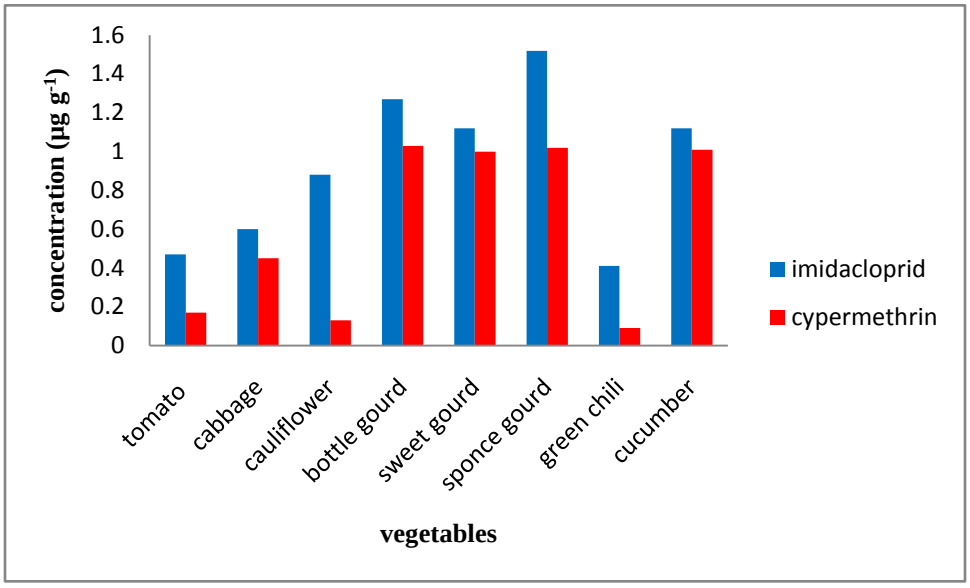


Fig 43: Comparison between the concentrations of imidacloprid and cypermethrin on 3rd day after application

Pandit Goutam K. (2016) studied on dissipation of imidacloprid residues in okra leaves, fruits and soil in northern region of West Bengal. He reported on the residual analysis on okra leaf, fruit and in field soil after application of imidacloprid. The halflife ($t_{1/2}$) ranged from 0.66 to 1.28 days in leaf and from 0.76 to 1.07 days in fruit. It was observed that imidacloprid is a safe insecticide to use in vegetables. The residual level went below MRL (0.5 mg kg^{-1} for okra) value within 7 days at recommended doses. The residue went below the detection limit after 1day after application.

The amount of cypermethrin on the 1st day of application in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber were 0.65 ± 0.09 , 1.61 ± 0.08 , 1.05 ± 0.08 , 1.17 ± 0.15 , 1.25 ± 0.06 , 1.60 ± 0.05 , 0.52 ± 0.03 and 1.87 ± 0.04 µg g^{-1},respectively (**Table 36-43**). The amount went below the MRL values after one day of application for tomato, cauliflower, green chili and cucumber with 73, 34, 82 and 47% dissipation from the initial value and after 3rd day

of application for cabbage, bottle gourd, sweet gourd and sponce gourd with dissipation percentage of 96, 96, 80 and 82% from initial concentration (**Table 44**).

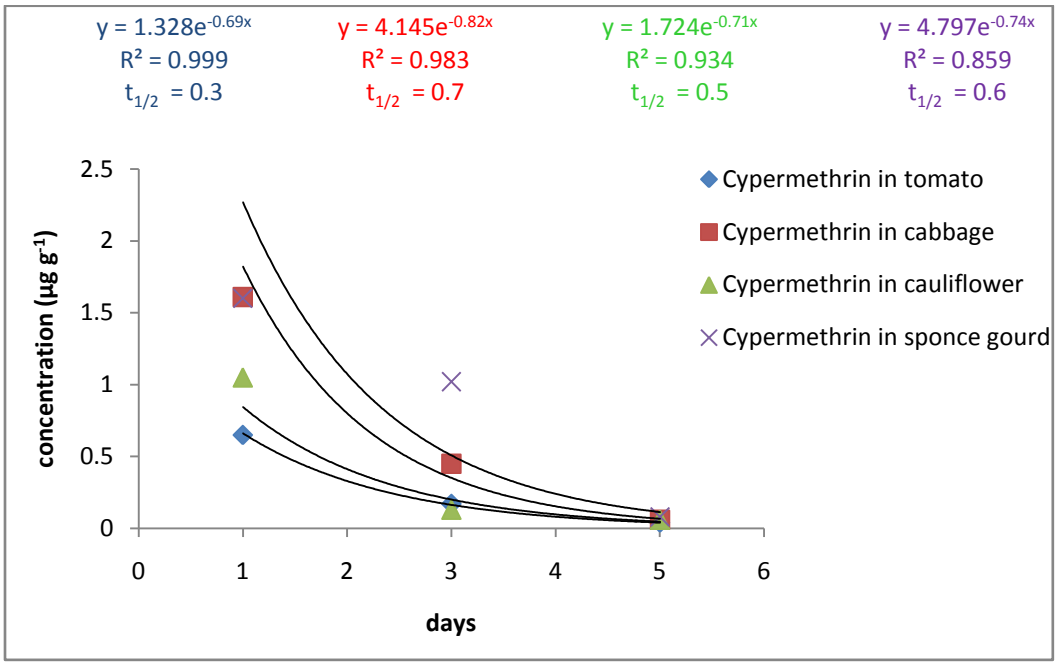


Fig 43: Dissipation curve of cypermethrin in tomato, cabbage, cauliflower and sponce gourd as a contituent of Double 50 EC

The residue of cypermethrin dissipated upto 100% on 5th day of application for tomato, cabbage, cauliflower, bottle gourd and cucumber and for chili, on 3rd day of application. Samples of 8th day of sweet gourd and sponce gourd could not be analyzed as these were rotten on that day. Therefore, it is suggested that the safe period for consuming cypermethrin treated tomato, cauliflower, green chili and cucumber is atleast after 1 day of application and for cabbage, bottle gourd, sweet gourd and sponce gourd, it is after 3 days of application.

Gupta et al., (2011), reported about the dissipation of cypermethrin in mix formulation with profenofos and chlorpyrifos, respectively, where it was observed that the residue persisited in fruits upto 7 days of application and half life of cypermethrin was about

0.8–1.6 days and 0.50–1.8 days of application in the formulation with profenofos and chlopyrifos, respectively.

Gagan Jyot (2013) reported on Estimation of chlorpyriphos and cypermethrin residues in chilli (*Capsicum annuum* L.) by gas–liquid chromatography. He suggested a waiting period of 1 day is suggested to reduce the risk before consumption of green chili.

These reports show much similarities with the result of the current study. Therefore, it can be said that the use of cypermethrin mixture at the recommended dosage does not seem to pose any hazards to the consumers if the safe period of consumption is maintained.

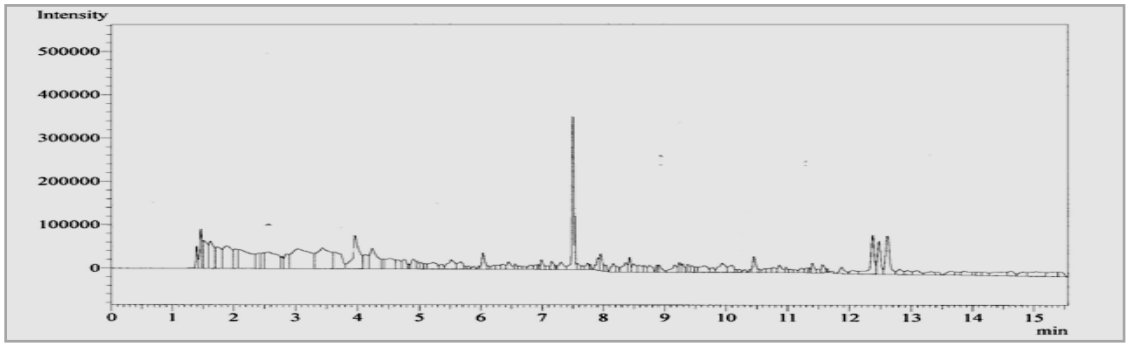


Fig 46: GC–ECD chromatograph of cabbage sample treated with Double 50 EC (1st day of application)

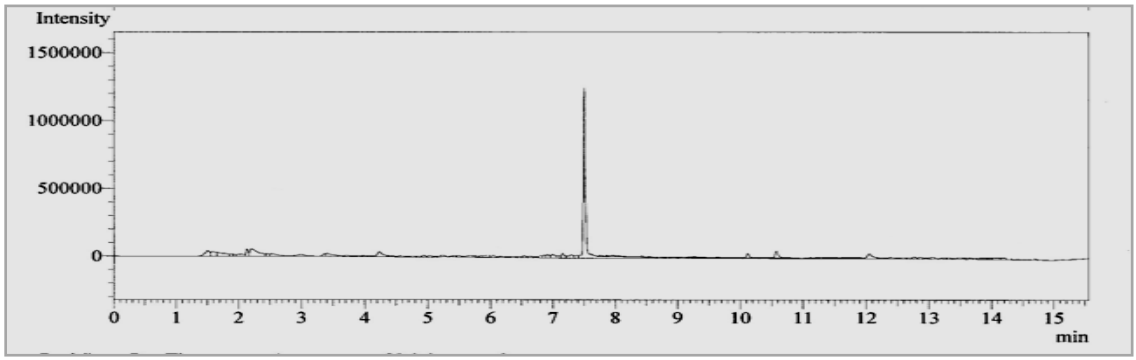


Fig 47: Chromatogram of certified standard of imidacloprid (0.125 µg g^{-1}) in Double 50 EC

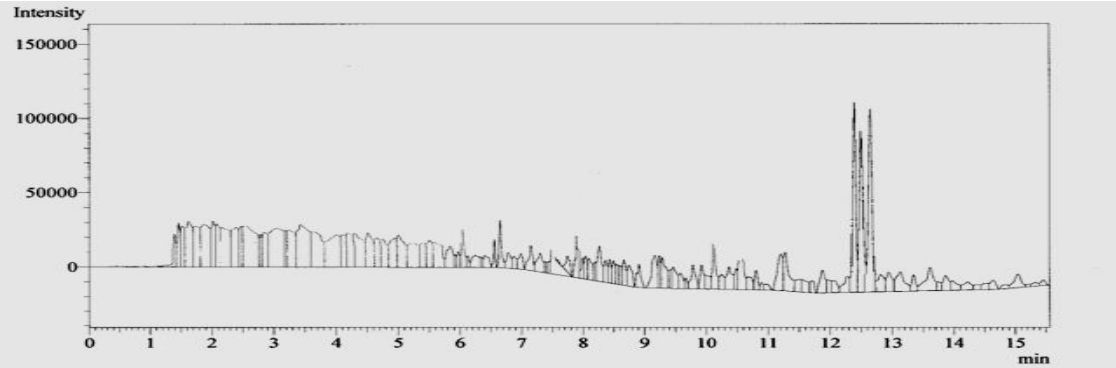


Fig 48: Chromatogram of certified standard of cypermetrin (0.612 µg g^{-1}) in Double 50 EC

3.3.5 Dissipation of Reeva 2.5 EC (lambda-cyhalothrin) in vegetables

Reeva 2.5 EC is the trade name for the commercial pesticide Lambda-cyhalothrin. It has been used widely to control sucking and chewing insects in field crops, beside its wide use in public health (Davey et al., 1992; Roberts et al., 1993; Dikshit et al., 2000; Mathirajan et al., 2000). It was sprayed on the eight kind of vegetables in the farmer's field, Nuritola, Comilla, Bangladesh at a dose of 1 mL per Liter of water/ ha and collected on 9th January, 2016. After proper sampling, extraction and clean up process, Reeva2.5 EC treated vegetables were analyzed by GC-ECD.

3.3.5.2 Calibration curve of certifiedlambda-cyhalothrin standards

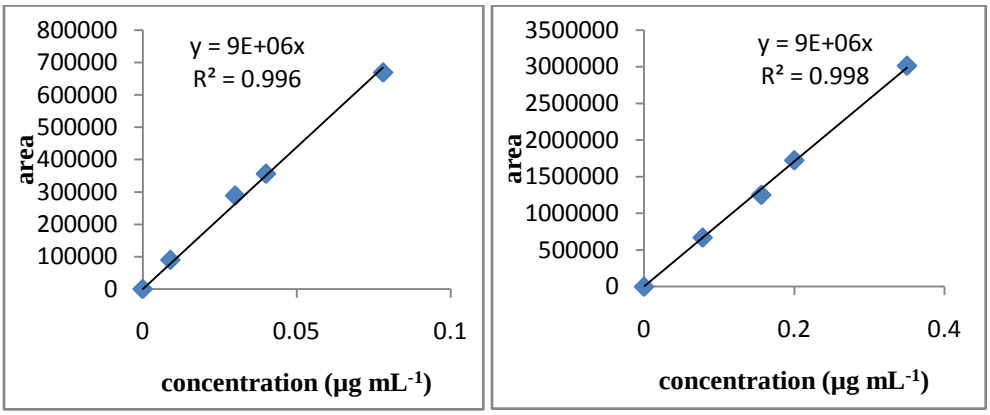
The linearity was evaluated by calibration curve plotted with concentration of standard lambda-cyhalothrin solution against area at those respective concentrations. It was obtained by plotting low to high concentrations vs. area, from 0.009 to 0.35 µg mL^{-1}(**Table 45**). Lower and higher concentration calibration curves showed the linear coefficient (r^2) 0.998 and 0.996 with linearity range of about 0.04– 0.08 µg mL^{-1} and 0.08– 0.30 µg mL^{-1}, respectively (**Table 45**).

3.3.5.3 Limit of detection and Limit of quantification

The LOD and the LOQ were found to be 0.009 µg mL^{-1} and 0.027 µg mL^{-1}, respectively.

Table45: Linearity range, correlation coefficients (r^2), LOD and LOQ

Pesticide	Calibration curve	Linearity range (µg g^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Lambda-cyhalothrin	higher	0.08 -0.30	0.998	0.009	0.027
	lower	0.04 – 0.08	0.996		



(a) **(b)**

Fig49 : Calibration curve of lower and higher concentration (a and b) of lambda-cyhalothrin standards

3.3.5.4 Recovery Experiments

Recovery percentages of lambda-cyhalothrin in spiked vegetable samples were done in two different concentration levels with three replications each. The recovery percentages were 78- 90% for tomato, 95%- 98% for cauliflower, 80-85% for cabbage, 74- 85% for bottle gourd, 86- 88% for sweet gourd, 79- 80% for sponce gourd, 80-82% for green chili and 79- 84% for cucumber with standard deviations less than and

equal to 3.0 The relative standard deviation (RSD) values ranged from 0.60 to 3.5 (**Table 3.40**).

Table 46: Recovery percentages of lambda- cyhalothrinin eight spiked vegetable samples

Vegetable	Spiking level ($\mu g\ mL^{-1}$)	Mean Recovery % ± SD	%RSD
Tomato	L 0.03	78 ± 1.2	1.5
	H 0.20	90 ± 1.2	1.3
Cauliflower	L 0.03	95 ± 0.5	0.4
	H 0.50	98 ± 1.0	1.3
Cabbage	L 0.03	85 ± 2.5	2.6
	H 0.50	80± 0.5	0.6
Bottle gourd	L 0.03	85± 3.0	3.5
	H 0.50	74± 2.5	3.4
Sweet gourd	L 0.03	86± 1.0	1.2
	H 0.50	88± 2.5	2.8
Sponce gourd	L 0.03	79± 1.0	1.1
	H 0.50	80± 1.5	0.5
Green chilli	L 0.03	82 ± 2.5	3.1
	H 0.20	80 ± 2.0	2.5
Cucumber	L 0.03	79 ± 1.0	1.3
	H 0.20	84 ± 1.5	1.8

L= Lower, H= Higher

3.3.5.5 Residual amount of lambda-cyhalothrin in vegetables

The vegetable samples were kept at ambient temperature and samples of 8^{th} and 10^{th} day were not analysed as all of them perished on those days. The residual amount of lambda-cyhalothrin was analyzed by GC-ECD.

Table 47:Amount of lambda-cyhalothrin (Av. ± SD, µg g^{-1}) in different vegetables

DAS	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chili	Cucumber
Control	bdl							
1	0.19±0.09	0.26±0.01	0.22 ± 0.10	0.24 ± 0.02	0.21± 0.03	0.23 ± 0.02	0.12 ± 0.05	0.16 ± 0.02
3	0.04±0.03	0.07±0.02	0.06 ± 0.05	0.05 ± 0.03	0.05 ± 0.05	0.06± 0.03	bdl	bdl
5	ND	bdl	bdl	bdl	ND	bdl	ND	ND
8	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND

bdl= below detection limit

ND=Not Detected

Table 48: Dissipation percentage of lambda-cyhalothrin

Days of spraying	Dissipation of lambda-cyhalothrin (%)							
	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chilli	Cucumber
3	78	73	70	73	76	78	100	100
5	100	100	100	100	100	100	-	-

3.3.5.6 Discussion for lambda-cyhalothrin

Recoveries of lambda-cyhalothrin at three different spiking levels validated the process adopted for extraction and analysis. The recoveries achieved were in between the range of about 74 - 98%, which was in an acceptable range (according to Codex, 2010) for extraction and analysis of lambda-cyhalothrin residues in the samples. The value obtained confirmed that the extractiondone with ethyl acetate and clean up with PSA coupled with the GC-ECD analysis is suitable for the residual determination of lambda-cyhalothrin in the vegetables. The relative standard deviation (RSD) values ranged from 0.60 to 3.5 (**Table 46**).

Good linearity was achieved for lambda-cyhalothrinfor over eight concentration levels ranging from 0.009 to 0.35 µg mL^{-1}, with excellent linear coefficient of 0.996 and 0.998 for lower and higher concentration calibration curves, respectively (**Table 45**).

Precision of the method was analyzed by relative standard deviation ($RSD < 20$ %) in the observed area. It can be said that lambda-cyhalothrin can be detected with good precision which confirmed the extraction procedure adopted good recoveries.

Specificity and selectivity of the method was achieved by obtaining same retention time for standard as well as spiked samples. The interferences were examined by comparing the chromatograms of standard and blank. Results showed no interfering endogenous peak. The limit of detection and quantification for lambda-cyhalothrin were 0.009 and 0.027 µg mL^{-1}, respectively.The obtained results indicated that the method was accurate and reproducible.

3.3.5.7 Safe period of consumption of lambda-cyhalothrin

A sharp decrease in residual amount of lambda-cyhalothrin in three days after application was observed. In the current study, results (**Table 48**) revealed that the residues had declined by a range of about 72 - 100% just after 1 day of application. There are certain factors which influence the persistence of pesticides in crops, including the pesticide character(stability either as parent molecule or metabolites, its volatility, solubility, formulation), and the method and citation (Aydinalp&Porca, 2004; Voutsas et al., 2005; Brady et al., 2006; Isenring&Madeley,2006; Cabras et al., 1989; Malhat et al., 2013). Additionally, dilution factor might have played important role (Cabras et al., 1990; Malhat et al., 2014). Most importantly to the treated surface and its weight (Khay et al.,2008; Cabras et al.,1990).

The maximum residue limit, MRL values of lambda-cyhalothrin for tomato, cabbage, cauliflower, green chili and cucumber are 0.05 µg $g^{-1}$and for sweet gourd and bottle gourd, it is 0.10 µg g^{-1} (according to FAO, 2010).On the 1st day of application, the initial value of lambda-cyhalothrin in tomato, cabbage, cauliflower, bottle gourd,

sweet gourd, sponce gourd, green chili and cucumber were0.19 ± 0.09 , 0.26 ± 0.01 , 0.22 ± 0.10 , 0.24 ± 0.02 , 0.21± 0.03 , 0.23 ± 0.02 , 0.12 ± 0.05 and 0.16 ± 0.02 µg g^{-1} (**Table 3.41**). After 1st day of application, the initial concentration declined very sharply and went under the MRL value for each vegetables. From the bar diagram (**Fig 50**) showed the results where the concentrations of lambda-cyhalothrin were slightly higher than the detection limit for each of the vegetables but the concentrations were below than the MRL values.

Farag Malhat (2016) studied on Dissipation pattern and risk assessment of the synthetic pyrethroid Lambda-cyhalothrin applied on tomatoes under dryland conditions, a case study. He reported about the rapid dissipation about the residual concentration of lambda-cyhalothrin.

Chuanshan Yu, (2015) reported on Representative commodity for five brassica vegetables based on the determination and dissipation of six pesticide residues where lambda-cyhalothrin was determined according to QuEChERS method and analyzed by GC-ECD. The results showed much similarity with the current study.

Kuariet al., (2003) studied onpesticides on 80 vegetable samples (cabbage, cauliflower, pea grains, brinjal, tomato, potato, and green chilly) from Hisar, Haryana, India during 1997-1998**.** Residue levels oforganophosphorouswere highest followed by carbamates, synntheticpyrethroids and organochlorines. 32% samples showed residue level of the pesticides above the corresponding MRL values.

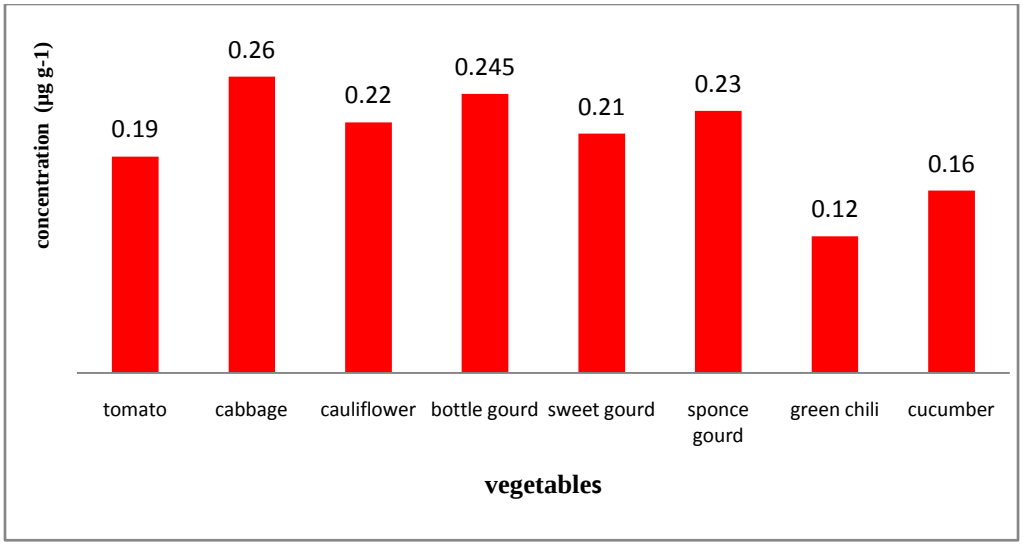


Fig 50: Residual amount of lambda-cyhalothrin on 1st day of application in eight vegetables

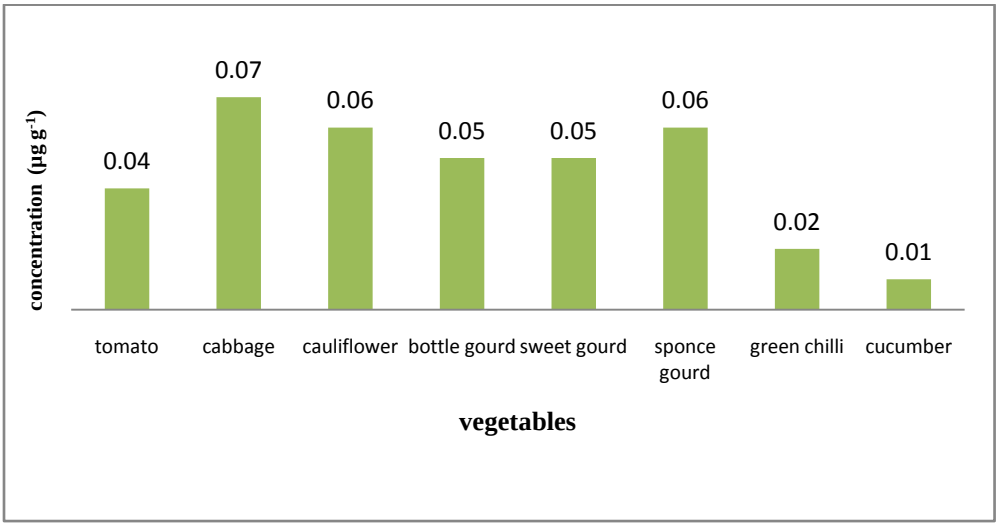


Fig 51: Residual amount of lambda-cyhalothrin on 3rd day of application in eight vegetables

Food safety is an area of growing concern worldwide. The presence of hazardous pesticide residues in food has beencausing a great concern among the consumers. Therefore, the samples were kept in ambient temperature to find out the persistence and dissipation pattern of the pesticides when vegetables are already harvested just

after one hour of application. The result will help the consumers to intake these vegetables when the pesticide residues decline below MRL and reach to the safe period of consumption. For lambda-cyhalothrin, it is safe to consume these vegetables atleast after 1 day of spraying.

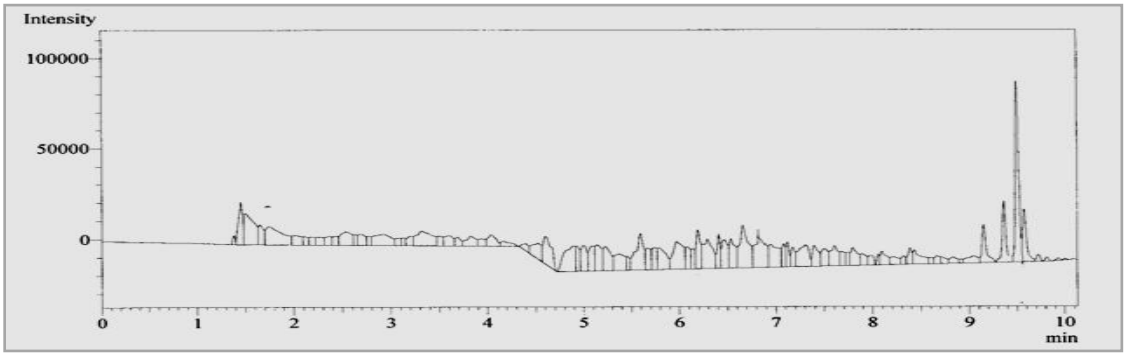


Fig 52: GC-ECD chromatogram of lambda-cyhalothrin treated cauliflower sample (1st day application)

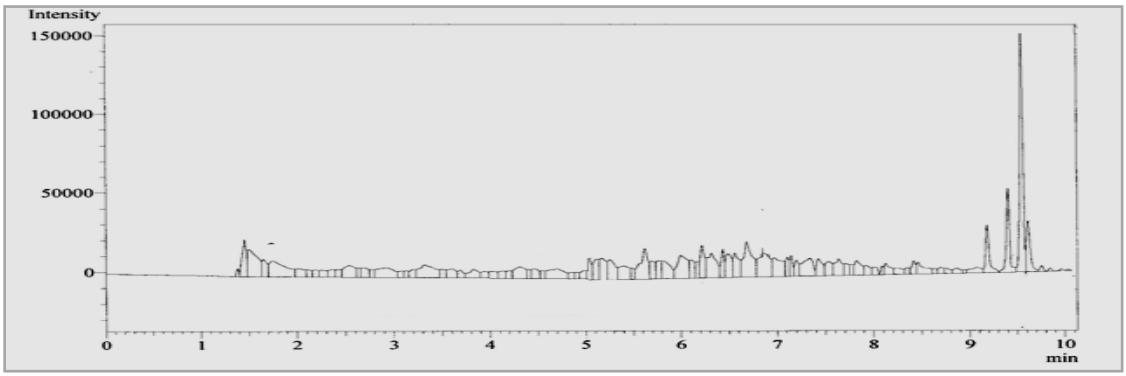


Fig 53: GC-ECD chromatogram of certified standard of lambda-cyhalothrin (0.625 µg g^{-1})

3.3.6 Dissipation of Asataf 75 SP treated vegetables

The active component of asataf 75 SP is acephate.In order to definitude environmental safety and establish safe application, residue dynamics of acephate was studied byQuEChERS method and analyzed by gas chromatography.Asataf 75 SP was sprayed

at a dose of 1 gm per Liter of water/ ha on 9th January, 2016. The residue of was analyzed by GC-ECD (Shimadzu, 2010). The retention time was at 6.9 minutes.

3.3.6.2 Calibration curve of certified acephate standards

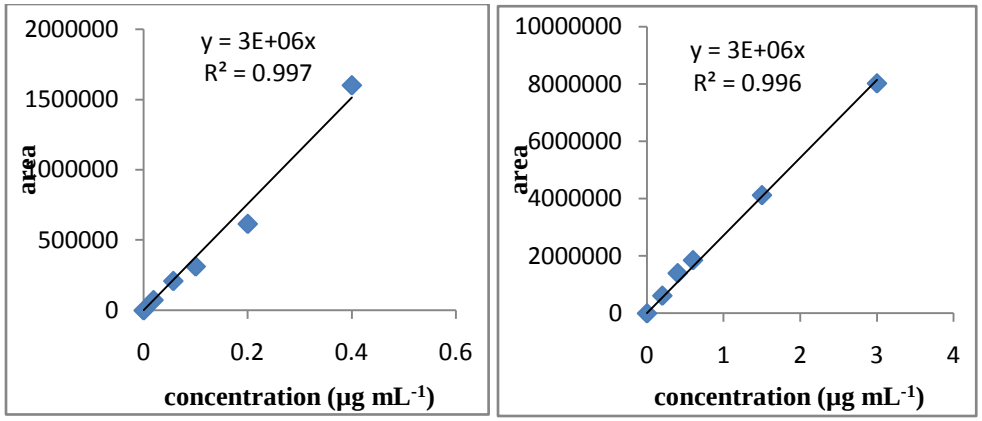
The linearity was evaluated by calibration curve plotted with concentration of standard acephate solution against area at those respective concentrations. It was obtained by plotting low to high concentrations vs. area, from 0.019 to 5.0 µg mL^{-1}. Lower and higher concentration calibration curves showed the linear coefficient (r^2) 0.997 and 0.996 with linearity range of about 0.10– 0.35 µg mL^{-1} and 0.80– 3.0 µg mL^{-1}, respectively.

3.3.6.3 Limit of detection and Limit of quantification

The LOD and the LOQ were found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1}, respectively (**Table 49**).

Table 49: Linearity range, correlation coefficients (r^2), LOD and LOQ

Pesticide	Calibration curve	Linearity range (µg mL^{-1})	Correlation coefficient(r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Acephate	higher	0.80 -3.0	0.996	0.019	0.057
	lower	0.10 – 0.35	0.997		



(a) **(b)**

Fig 54: Calibration curve of lower and higher concentration (a and b) of acephate standards

3.3.6.4 Recovery Experiments

Recovery percentages of acephaet in spiked vegetable samples were done in two different concentration levels with three replications each. The recovery percentages were in between the range of 75 - 105% with standard deviations less than and equal to 3.0. The relative standard deviation (RSD) values ranged from 0.5 to 3.6 (**Table 50**).

3.3.6.5 Residual amount of acephate in vegetables

Vegetable is a perishable item and as the samples were kept at ambient temperature, therefore, samples of 8th and 10th day could not be detected as they were rotten. The residues of acephate were detected by GC-ECD, which are shown in **Table 51**.

Table 50: Recovery percentages of acephate in eight spiked vegetable samples

Vegetable	Spiking level ($\mu g\ mL^{-1}$)	Mean Recovery % ± SD	%RSD
Cauliflower	L 0.05	105 ± 1.5	1.9
	H 2.00	95 ± 2.5	2.8
Cabbage	L 0.05	83 ± 1.2	1.4
	H 2.00	96 ± 0.5	0.5
Bottle gourd	L 0.05	80 ± 1.5	1.8
	H 2.00	75 ± 1.0	1.3
Sweet gourd	L 0.05	88 ± 1.0	1.1
	H 2.00	78 ± 2.3	2.9
Sponce gourd	L 0.05	80 ± 1.5	1.4
	H 2.00	76 ± 1.0	1.1
Green chilli	L 0.05	86 ± 2.8	3.2
	H 1.00	95 ± 3.5	3.6
Cucumber	L 0.05	77 ± 2.5	3.2
	H 1.00	80 ± 1.0	1.2
Tomato	L 0.05	99 ± 1.5	1.6
	H 1.00	94 ± 2.5	2.5

L= Lower, H= Higher

Table 51:Amount of Acephate (Av. ± SD, $\mu g\ g^{-1}$) in different vegetables

DAS	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chili	Cucumber
Control	bdl							
1	1.85±0.03	2.75 ± 0.04	2.61 ± 0.06	2.03±0.03	2.1 ± 0.08	2.44±0.03	1.69±0.01	1.71 ± 0.05
3	0.6 ± 0.01	2.12 ± 0.05	2.01 ± 0.05	1.27±0.02	1.28±0.05	2.0± 0.07	0.88±0.05	0.96 ± 0.07
5	0.07±0.02	0.31 ± 0.02	0.14 ± 0.05	0.32±0.02	0.08±0.03	1.17±0.01	0.06±0.03	0.15 ± 0.02
8	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND

bdl= below detection limit

ND= Not Detected

Table52: Dissipation percentage of Acephate

Days of spraying	Dissipation percentage of Acephate(%)							
	Tomato	Cabbage	Cauliflower	Bottle gourd	Sweet gourd	Sponce gourd	Green chilli	Cucumber
3	64	26	23	37	40	28	47	43
5	100	89	95	85	ND	54	ND	92
8	ND	ND	ND	ND	ND	ND	ND	ND

ND= Not Detected

3.3.6.6 Discussion on acephate

Recovery experiments (**Table 50**) were done at a lower and higher spiking level of 0.05 and 1.0 µg mL^{-1} for tomato, cucumber and green chili; again 0.05 and 2.0 µg mL^{-1} for cauliflower, cabbage, bottle gourd, sweet gourd and sponce gourd. The average recoveries of acephate ranged from 75 to 105% of the spiked control samples, with relative standard deviation less than and equal to 3, which is in between the range of 70% - 120%, acceptable range for a acceptable recovery experiment, reported by Codex.

Two individual calibration curves were made to cover different range of concentrations of acephatein the vegetables from 1st day to 5th day. Lower and higher concentration calibration curves showed the linear coefficient (r^2) 0.997 and 0.996 with linearity range of about 0.10– 0.35 and 0.80– 3.0 µg mL^{-1}.

Sensitivity of instrument was evaluated by determining LOD and LOQ. The LOD and the LOQ were found to be 0.019 and 0.057 µg mL^{-1}, respectively.

No substrate interferences were observed as evidenced by the control sample analysis. These data indicates that the extraction method is satisfactory for the requirement of acephate residue analysis.

3.3.6.7 Safe period of consumption of acephate

According to Codex alimentaris commission , April 2000, the MRL value of acephate in tomato, cucumber and green chili is 1 mg kg^{-1} and cabbage, cauliflower and sweet

gourd is 2 mg kg^{-1}.The initial value of acephate in all the vegetables were slightly higher than the MRL value on 1st day which is shown by bar diagram, in **Fig. 55.**

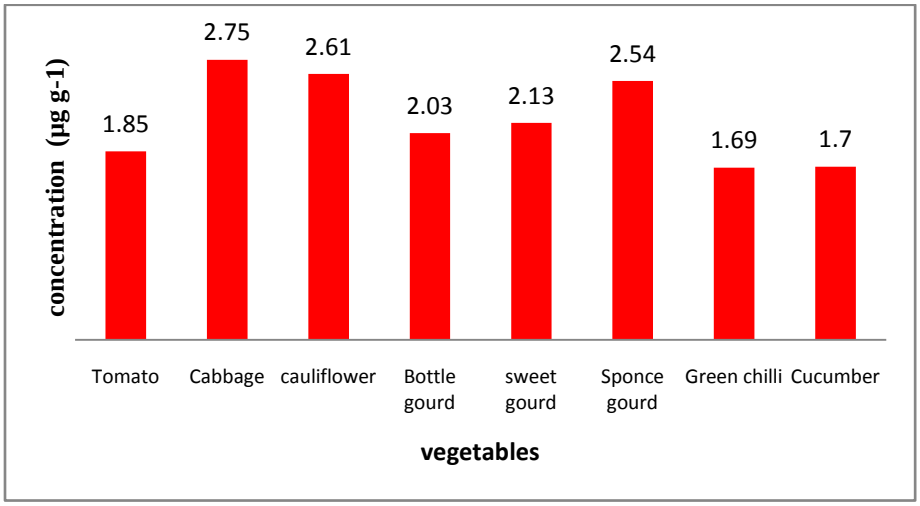


Fig 55: Residual amount of acephate on 1st day in eight vegetables

The initial amountsof acephatewere declined (**Table 52**) by 64, 26, 23, 37, 40, 18, 47, 47 and 43% to the amount of 3rd day, in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber, respectively; and the results showed the value 0.65 ± 0.01 , 2.12 ± 0.05 , 2.01 ± 0.05 , 1.27 ± 0.02 , 1.28 ± 0.05 , 2.01± 0.07 , 0.88 ± 0.05 and 0.96 ± 0.07 µg g^{-1} (Table 3.44). In **Fig 56**, it is shown that all the samples had residual amount of acephate less than their MRL value except cabbage and cauliflower. That means, acephate have more persistency in vegetables like; cabbage and cauliflower (brassica vegetables).In case of brassica vegetables, the pesticide reaches to the target point properly. They grow in upper direction and its surface is large and non-homogeneous. So, the pesticide has much space to accommodate.

Rajinder Kumar, analyzed on bioefficacy and persistence of acephate in mungbean*Vignaradiata* (L.). Wilczek, (2016) where he reported that the half life of acephate in mungbean is about 2.98 days and in soil its about 1.86 days of application.

He also reported that the persisitency of acephate is higher in greenhouse plants which indicate about the effect of heat light, wind and heat.

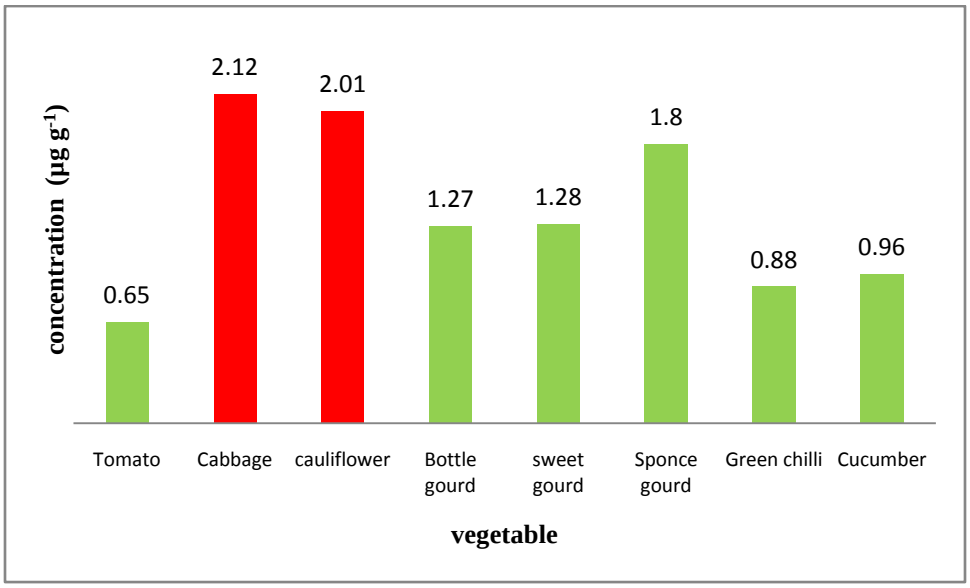


Fig 56: Residual amount of acephateon 3rd day in eight vegetables

Acephate, applied on open field vegetables, persist for short time when come in contact with air, wind and heat, reported by HONG Wen-ying an earlier study, Residue Dynamics of Acephate and Its Metabolite Methamidophos in Pakchoi. The rapid dissipation of originally applied pesticide is dependent on a variety of environmental factors such as sunlight and temperature (Lichtenstein, 1972). However, high temperature is reported to the major factor in reducing the pesticides from plant surfaces (Awad et al., 1967). Light plays an important role in the behaviour of pesticide in the environment (Zepp and Cline, 1977).

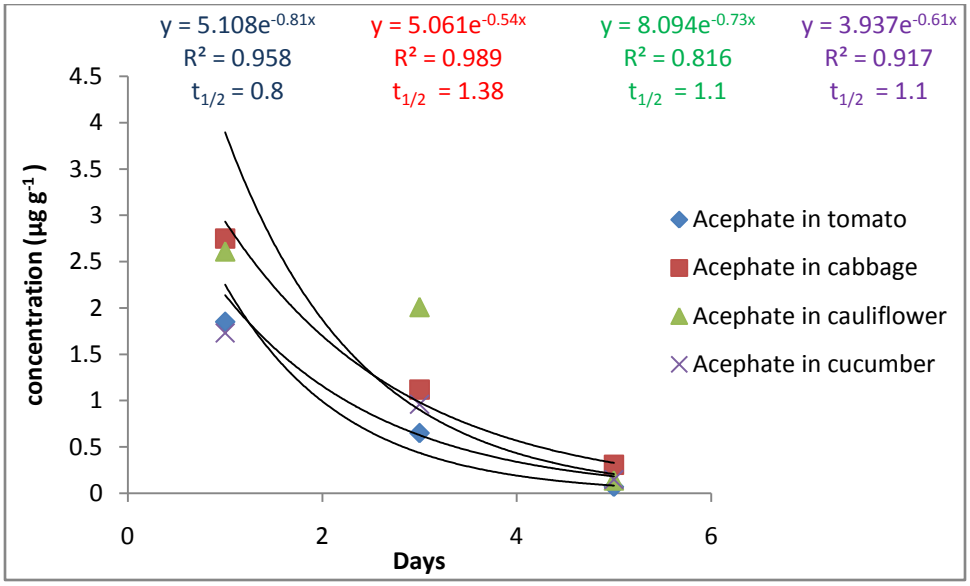


Fig 57: Dissipation curve of acephate in tomato, cabbage, cauliflower and bottle gourd in Asataf 75 SP

The half-lives value of acephate were 0.80, 1.38, 1.10, 0.94, 0.88, 0.84, 0.75 and 1.1 days for tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber, respectively (**Fig 57**). Chaiet al., (2009) studied on dissipation of acephate, chlorpyrifos and cypermethrinand their metabolites in a humid-tropical vegetable production system, according to his analysis, average half life of acephate during dissipation is 1.1 days, which is much similar to that of value of current study. From the current study, it can be suggested that the safe level of consuming acephate treated tomato, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber is atleastafter 1 day of application and for cabbage and cauliflower, safe period of consuming is after 3 days of application.

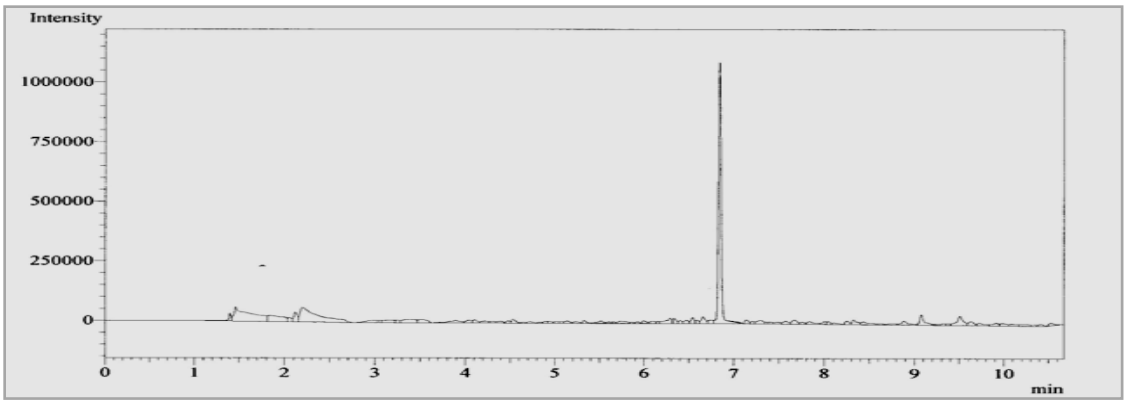


Fig 58: GC-ECD chromatogram of acephate treated cabbage sample (1st day of application)

3.3.8 Storage stability of the five pesticides

To analyze the storage stability of the pesticides at freezing condition, control vegetable samples were fortifiedwith higher concentrations of the five pesticides with two replicates each and the samples were then stored in a freezer. The vegetable samples fortified with chlorpyrifos, cypermethrin and imidacloprid, were freezed on 12th January, 2016 and kept at freezer for about 35 days. Control vegetable samples spiked with lambda-cyhalothrin and acephate were freezed for about 39 days, from 18th February to 27th March, 2016. Then these spiked samples were extracted, cleaned-up and analyzed following the same procedure to find out the stability of the pesticidal residues at freezing condition.

Table 53: Recovery percentages for storage stability test

Samples	Pesticide	Recovery% ± SD	% RSD
Tomato	Chlorpyrifos	92 ± 1.5	1.6
	Cypermethrin	88 ± 3.5	3.9
	Imidacloprid	90 ± 3.8	4.2
	Lambda-cyhalothrin	85 ± 1.5	1.7
	Acephate	87 ± 2.5	2.9
Cabbage	Chlorpyrifos	86 ± 2.0	2.3
	Cypermethrin	88 ± 2.5	2.8
	Imidacloprid	95 ± 1.5	1.8
	Lambda-cyhalothrin	86 ± 3.5	4.1
	Acephate	90 ± 1.0	1.1
Green chili	Chlorpyrifos	85 ± 3.0	3.5
	Cypermethrin	88 ± 2.8	3.1
	Imidacloprid	90 ± 3.0	3.3
	Lambda-cyhalothrin	84 ± 1.8	2.1
	Acephate	92 ± 2.5	2.6
Cucumber	Chlorpyrifos	90 ± 1.2	1.3
	Cypermethrin	87 ± 2.8	3.2
	Imidacloprid	93 ± 3.6	3.8
	Lambda-cyhalothrin	88 ± 3.0	3.4
	Acephate	85 ± 2.5	2.9

The average recoveries of the pesticides were found (**Table 53**) in the range of 84 - 93% with RSD value ranging from 1.1 to 4.2 which indicated that pesticidal residues were quite stable at -20 °C storage condition because the recovery results were consistent with the range listed in the Codex guidelines (2010). The storage stability results showed that the pesticides were quiet persistent at freezing condition and not degraded during the period of this test.

SUMMARY

Analysis of Imidacloprid in Rice and Wheat Flour and its Toxicity Test against Rice Weevils

In many countries of the world, rice and wheat are considered as the main food and consume them regularly. But their production is also prone to losses due to insect pests. Imidacloprid is routinely used to protect rice from insect pest attack. Imidacloprid acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system. Following binding to the nicotinic receptor, nerve impulses are spontaneously discharged at first, followed by failure of the neuron to propagate any signal. Sustained activation of the receptor results from the inability of acetyl cholinesterases to break down the pesticide. The application of any insecticide to food crop draws concerns over food safety and requires a careful monitoring of the residue. In parts of the world, where imidacloprid is usedwithout due regards to dosing and operators are not adequately trained, there is a likelihood that the residue of imidacloprid may exceed the threshold limit. The study of determination of the residue of imidacloprid in rice and wheat flour is therefore important from food safety point of view. The current study was carried out for developing a method for quantitative analysis of imidacloprid in boiled and parboiled rice samples and in wheat flour using HPLC and also to find out the toxicity level of imidacloprid in rice.

The residues of imidacloprid were detected and quantified by HPLC while maintaining the column temperature at 30 °C. The sample extracts were injected manually by using a 20 μL sample loop and injection volume was 60 μL. Mobile phase was an isocratic elution of acetonitrile: water, (70:30). The flow rate was set at 0.5 mL min^{-1} using a UV wavelength of 280 nm (wavelength). Retention time (t_R) of imidacloprid under these conditions was 6.1 mins. The calibration curve of certified imidacloprid standards showed peak area with a very good linear correlation (r^2= 0.996) when compared with absolute values at different concentrations. The LOD and LOQ were found to be 0.009 and 0.027 µg mL^{-1}. Extraction was carried out using acetone-methanol mixture(1:1) solvent and partitioning was done using DCM. Clean-up was performed using a silica gel column. The cleaned –up extract was analyzes by LC-PDA.

Imidacloprid residues in rice samples

In total, thirty samples were purchased to analyze the experiment. Among them, fifteen samples were unpacked, bought from various open markets in Dhaka and fifteen were packed and branded rice samples. In these samples both fragrant, such as kaligira, chinirura and nonfragrant, such as minikate, najirshail were present. All the rice samples were stored in a freezer at -20 $^{\circ}$C until analysis.

The recovery experiments of imidacloprid residue in spiked rice samples were carried out in five different levels with five replications; 0.625, 1.25, 2.5, 5 and 10 µg mL^{-1} and the average recoveries were 72.6, 79.3, 80.3, 81.0 and 84.2 % with RSD values of 2.85, 3.14, 3.13, 3.7 and 6.22, respectively which were in acceptable range (Codex Alimentarius, 2010). A widely accepted criterion for the acceptability of performance of an analytical method is its capability of providing average recovery within the range of 70 -120%.

Among 30 rice samples, 9 (fragrant) packed samples were found to contain imidacloprid residues in the range 1.59 – 4.51 µg g^{-1} which was greater than the MRL value (1.5 µg g^{-1} in rice, EPA 2010) and 9 (fragrant) unpacked samples were found to contain imidacloprid residues in the range 0.06 – 1.10 µg g^{-1} which was lower than the MRL value. The imidacloprid residue in non-fragrant rice samples were found below detection limit. In boiled rice samples, no trace of imidacloprid was observed. Among 15 wheat flour samples, imidacloprid residues were observed only in 8 samples, ranging from 0.03 – 0.44 µg g^{-1} which were lower than the MRL value (1.5 µg g^{-1} in wheat, EPA 2010). The most interesting part of the analysis was that there were no traces of imidacloprid in rice samples when the sample was boiled. As we consume rice after cooking, so it is safe for us to intake it without the risk of health hazard.

Imidacloprid residues in wheat flour samples

A total of fifteen wheat flour samples were purchased to analyze the experiment. Among them, one is control, ten were branded packed samples and five were unpacked samples brought from various markets in Dhaka. All the wheat samples were stored in a freezer at -20°C until analysis.

Recovery experiments for wheat samples were carried out in three levels with five replications; 1.25, 2.5 and 5 µg g^{-1} and the recoveries were in the range of 83 - 90% with RSD value of 3.06, 2.53 and 3.11, respectively

Among the 15 wheat flour samples, only few showed traces of imidacloprid above the detection limit and all the samples showed small amount of imidacloprid in the samples which were below the MRL value (according to European Food Safety Authority, 2000; MRL value for wheat is 1.5 µg g^{-1}), ranged from 0.03 – 0.44 µg g^{-1}.

Toxicity test of imidacloprid

Imidacloprid is rated as "moderately toxic" on an acute oral basis to mammals and low toxicity on a dermal basis by the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA). Relative to human health, exposure of imidacloprid to high doses may be associated with degenerative changes in the testes, thymus, bone marrow and pancreas. The primary effects of longer term, lower-dose exposure to imidacloprid are on the liver, thyroid, and body weight (reduction). Low to miId dose oral exposures have been associated with reproductive toxicity. The current study presents an improved method for analyzing the toxicity of imidacloprid in rice samples which we consume every day, which was performed against rice weevil, *Sitophylus oryzae,* that followed a standard protocol and was evaluated with the collaboration of Bangladesh Institute of Nuclear Agriculture, Mymensingh.

Rice sample (parboiled) was ground into powder and a mould was made from the powder by adding water. Six disks were made. One was used as control, one was blank and the rests (n=4) were spiked with four different concentrations of imidacloprid by adding 150 µL per disk. The solvent was allowed to evaporate for 24 hrs. Adult rice weevils (n=10), were then placed per petri dish to find out the effect of the pesticide on the insects. Mortality of the insect was counted after 24, 48 and 72 hours after treatment. The observed mortality was corrected by Abbott's formula (1987). Then it was analysed by Probit analysis using MSTAT-C Statistical Software in a computer. Disk flour bioassay was carried out to evaluate toxic action by ingestion. the average mortality of adult weevils was 30, 55, 61 and 74% while spiking the control rice with a concentration level of 1.25, 2.5, 5.0 and 10 µg g^{-1}. The mortality of adult weevils was 57% at 72 hours while spiking the control rice with a concentration level 1.25 µg g^{-1}, therefore, a concentration lower than this value would be enough to control the growth of weevils during storage. Therefore, the results of residual analysis in rice indicate indiscriminate use of imidacloprid in the market samples during storage.

Dissipation of Quinalphos in tomato, cauliflower and beans

Quinalphos, *O,O*-diethyl-*O*-quinoxalin-2-yl-phosphorothioate is an insecticide and is used for the management of diamond-back moth and tobacco caterpillar on vegetables. Accumulation of quinalphos residues in vegetables may pose human health hazards as quinalphos is very toxic chemical. Quinalphos is still not a registered pesticide in Bangladesh, therefore, the result of the present study is very important to determine the dissipation of quinalphos under the weather condition and soil property of our country and will help the government for having a decision about registering quinalphos as a regular insecticide.

Therefore, dissipation pattern of quinalphos was studied in tomato, bean and cauliflower which were grown in the experimental field of Bangladesh Agricultural Research Institute (BARI). Quinalphos, 0.03 percent aqueous emulsion prepared from Convoy 25 EC was sprayed at a recommended dose of 1 mL per Litre of water, in experimental field of bean, tomato and cauliflower on 24 december, 2014. Before spraying, control samples (untreated) were collected, then three replicate samples from each bed of three vegetables were collected from 0 day (after 2 hours of spraying) upto 15 days after spraying. The samples were taken in jeep-locked bags and immediately kept in a freezer below -20°C.

Extraction of quinalphoswas carried out following QuEChERS method using ethyl acetate as solvent and clean-up was performed using PSA.

Due to the vast variation in the residual amount in the field samples, three calibration curves were prepared (higher, middle and lower levels) in order to cover the range. High, middle and lower concentration calibration curves were obtained with linear correlation coefficient (r^2) 0.998, 0.995 and 0.993, respectively, the linearity range for high, middle and lower concentration calibration curves were 2.5- 6.5, 1.0 -2.5 and 0.06 - 0.13 µg mL^{-1}, respectively. LOD and LOQ were found to be 0.009 and 0.027 µg mL^{-1}, respectively. The percentages of the recovery of quinalphos residues in spiked vegetable samples at three different levels (0.625, 2.5 and 5.0 µg mL^{-1}) were in the range of 74 -86%.

On 0 day (2 hrs after spraying), the residual amounts of quinalphos were as; Cauliflower (6.31 ± 0.15 µg g^{-1}) > Tomato (3.10 ± 0.18 µg g^{-1}) > Bean (6.51 ± 1.06 µg g^{-1}). On 3rd day, the residual amounts of quinalphos were as; Cauliflower (2.02 ± 0.25 µg g^{-1}) > Tomato (0.88 ± 0.08 µg g^{-1}) > Bean (3.43 ± 0.11 µg g^{-1}) and on 7th day, the residual amounts were as; Cauliflower (0.10 ± 0.06 µg g^{-1}) > Tomato (0.21 ± 0.02 µg g^{-1}) > Bean (0.81 ± 0.19 µg g^{-1}).

The amount of quinalphos residue in tomato, bean and cauliflower were in the range of 6.31 ± 0.35 – 0.05 ± 0.09, 3.10 ± 0.18– 0.05 ± 0.07 and 6.5 ± 1.06– 0.06 ± 0.03 μg g^{-1} and went bdl after 11, 10 and 11 days, respectively. As quinalphos treatment on these three vegetables took place on the same day and in same climate area, therefore it can be said that the variation of the dissipation rate depends on accumulation criteria on these three vegetables and also on the biological, chemical or physical differences of these respective vegetables.The average half-life ($t_{1/2}$) of quinalphos was calculated to be 1.6 days. The residue dissipated over MRL value within 7, 6 and 4 days after final application for cauliflower, tomato and bean, respectively. Therefore, it is suggested not to spray quinalphos twice with an interval of at least 11 days after final application.

Determination of the dissipation of five pesticides in eight different vegetables

Farmers of our country use pesticides in agricultural fields, without any self protection and most of the times, they overdosed the crops with pesticides. As a result, these pesticides persist above MRL value. It is a duty of the advanced citizen to maintain the safe consumption period before intaking food grains, vegetables and fruits. Therefore, it is very important to publish the MRLs of each pesticide on food crops and the safe periodof consumption of pesticide treated vegetables. Vegetables are being also sprayed with numerous numbers of pesticides. The safe period of consumption and the dissipation pattern of five different pesticides; Vitaban 48 EC (chlorpyrifos), Double 50 EC (mix formulation of imidacloprid and cypermethrin), Nitro 505 EC (mix formulation of chlorpyrifos and cypermethrin), Asataf 75 SP (acephate) and Reeva 2.5 EC (lambda-cyhalothrin), in eight different vegetables (cabbage, cucumber, bottle gourd, sweet gourd, sponce gourd, green chili, cauliflower and tomato) were studied. The pesticides were applied in the agricultural field of Nuritola, Comilla, Bangladesh. After one hour of pesticides application, all the eight vegetable samples (weighed about 5 kg each) were collected and then transferred to the laboratory as soon as possible. The samples were kept at ambient temperature, so that they could be in a contact with air, wind, light and heat. From these samples, working samples were chopped, homogenized by kitchen blender and then extracted and cleaned-up on consecutive 1, 3, 5, 8 and 10 day of application.

Extraction of the pesticides followed QuEChERS method and clean-up was performed using PSA.

The quantitative analysis of pesticide residues for this part was conducted by gas chromatograph GC- ECD, Shimadzu 2010).

Dissipation of Vitaban 48 EC (chloropyrifos)

The active component of Vitaban 48 EC is Chlorpyrifos and was sprayed on the eight kind of vegetables in the farmer's field, Nuritola, Comilla, Bangladesh at a dose of 3mL per Liter of water/ ha. Higher and lower concentration calibration curves of certified chlorpyrifos standards showed the linear coefficient (r^2) 0.995 and 0.997 with linearity range of about 1.0– 3.2 µg mL^{-1} and 0.10 – 0.30 µg mL^{-1}. The recovery percentages were in between the range of 73 - 99% with standard deviations less than and equal to 4.2. The RSD values ranged from 0.50 to 4.24.

The initial residual amount of chlorpyrifos on 1st day, in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber were 3.12±0.8, 4.30±08, 2.94±0.18, 2.75±1.01, 4.23±1.03, 4.11±0.33, 5.14 ±1.33 and 4.21±1.01 µg g^{-1}, respectively.

The residues in tomato, bottle gourd, sweet gourd and green chili, treated with Chlorpyrifos went below MRL value after 5 days of application, on the other hand, cabbage, cauliflower, sponce gourd and cucumber, treated with chlorpyrifos went under MRL value after 8 days.The average half life of Chlorpyrifos in the vegetables was found to be 1.14 days.

Dissipation of Nitro 505 EC

Nitro 505 EC or Nitro was sprayed at a dose of 1 mL per Liter of water/ ha. Nitro 505EC is a mix formulation of chlorpyrifos (50%) and cypermethrin (5%). After extraction and clean up, Nitro treated vegetables were analyzed by GC-ECD. Higher and lower concentration calibration curves showed the linear coefficient (r^2) 0.995 and 0.994 with linearity range of about 1.5– 3.5 and 0.05 – 0.20 µg mL^{-1}.The LOD and LOQ were found to be 0.019 µg mL^{-1} and 0.057 µg mL^{-1}, respectively. The recovery percentages were in between the range of 73 - 102% with standard deviations less than and equal to 4.2. The RSD values ranged from 0.50 to 4.24.

The initial amount of chlorpyrifos and cypermethrin in all the vegetables on first day were much higher than the MRL values of both pesticides. A dissipation of 100% of chlorpyrifos occurred on 5th day after spraying for green chili and on 8th day after spraying for rest of the vegetables. For tomato, the initial amount declined by 43% upto 3rd day after application and went below MRL value. For cabbage and cauliflower, the initial amount declined by 95% and 94% upto 8th

day after application and went below MRL. For bottle gourd and sweet gourd, the safe period of consumption were obtained after 3 day of application and dissipates by 34 and 19%, respectively and for sponce gourd, green chili and cucumber, it is after 5 days of application, where 78, 100 and 99% dissipation occurs.

The residual concentration of cypermethrin in Nitro 505 EC on 1st day in tomato, cabbage, cauliflower, sweet gourd, sponce gourd, green chili and cucumber were 1.22 ± 0.08, 1.17 ± 0.15, 1.01 ± 0.08, 1.32 ± 0.02, 1.07 ± 0.06, 1.94 ± 0.02 and 1.02 ± 0.20 µg g^{-1}, respectively. Cypermethrin in tomato, cauliflower, sweet gourd, sponce gourd, green chili and cucumber went below MRL value after 1st day of application, in case of cabbage and sponce gourd, the initial concentration went below the MRL value after 3rd day of application. Therefore, it can be suggested from the current study that it is safe to consume cypermethrin treated tomato, cauliflower, sweet gourd, green chili and cucumber atleast after 1 day of application and for cabbage and sponce gourd, the safe period of consumption was 3 days after application at ambient temperature.

Dissipationof Double 50 EC

Double 50 EC or Double was sprayed at a dose of 800 mL/ ha. The active component of Double is imidacloprid and cypermethrin. The residue of Double in the vegetables was determined by QuEChERS method and cleaned up with PSA, then the residue was analyzed by GC-ECD (Shimadzu, 2010). Higher and lower concentration calibration curves showed the linear coefficient (r^2) 0.998 and 0.994 with linearity range of about 1.0– 2.0 and 0.03 – 0.08 µg mL^{-1}.The LOD and the LOQ were found to be 0.009 and 0.027 µg mL^{-1}, respectively.The recovery percentages were in between the range of 71 -104% with standard deviations less than and equal to 3.5. The relative standard deviation (RSD) values ranged from 0.8 to 4.2.

The initial value of imidacloprid went below MRL after 1day of application for tomato, green chili and after 3 days of application for cabbage, cauliflower, sweet gourd, bottle gourd and cucumber, after 5days of application for sponce gourd. The amount of imidacloprid went below detetion limit after 8 days of application for all the vegetables.

The initial amount of cypermethrin went below the MRL values after one day of application for tomato, cauliflower, green chili and cucumber with 73, 34, 82 and 47% dissipation and after 3rd

day of application for cabbage, bottle gourd, sweet gourd and sponce gourd with dissipation percentage of 96, 96, 80 and 82% from initial concentration.

The average half-lives were found to be 0.62 and 0.54 day for imidacloprid and cymermethrin, respectively.

Dissipation of Reeva 2.5 EC

Reeva 2.5 EC is the trade name for the commercial pesticide Lambda-cyhalothrin. It was sprayed at a dose of 1 mL per Liter of water/ ha. After proper sampling, extraction and clean up process, Reeva 2.5 EC treated vegetables were analyzed by GC-ECD.Lower and higher concentration calibration curves showed the linear coefficient (r^2) 0.998 and 0.996 with linearity range of about 0.04– 0.08 and 0.08 – 0.30 µg mL^{-1}, respectively. LOD and LOQ were found to be 0.009 and 0.027 µg mL^{-1}, respectively.

The recovery percentages were in between the range of 74 - 98% with standard deviations less than and equal to 3.0 The RSD values ranged from 0.60 to 3.5. A sharp decrease in residual amount of lambda-cyhalothrin in three days after application was observed. In the current study, results described that the residues had declined by a range of about 72 - 100% just after 1 day of application. The maximum residue limit, MRL values of lambda-cyhalothrin for tomato, cabbage, cauliflower, green chili and cucumber are 0.05 µg g^{-1} and for sweet gourd and bottle gourd, it is 0.10 µg g^{-1} (according to FAO, 2010). On the 1st day of application, the initial value of lambda-cyhalothrin in tomato, cabbage, cauliflower, bottle gourd, sweet gourd, sponce gourd, green chili and cucumber were 0.19 ± 0.09, 0.26 ± 0.01, 0.22 ± 0.10, 0.24 ± 0.02, 0.21± 0.03, 0.23 ± 0.02, 0.12 ± 0.05 and 0.16 ± 0.02 µg g^{-1}.

After 1 day of application, the initial concentration declined very sharply and went below the MRL value for each vegetables. The result will help the consumers to intake these vegetables when the pesticide residues decline below MRL and reach to the safe period of consumption. For lambda-cyhalothrin, it is safe to consume these vegetables atleast after 1 day of spraying.

Dissipation of Asataf 75 SP

Asataf 75 SP was sprayed at a dose of 1 gm per Liter of water/ ha. The active component of asataf 75 SP is acephate. The residue of was analyzed by GC-ECD (Shimadzu, 2010).

Lower and higher concentration calibration curves showed the linear coefficient (r^2) 0.997 and 0.996 with linearity range of about 0.10– 0.35 and 0.80 – 3.0 µg mL^{-1}, respectively. The LOD and LOQ were found to be 0.019 and 0.057 µg mL^{-1}, respectively. Recovery percentages of acephaet in spiked vegetable samples were done in two different concentration levels with three replications each. The recovery percentages were in between the range of 75 - 105% with standard deviations less than and equal to 3.0. The relative standard deviation (RSD) values ranged from 0.5 to 3.6.

The residual amount of acephate went below the MRL value after 5 days of application for cabbage and cauliflower and after 3 days of application for the rest of the samples. That means, acephate has more persistency in vegetables like; cabbage and cauliflower (brassica vegetables). The average half-life value of acephate was 1.2 days.

In case of each pesticides, the initial value were much higher than the MRL value, which indicates that it is highly risky to consume these pesticide treated vegetables on the 1st day of application, as that may cause acute toxicity and severe health hazards. The residual amount of these synthetic pesticides dissipated upto the MRL value within a maximum period of 3 days after pesticide application (average) and went below the detection limit rapidly. If farmers wait atleast one day after spraying; wait in market for minimum a day or twice, and then consumers keep them at ambient temperature at home for atleast one day before taking them as meal, then these vegetables become completely safe to consume.

Storage stability of the five pesticides

The storage stability was determined by spiking the control vegetable samples with the five pesticides and then storing them in a freezer at -20 ^{0}C. The control vegetables were spiked with chlorpyrifos, cypermethrin and imidacloprid at a concentration level of 1.0 µg mL^{-1} and with lambda-cyhalothrin and acefate at a concentration level of 0.5 and 2.0 µg mL^{-1},respectively. Then these spiked samples were stored in freezer for 35 days. These samples were then extracted following QuChERS method, cleaned-up using PSA and analyzed by GC-ECD. The average recoveries of the pesticides were found in the range of 84 - 93% with RSD value ranging from 1.1 to 4.2 The results showed that the pesticides were quiet persistent at freezing condition and not degraded during the period of storage.

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