

Competitive Research Grant Sub-Project Completion Report

On

**Determination of residue level of
commonly used pesticides in tea**

Project Duration

May 2017 to September 2018

Bangladesh Tea Research Institute (BTRI)

Srimangal-3210, Moulvibazar



Submitted to
Project Implementation Unit-BARC, NATP-2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215



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Citation:

Determination of Residue Level of Commonly Used Pesticides in Tea
Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka - 1215
Bangladesh

Edited and Published by:

Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka - 1215
Bangladesh

Acknowledgement

The execution of CRG sub-project has successfully been completed by Bangladesh Tea research Institute (BTRI) using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grand fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

Published in: September 2018

Printed by: pdf version

Acronyms

ADI	:	Acceptable Daily Intake
BARC	:	Bangladesh Agricultural Research Council
BARI	:	Bangladesh Agricultural Research Institute
BTB	:	Bangladesh Tea Board
BTRI	:	Bangladesh Tea Research Institute
Co-PI	:	Co-Principal Investigator
CIPAC	:	Collaborative International Pesticides Analytical Council
CRG	:	Competitive Research Grant
CTC	:	Crushing Tearing and Curling
DDT	:	Dichlorodiphenyltrichloroethane
EC	:	Emulsifiable Concentrate
ECD	:	Electron Captured Detector
EPA	:	Environmental Protection Agency
EU	:	European Union/Commission
FAO	:	Food and Agriculture Organization
FTD	:	Flame Thermoionized Detector
GAP	:	Good Agricultural Practice
GC	:	Gas Chromatography
ISO	:	International Organization for Standardization
IUPAC	:	International Union of Pure and Applied Chemistry
ITC	:	International Tea Committee
MRL	:	Maximum Residue Limit
MTF	:	Mini Tea Factory
NATP	:	National Agricultural Technology Program
ND	:	Not Detected
NOAEL	:	No-observed-Adverse Effect Level
PCR	:	Project Completion Report
PHI	:	Pre Harvest Interval
PI	:	Principal Investigator
PIU	:	Project Implementation Unit
QuEChERS	:	Quick Easy Cheap Effective Rugged Safe
RCBD	:	Randomized Complete Block Design
WHO	:	World Health Organization
WTO	:	World Trade Organization

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Executive Summary

A series of experiments were carried out at Bangladesh Tea Research Institute (BTRI) during 2017-18 to find out the residue levels both in green leaf and black tea of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin at different plucking intervals after spraying and their degradation. Pesticides were applied in the experimental plots at BTRI main farm following BTRI recommended dose. Every time of plucking, 250g green leaves were separated and preserved in -20°C for leaf analysis. Another 2 kg leaves were plucked and processed in the Mini Tea Factory (MTF) of the Institute following Crushing Tearing and Curling (CTC) method. The residue of selected pesticides from tea samples (green leaf and black tea) were analyzed at pesticide analytical laboratory (ISO/IEC 17025 accredited lab), Entomology Division, Bangladesh Agricultural Research Institute (BARI). The QuEChERS extraction technique was used for the extraction and clean-up of samples. Acetonitrile was used as extracting reagents and anhydrous MgSO₄ as clean-up reagent. Analysis was done by GC-2010 (Shimadzu). Chlorpyrifos and Quinalphos were determined by FTD (Flame Thermoionized Detector) with ATTM-1 capillary column (30m x 0.25mm x 0.25µm). For detection of Cypermethrin and Deltamethrin Electron Capture Detectors (ECD) were used with Optima-1 capillary column (130m x 0.25mm x 0.25µm). Helium was used as carrier and make up gas in FTD and in case of ECD, it was Nitrogen. In the experiment on Chlorpyrifos, the initial (0 day) deposits of residues were 7.822 and 0.893 mg/kg in green leaf and black tea respectively. Those declined to not detectable level in green leaf on 10th day and 0.013 mg/kg on the 7th day in black tea. In case of Quinalphos, residue levels were found initially (0 day) 4.973 and 0.193 mg/kg in green leaf and black tea respectively which declined to not detectable level on 10th and 5th day in green leaf and black tea respectively. In the experiment on Cypermethrin, the initial deposits of residues at 0 days were 3.335 and 0.276 mg/kg in green leaf and black tea respectively. Those declined to 0.034 and 0.050 mg/kg respectively on the 10th and 5th day. The residue level of Deltamethrin on 0 day was 2.262 mg/kg and 1.499 mg/kg which dropped down to 0.051 mg/kg and 0.254 mg/kg on the 10th and 3rd day in green leaf and black tea respectively. The degradation rate of residues of these pesticides with the increase of plucking days was not uniform. According to Good Agricultural Practice (GAP), interval between the last application of pesticide and harvest of crops (PHI) is 7 days. Residue levels of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin at this PHI in the green leaf were 0.164, 0.022, 0.102, and 0.138 mg/kg respectively which are below the Maximum Residue Limit (MRL) fixed by EU, FAO/WHO, EPA and German Law. Where as in black tea, the residue levels of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin on the 7th day were 0.013, ND, ND and ND mg/kg respectively. None of the analyzed black tea samples of Pre-Harvest Interval (PHI) at 7 days dose exceeded the MRLs imposed by the above international organizations. It is found that the residue of pesticides was degraded significantly in manufacturing process. The average pesticide residue degradation of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin were 80.10, 95.47, 91.506 and 29.935% respectively.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project:

Determination of residue level of commonly used pesticides in tea

2. Implementing organization:

Bangladesh Tea Research Institute (BTRI), Srimangal.

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

Principal Investigator (PI):

Shovon Kumar Paul, Scientific Officer, Entomology Division, Bangladesh Tea Research Institute, Srimangal-3210, Moulvibazar, Phone no.: 08626-71225-Ext 107 (Off), Mobile: 01556342527, E-mail: shovonbtri@gmail.com

Co-Principal Investigator (Co-PI):

Md. Jahangir Alam, Scientific Officer, Entomology Division, Bangladesh Tea Research Institute, Srimangal-3210, Moulvibazar, Phone no.: 08626-71225-Ext 107 (Off), Mobile: 01723316421, E-mail: anikbau52@gmail.com

4. Sub-project budget (Tk):

4.1 Total: Tk.20,00,000.00 (Twenty Lakh Only)

4.2 Revised (if any): N/A

5. Duration of the sub-project:

5.1 Start date (based on LoA signed): 11 May 2017

5.2 End date: 30 September 2018

6. Justification of undertaking the sub-project:

Tea is the most widely used non-alcoholic beverage after water all over the world, prepared from the tender shoot of tea plant, *Camellia sinensis*(L) O. Kuntze. The global production of tea is about 5,305 million kg (ITC, 2016), produced in more than 50 countries of the world (Roy *et al.*, 2014). Tea is a long established plantation crop of enormous economic importance to Bangladesh meeting the entire domestic demand of

this cheapest health beverage. The position of Bangladesh in respect of production is 10th (ITC, 2016).

About 15% of its crop could be lost by various pests particularly insects, mites, nematodes if adequate control measures are not taken in time (Ahmed, 2005). Every part of tea plant is potential target for attack of wide spectrum of pests. The prevailing warm condition favours the pests like tea mosquito bug, thrips, jassid, aphid, mites, termites etc. (Sana, 1989). Therefore, managing these pest populations within economic threshold level is important for which application of pesticides becomes imperative in Integrated Pest Management system. It is established that tea-growing countries of the world are being benefited from the chemical control of pests. In this perspective, chemical control of pests is a dominating feature in Bangladesh tea (Alam, 1999). To combat pests problem different group of pesticides like Organophosphate, Pyrethroids, Carbamates, Avermectin, Neonicotinoid and some unclassified group have been used in the tea fields since 1960 (Ahmed, 2007). Due to the complex pests' situation, in tea cultivation a total avoidance of pesticides is not feasible. So, fixation of MRL deserves importance. Otherwise use of pesticides on food crops can result in harmful residues in their food products. But the proper use of pesticides knowing the PHI can minimize harmful residues.

Now-a-days pesticide residue is a burning issue in the world. Pesticide residue in made tea means what is left over or remains of a pesticide in the made tea after the application of a pesticide to tea bushes in the field prior to plucking and subsequent manufacturing. As tea is a consumable commodity, the effect of residue of pesticides in made tea is harmful to human health (Dharmadi, 2007; Zhongmao, 2013). Tea produced in Bangladesh is exported to different countries of the world particularly West and East European as well as Middle Eastern countries. The consumers of these countries have become more conscious about the residue of the pesticides. In this perspective, EPA/ Codex Commission/ FAO/ WHO, German Law etc. have given restrictions on producing and procuring tea having pesticide residue (Ahmed, 2007). Since Bangladesh

is one of the signatories of FAO Code of Conduct on the distribution and use of pesticide. It is endeavoring to implement role of pesticide in the IPM particularly as defined in the FAO code for ensuring scientifically integrated usage as low volume crop protection agents to ensure friendly environment. Moreover, Government has already banned some highly toxic pesticides.

BTRI under Bangladesh Tea Board has Pesticide Residue Analytical Laboratory equipped with Gas Chromatograph (Shimadzu GC-14B) with other necessary equipments. The Institute estimated to analyze initially at least a good number of tea samples per annum at different levels eg. field application, made tea at factory, experimental samples, auction point & export point and random samples in the market at our own initiative as well as requests from outside and industry (Ahmed, 2005). Previously the Institute determined the safe harvest interval and also developed the protocols for the residue analysis of some pesticides (Ahmed *et al.*, 2010). New group of pesticides are developing for the better control of pests and these are also being registered for tea pests management. Besides, the regular monitoring of commonly used pesticide residues by the tea growers will give them a feedback on the presence of residues in their teas and also enable them to adopt safer practices in the usage of plant protection chemicals. Considering the above facts, an attempt has to be made to determine the residue level of the aforesaid pesticides in tea.

7. Sub-project goal: Production of safe tea with minimizing the risk of pesticide on human health and environment

8. Sub-project objective (s):

- a) To determine the residue of commonly used pesticides in tea
- b) To fix the safe harvest interval after spraying of pesticides in tea
- c) To know the degradation of pesticide residue from green leaf to made tea during processing.

9. Implementing location (s): BTRI main farm (Field experiments), Pesticide Residue Laboratory, Entomology Division, BTRI & Pesticide Analytical Laboratory, Entomology Division, BARI.



C section



A2 section

Plate 1. Experimental Field at different locations of BTRI Main Farm

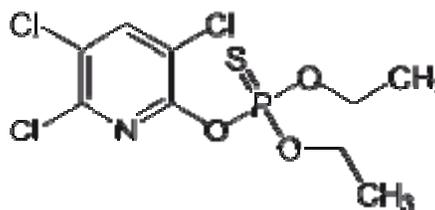
10. Materials and Methods

10.1 Determination of Chlorpyrifos residue in green leaf and made tea collected from supervised field trial

To determine residue as well as fixing the safe harvest interval (PHI), the experiment on Chlorpyrifos (Gola 48 EC) was carried out on the mature tea at BTRI main farm during 2017-18. The experiment was laid out in RCBD and replicated thrice. Chlorpyrifos was applied in all the plots at the approved dose of 1.0 lit. per ha in 500 lit. of water.

Physicochemical properties of Chlorpyrifos

ISO common name	:	Chlorpyrifos
Chemical name		
IUPAC	:	diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy- λ^5 -phosphane
CAS	:	0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate
CAS registry No.	:	2921-88-2
CIPAC No.	:	221
Synonyms and trade names	:	Chlorpyrifos, Pyrifos, Pyrinex, Lorsban, Gola
Structural formula	:	C ₉ H ₁₁ Cl ₃ NO ₃ PS



Molecular weight	:	350.6
Appearance	:	white or colorless crystals, mild mercaptan odor
Vapour pressure in mPa	:	1.87 x 10 ⁻⁵ mmHg at 25 °C
Melting point	:	42 °C
Octanol-water partition coefficient (log K _{ow})	:	4.70
Solubility		
Water	:	1.4 mg/L at 25 °C
Organic solvents		
Acetone	:	650 g/100 g
Xylene	:	400 g/100 g
Chloroform	:	630 g/100 g
Ethanol	:	63 g/100 g
Relative density	:	1.44 (20°C)
Hydrolysis	:	rapid hydrolysis under alkaline conditions

compared to neutral or acidic conditions

<https://pubchem.ncbi.nlm.nih.gov/compound/Chlorpyrifos>

http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/chlorpyrifos08/pdf

Metabolism and environmental fate (<http://npic.orst.edu/factsheets/chlorpge.html>)

Plant metabolism:Chlorpyrifos may be taken up by plants through leaf surfaces, much of the applied chlorpyrifos is usually lost from volatilization, and very little is translocated throughout the plant.

Environmental fate in soil:Chlorpyrifos is stable in soils with reported half-lives ranging between 7 and 120 days. Chlorpyrifos is less persistent in soils with a higher pH.

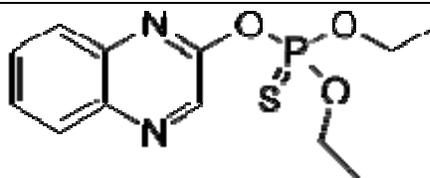
Environmental fate in water:Volatilization of chlorpyrifos from water is the most likely route of loss for chlorpyrifos, with volatilization half-lives of 3.5 and 20 days estimated for pond water. The rate of hydrolysis for chlorpyrifos increases with temperature and alkalinity.

10.2 Determination of Quinalphos residue in green leaf and made tea collected from supervised field trial

To determine residue as well as fixing the safe harvest interval (PHI), the experiment on Quinalphos (Quicklac 25EC) was carried out on the mature tea at BTRI main farm during 2017-18. The experiment was laid out in RCBD and replicated thrice. Quinalphos was applied in all the plots at the approved dose of 0.5 litre in 500 litre water per hectare.

Physicochemical properties of Quinalphos

ISO common name	:	Quinalphos
Chemical name	:	
IUPAC	:	diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ ⁵ -phosphane
CAS	:	O,O-diethyl O-2-quinoxalanyl phosphorothioate
CAS registry No.	:	13593-03-8
CIPAC No.	:	449
Synonyms and trade names	:	Quinalphos, Bayrusil, Ekalux, Quinaltaf, Quicklac
Structural formula	:	C ₁₂ H ₁₅ N ₂ O ₃ PS



Molecular weight	: 298.297 g/mol
Appearance	: Colourless crystals
Vapour pressure in mPa	: 0.346 mPa at 20 °C
Melting point	: 31.5°C
Octanol-water partition coefficient (logP)	: 4.44
Solubility	
Water	: 17.8 mg/L at 20 °C
Organic solvents	
Hexene	: 250 g/L at 20 °C
Relative density	: 1.235

<https://pubchem.ncbi.nlm.nih.gov/compound/quinalphos>

<https://sitem.herts.ac.uk/aeru/iupac/Reports/205.htm>

Metabolism and environmental fate

Plant metabolism:Metabolism occurs principally by oxidation and hydrolysis by esterases and by reaction with glutathione. Demethylation and glucuronidation may also occur. Oxidation of organophosphorus pesticides may result in more or less toxic products to plant. In plants one-third is absorbed by the leaf surface and penetrates into the plants; whilst two-third disappears by evaporation within 14 days

Environmental fate in soil:In soil, it is rapidly degraded under aerobic conditions. The hydrolysis product hydroxyquinoxaline does not accumulate in the soil but is further broken down to polar metabolites and carbon dioxide. The half-life range is between 10 to 16 days in soil, less than 24 hours in water.

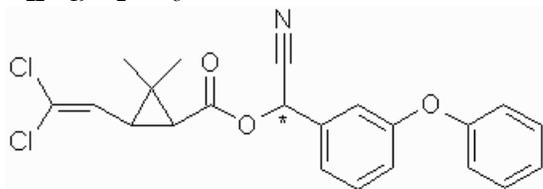
Environmental fate in water:Recent evidences indicate that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation.

10.3 Determination of Cypermethrin residue in green leaf and made tea collected from supervised field trial

To determine residue as well as fixing the safe harvest interval (PHI), the experiment on Cypermethrin (Ripcord 10EC) was carried out on the mature tea at the Institute Farm during 2017-18. The experiment was conducted in RCBD with three replications. Cypermethrin was applied in all the plots at the approved dose of 0.5 litre in 500 litre water per hectare.

Physicochemical properties of Cypermethrin

ISO common name	:	Cypermethrin
Chemical name		
IUPAC	:	(<i>RS</i>)- α -cyano-3-phenoxybenzyl (1 <i>RS</i>)- <i>cis</i> - <i>trans</i> -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS	:	cyano (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS registry No.	:	52315-07-8
CIPAC No.	:	332
Synonyms and trade names	:	Simper, Ripcord, Cymbush
Structural formula	:	$C_{22}H_{19}Cl_2NO_3$



Molecular weight	:	416.3
Appearance	:	Odourless crystals
Vapour pressure in mPa	:	2.0×10^{-4} mPa (20°C)
Melting point	:	61-83 °C
Octanol-water partition coefficient (K_{ow})	:	6.60
Solubility		
Water	:	0.004 mg/L
Organic solvents		
Acetone, Xylene, Chloroform	:	all 450 g/L at 20 °C
Ethanol	:	337 g/L at 20 °C
Hexane	:	103 g/L at 20 °C
Relative density	:	1.24 (20 °C)
Hydrolysis	:	Hydrolyzed in Alkaline media
Photolysis	:	Relatively stable to light in field situations.

<http://www.fao.org/3/W4601E/w4601e07.htm>

<https://apps.who.int/iris/bitstream/handle/10665/40017/9241542829-eng.pdf>

Metabolism and environmental fate(<http://npic.orst.edu/factsheets/cypermethrin.pdf>)

Plant Metabolism:When applied topically to cabbage and lettuce plants, cypermethrin is rapidly metabolized to α -cyano-3-phenoxybenzyl alcohol.

Environmental fate in soil:Cypermethrin has a moderate persistence in soils. Under laboratory conditions, cypermethrin degrades more rapidly on sandy clay and sandy loam soils than on clay soils, and more rapidly in soils low in organic material. Cypermethrin is not soluble in water and has a strong tendency to adsorb to soil particles that causes groundwater contamination.

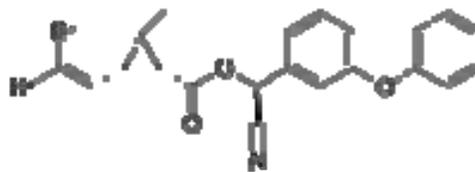
Environmental fate in water:Under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life of greater than 50 days and to photo-degradation with half-life of greater than 100 days.

10.4 Determination of Deltamethrin residue in green leaf and made tea collected from supervised field trial

To determine residue as well as fixing the safe harvest interval (PHI), the experiment on Deltamethrin (Decis 2.5EC) was carried out on the mature tea at the Institute Farm during 2017-18. The experiment was conducted in RCBD with three replications. Deltamethrin was applied in all the plots at the approved dose of 0.5 litre in 500 litre water per hectare.

Physicochemical properties of Deltamethrin

ISO common name	:	Deltamethrin
Chemical name	:	
IUPAC	:	[(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate
CAS	:	(S)-cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromoethenyl)-2,2-imethylcyclopropanecarboxylate
CAS registry No.	:	52918-63-5
CIPAC No.	:	333
Synonyms and trade names	:	Deltamethrin, Butox, Decis
Structural formula	:	$C_{22}H_{19}Br_2NO_3$



Molecular weight	: 505.2 g/mol
Appearance	: colorless or white to light beige & odorless crystals
Vapour pressure in mPa	: 1.5×10^{-8} mmHg at 25 °C
Melting point	: 100 °C
Octanol-water partition coefficient (log K_{ow})	: 6.20
Solubility	
Water	: 0.0002 mg/L
Organic solvents	
Acetone	: 500 g/L at 20 °C
Xylene	: 250 g/L at 20 °C
Methanol	: 8.15 g/L at 20 °C
Relative density	: 1.5 g cm^{-3}
Commercially available formulations	: EC

<https://pubchem.ncbi.nlm.nih.gov/compound/Deltamethrin>

http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Deltamethrin_2012

Metabolism and environmental fate (<http://npic.orst.edu/factsheets/archive/Deltatech.html>)

Plant Metabolism: Deltamethrin is not likely to adsorb as strongly to leafy components of vegetation, so volatilization from these surfaces may be higher compared to soil. The half-life of deltamethrin on vegetative surfaces ranges from 5.9-17.0 days, depending on the plant species.

Environmental fate in soil: Reported half-lives under aerobic laboratory conditions for deltamethrin in sandy loam or silt loam soil ranges from 11-72 days. In anaerobic soil conditions, the half-life of deltamethrin ranges from 31-36 days. Deltamethrin has little potential to leach into groundwater due to its strong tendency to bind to soil organic matter.

Environmental fate in water: Deltamethrin is stable to hydrolysis in solutions of pH 5 and 7. In a pH 9 solution, the average half-life is 2.5 days. Deltamethrin, have been found in aquatic sediment.

Use pattern

Chlorpyrifos and Quinalphos are organophosphate (OP) insecticide with contact and stomach action. On the other hand Cypermethrin and Deltamethrin are synthetic pyrethroid insecticide effective against insects via ingestion and direct contact. In Bangladesh, these pesticides are used for the control of sucking pest in tea like tea mosquito bug, aphid, jassid, thrips, looper caterpillar.

Collection, processing and preservation

Sampling

Green leaf samples were collected from Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin treated plots of about 3 kg at 0 (3 hours), 1st, 3rd, 5th, 7th, and 10th day after application of pesticides (Anonymous, 2009). Leaf samples for 0 day were collected after 3 hours of application and the samples for other days were plucked at respective interval after pesticide application. The sample comprised of minimum 70% two leaves and a bud. Every time of plucking, 250g green leaves were separated and preserved in -20°C for leaf analysis. Another 2 kg leaves were manufactured in the MTF of the Institute following CTC black tea manufacturing process. The manufacturing process involved withering, rolling, oxidation (fermentation) and drying. For blank sample, leaves from the plots were collected before spraying and processed in the MTF adopting the same method.



A2 section



Salon section

Plate 2. Collection of green leaf sample from experimental plots



Plate 3. Processing of green leaf samples and Preservation of made tea

Extraction and Clean up

The residue of pesticide from green leaf and black tea samples were analysed at pesticide analytical laboratory (ISO/IEC 17025 accredited lab), Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. The QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhane *et al.* (2015).

The samples were grounded thoroughly with the fruit blender. A representative 10 g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30s by the use of a vortex mixer. Then, 4 g of anhydrous MgSO₄ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates.

Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous MgSO₄, 150 mg charcoal and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30s and centrifuged for 5 minutes at 4000 rpm (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter and then it was taken in a clean GC vial for injection.

Detection and Quantification residues of the selected pesticides in samples

Chlorpyrifos and Quinalphos

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) in FTD detector with ATTM-1 column: length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm. Helium was used as carrier and make up gas.

Cypermethrin and Deltamethrin

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) in ECD detector with Optima-1 column: length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm. Nitrogen was used as carrier and make up gas.

The instrument parameters for detecting residues of the selected pesticides were as follows.

Instrument parameters	Chlorpyrifos&Quinalphos	Cypermethrin&Deltamethrin
Machine	GC-2010	GC-2010
[Injection Port SPL]		
Injector (Auto)	AOC 20i	AOC 20i
Injection Mode	Split	Split
Temperature	260°C	280°C
Carrier Gas	He	N ₂
Flow Control Mode	Linear velocity	Linear velocity
Linear Velocity	40.0 cm/sec	40.0 cm/sec
Purge Flow	3.0 mL/min	3.0 mL/min
Split Ratio	20.0	10.0
Injection Volume	1.0 µL	1.0 µL
[Column Oven Temperature]		
Initial Temperature	160°C	160°C
Final Temperature	240°C	270°C
Equilibrium Time	1.0 min, Hold time: 2	1.0 min, Hold time: 6
Total Program Time	12.0 min	18.0 min
[Column Information]		
Column Name	ATTM-1	Optima-1
Column Length	30.0 m	30.0 m
Film Thickness	0.25 µm	0.25 µm
Inner Diameter	0.25 mm	0.25 mm
[Detector Channel 1]		
Detector	FTD	ECD
Temperature	280°C	300°C
Stop Time	12.0 min	18.0 min
Current	0.5 pA	1.0 pA
Makeup Gas	He	N ₂
Makeup Flow	30.0 mL/min	30.0 ml/min
H ₂ Flow	105 mL/min	-
Air Flow	145.0 mL/min	-

The standard for Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin were obtained from Sigma-Aldrich Laborchemikalien, Seelze, Germany via S. F. Scientific Pvt.

Ltd. Dhaka, Bangladesh. Standards of these insecticides contained 99.6% purity. The purity of formulated insecticides were also tested and found to be 100% pure.

Foregoing to the injection of the sample extract, standard solutions of different concentrations of Chlorpyrifos and Quinalphos were prepared and injected with the above instrument parameters separately. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution of concerned insecticide. Each peak was characterized by its retention time. Sample results were expressed in mgkg^{-1} automatically by the GC software which represented the concentration of the final volume injected. From this value the actual amount of Chlorpyrifos and Quinalphos residue present in the sample was determined by using the following formula:

Residue in sample (mgkg^{-1})

$$\text{Conc. obtained in injected volume (mgkg}^{-1}\text{)} \times \text{Quantity of final volume (L)} \\ = \frac{\text{-----}}{\text{Amount of sample taken (kg)}}$$



Plate 4. Pesticide residue analytical laboratory, BTRI (a) & BARI (b).

11. Results and discussion:

The analytical results of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin residue found in the green leaf of tea and made tea samples are summarized in the Table 1-8.

Chlorpyrifos in Tea

Table 1. The Level of Chlorpyrifos (Gola48 EC) residue (mg/kg) estimated from green leaf of tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	7.822	0.1
1	10	1	1.975	
3	10	1	0.645	
5	10	1	0.446	
7	10	1	0.164	
10	10	1	ND	

Residue of Chlorpyrifos in green leaf of tea was detected up to 7 DAS and the quantities were above MRL up to 7 DAS and these were 7.822 mg/kg, 1.975 mg/kg, 0.645 mg/kg, 0.446 mg/kg and 0.164 mg/kg at 0, 1, 3, 5, and 7 DAS, respectively (Table 1).

Table 2. The Level of Chlorpyrifos (Gola 48 EC) residue (mg/kg) estimated from made tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	0.893	0.1
1	10	1	0.531	
3	10	1	0.189	
5	10	1	0.107	
7	10	1	0.013	

Residue of Chlorpyrifos in made tea was detected up to 7 DAS and the quantities were above MRL up to 5 DAS and these were 0.893 mg/kg, 0.531 mg/kg, 0.189 mg/kg and 0.107 mg/kg at 0, 1, 3, and 5 DAS, respectively (Table 2). While sample of 7 DAS contained 0.013 mg/kg residue which was below MRL set by EU/FAO-WHO.

Quinalphos in Tea

Table 3. The Level of Quinalphos (Quicklac 25EC) residue (mg/kg) estimated from green leaf of tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	4.973	0.05
1	10	1	1.364	
3	10	1	0.64	
5	10	1	0.058	
7	10	1	0.022	
10	10	1	ND	

The results revealed that residue of Quinalphos could be detected up to 7 DAS in green leaf of Tea. The quantities of residue were above MRL up to 5 DAS and these were 4.973 mg/kg, 1.364 mg/kg, 0.64mg/kg and 0.058 mg/kg at 0, 1, 3 and 5 DAS, respectively (Table 3). Sample of 7 DAS contained 0.022 mg/kg, which was below the EU-MRL (0.05) set by European Union (European commission,2005). No residue was detected at 10 DAS. So, the PHI of Quinalphos for green leaf can be selected at 7 DAS.

Table 4. The Level of Quinalphos (Quicklac 25EC) residue (mg/kg) estimated from made tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	0.193	0.05
1	10	1	0.079	
3	10	1	0.025	
5	10	1	ND	
7	10	1	ND	

The results revealed that residue of Quinalphos could be detected up to 3 DAS in made tea sample. The quantities of residue were above MRL up to 1 DAS and these were 0.193 mg/kg and 0.079 mg/kg at 0 and 1 DAS, respectively (Table 4). Sample of 3 DAS contained 0.025 mg/kg, which was below the EU-MRL (0.05) set by European Union (European commission,2005). No residue was detected at 5 and 7 DAS.

Cypermethrin in Tea

Table 5. The Level of Cypermethrin (Ripcord 10EC) residue (mg/kg) estimated from green leaf of tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	3.335	0.5
1	10	1	2.844	
3	10	1	1.151	
5	10	1	0.300	
7	10	1	0.102	
10	10	1	0.034	

The results revealed that residue of Cypermethrin could be detected up to 10 DAS in green leaf of Tea. The quantities of residue were above MRL up to 3 DAS and these were 3.335mg/kg, 2.844mg/kg and 1.151mg/kg at 0, 1 and 3 DAS, respectively. Sample of 5 DAS contained 0.300mg/kg, which was below the EU-MRL (0.5) set by European Union (European commission,2005). So, the PHI of Cypermethrin for green leaf can be selected at 7 DAS.

Table 6. The Level of Cypermethrin (Ripcord 10EC) residue (mg/kg) estimated from made tea

Days after spraying	Sample weight (g)	Injected volume (μ L)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	0.276	0.5
1	10	1	0.244	
3	10	1	0.103	
5	10	1	0.050	
7	10	1	ND	

The results revealed that residue of Cypermethrin could be detected up to 5 DAS in made tea sample. The quantities of residue were above MRL up to 3 DAS and these were 0.276 mg/kg, 0.244mg/kg and 0.103mg/kg at 0, 1and 3 DAS, respectively. Sample of 5 DAS contained 0.050mg/kg, which was below the EU-MRL (0.05) set by European Union (European commission,2005). No residue was detected at 7 DAS.

Deltamethrin in Tea

Table 7. The Level of Deltamethrin (Decis 2.5 EC) residue (mg/kg) estimated from green leaf of tea

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	2.262	5
1	10	1	1.592	
3	10	1	0.344	
5	10	1	0.231	
7	10	1	0.138	
10	10	1	0.051	

The results revealed that residue of Deltamethrin could be detected up to 10 DAS in green leaf of Tea. But, the initial quantity of residue was 2.262mg/kg at 0 DAS, which was below the EU-MRL (5) set by European Union (European commission,2005). So, the PHI of Deltamethrin for green leaf can be selected at 7 DAS.

Table 8. The Level of Deltamethrin (Decis 2.5 EC) residue (mg/kg) estimated from made tea

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	1.499	5
1	10	1	1.079	
3	10	1	0.254	
5	10	1	ND	
7	10	1	ND	

The results revealed that residue of Deltamethrin could be detected up to 3 DAS in made tea sample. Sample of 0 DAS contained 1.499mg/kg, which was below the EU-MRL (5) set by European Union (European commission,2005). No residue was detected at 5 and 7 DAS.

Degradation of pesticide residue from green leaf to black tea

The residue of tested pesticides was degraded in subsequent plucking interval both in green and black tea (Fig. 1-4). In case of green leaf, it was found that the pesticide residue is higher than MRL even in 7 days after spraying. But in black tea, the residue decrease significantly compared to green leaf. That means manufacturing process plays a vital role in reducing pesticide residue in black tea from green leaf. The residues of selected pesticides in black tea were decreased below MRL within 7 days. The average pesticide residue degradation of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin were found 80.10, 95.47, 91.506 and 29.935% respectively. The highest percent degradation of residue (95.47%) was observed in Quinalphos (Table 9) because *Bacillus thuringiensis* grew on quinalphos with a generation time of 28.38 min or 0.473 h in logarithmic phase. Maximum degradation of quinalphos was observed with an inoculum of 1.0 OD, an optimum pH (6.5–7.5), and an optimum temperature of 35–37 °C. Among the additional carbon and nitrogen sources, the carbon source—sodium acetate and nitrogen source—a yeast extract marginally improved the rate of degradation of quinalphos (Gangireddygar VSR *et al.* 2017).

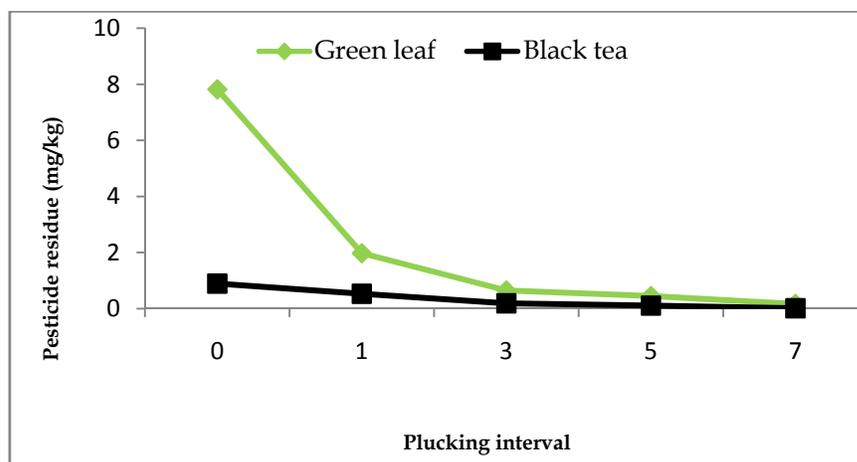


Fig. 1: Degradation of Chlorpyrifos residue in green leaf and black tea at different plucking interval

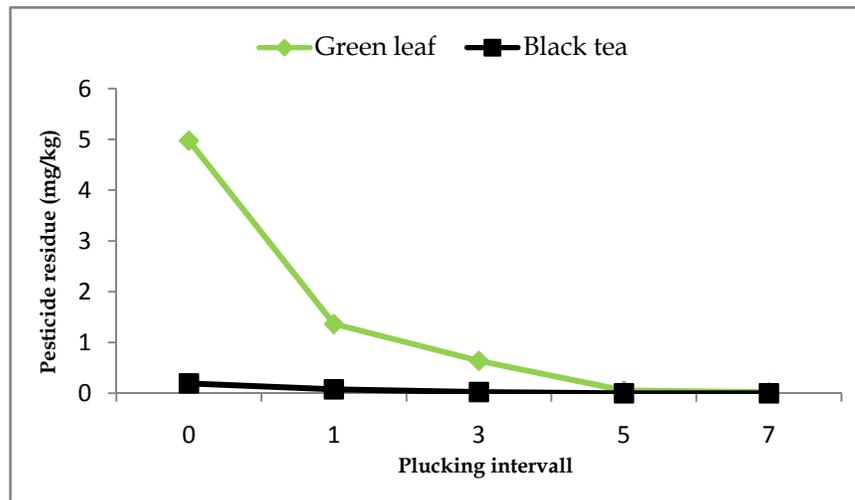


Fig. 2: Degradation of Quinalphos residue in green leaf and black tea at different plucking interval

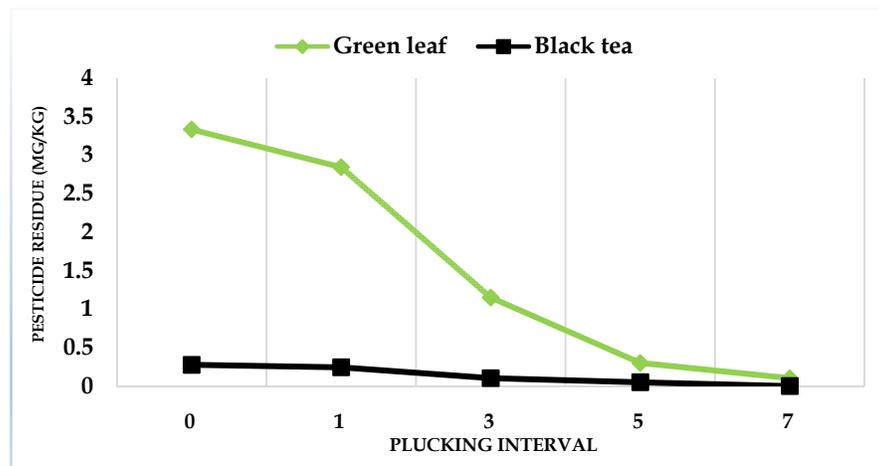


Fig. 3: Degradation of Cypermethrin residue in green leaf and black tea at different plucking interval

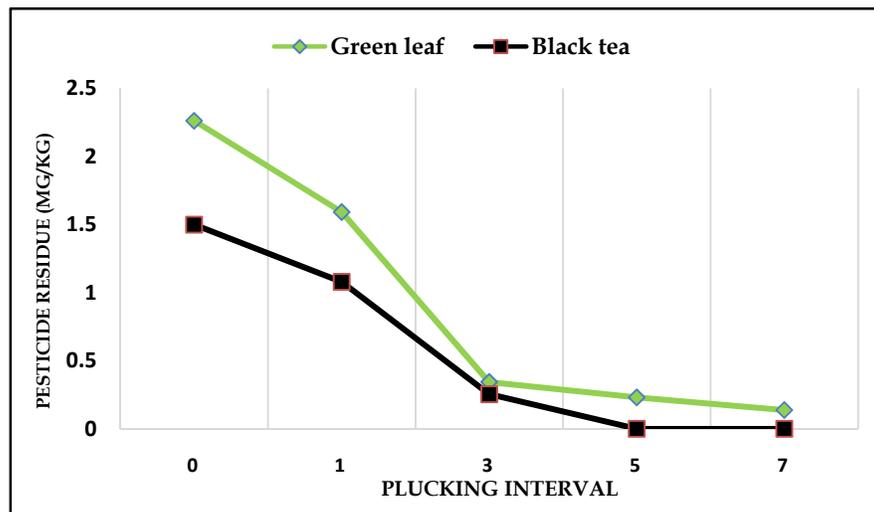


Fig. 4: Degradation of Deltamethrin residue in green leaf and black tea at different plucking interval

Table 9. The percent degradation of pesticide residue from green leaf to black tea at different plucking interval

Days after spraying	Chlorpyrifos			Quinalphos			Cypermethrin			Deltamethrin		
	Green leaf	Black tea	% degrad	Green leaf	Black tea	% degrad	Green leaf	Black tea	% degrad	Green leaf	Black tea	% degrad
0	7.822	0.893	88.58	4.973	0.193	96.12	3.335	0.276	91.724	2.262	1.499	33.731
1	1.975	0.531	73.11	1.364	0.079	94.21	2.844	0.244	91.421	1.592	1.079	32.224
3	0.645	0.189	70.70	0.64	0.025	96.09	1.151	0.103	91.051	0.344	0.254	26.163
5	0.446	0.107	76.01	0.058	ND	-	0.3	0.05	83.333	0.231	ND	-
7	0.164	0.013	92.07	0.022	ND	-	0.102	ND	-	0.138	ND	-

Safe harvest interval

It is customary to harvest tea leaves at different intervals after re-growth from previous plucks. From the above findings it is revealed that the closer the harvest interval the higher is the residue content or wider the interval the lesser is the residue level (Table 10).

Table 10. Safe harvest intervals for the tested pesticides in Bangladesh tea

Chemical	Dose /ha	Residue level* (ppm)	Safe harvest interval (days)
Chlorpyrifos	1.0 lit.	0.013	7
Quinalphos	0.5 lit.	ND	7
Cypermethrin	0.5 lit	ND	7
Deltamethrin	0.5 lit.	ND	7

* black tea at 7 PHI (William, 2013; Gurusubramanian *et al.*, 2008)

The residue level of the selected pesticides in Bangladesh tea compared to different international organizations is presented in Table 11. The residue level of above mentioned pesticides at 7 days after plucking is below the MRL fixed by EU, EPA, Codex Commission/FAO, German Law and Japan.

Table 11. Residue level of some commonly used chemicals in Bangladesh tea in comparison with the international organizations

Pesticides	MRL [mg/kg (ppm)] fixed by different organizations					
	EU	FAO/WHO	EPA	G. Law	Japan	Bangladesh
Chlorpyrifos	0.1	0.1	-	0.1	3.0	0.013
Quinalphos	0.05	-	-	0.1	0.1	ND
Cypermethrin	0.5	20	20	-	20	ND
Deltamethrin	5.0	10	-	-	10	ND

**Indicates lower limit of analytical determination. N. B. Lower the MRL value higher the toxicity level (William, 2013; Gurusubramanian et al., 2008; <http://www.upasitearesearch.org/pesticide-residue>)*

Interval between the last application and harvest of crop (PHI) is very important in respect of level of pesticide residue, quality and yield of tea. According to the GAP (Good Agricultural Practice), PHI is 7 days (Chaudhury, 2008). Maximum level of residues (MRLs) in made tea is accounted following the approved PHI.

Discussion

In the present study, none of the detected pesticides does not exceed the maximum residue levels set by EU, FAO/WHO, EPA, German Law and Japan which are supported by the findings of Seenivasan and Muraleedharan (2011), Bishnuet al. (2009), Amaraweera and Wickramasinghe (2019) and Amirahmadiet al. (2013).

Seenivasan and Muraleedharan (2011) made a large scale survey of produced tea in the factories of south India for a period of three years and 912 samples were analysed for the residues of different pesticide group like Organochlorine, Organophosphate and synthetic Pyrethroid. The analytical data showed that only 0.5% of samples had residues of interested pesticides. The residues of rest of the samples were below their maximum limits in tea, fixed by EU, FAO/WHO and Prevention of Food Adulteration Act of Govt. India. Bishnuet al. (2009) quantified the residues level of chlorpyrifos, cypermethrin, deltamethrin, ethion, heptachlor, dicofol and endosulfan in tea ecosystems of Doars regions of West Bengal, India. They found that the residues of banned pesticides like heptachlor in made tea may pose health hazard to the consumer.

In Sri Lanka, Amaraweera and Wickramasinghe (2019) recently analysed 15 tea samples from the major tea exporters in the country based on of 5 black tea grades (OP, BOP, FBOP, BOPF and PEKOE). Among the analyzed samples, some were found with detectable amounts of pesticide residues. But none of the sample exceeded the Maximum Residue Levels established by Codex, EU and Japan. Endosulfan and bifenthrin was the main focus on the study and only one tea grade had residues more than 0.005 ppm. Other than that Chlorpyrifos, Cypermethrin, Methyl parathion, O,P-DDT were detected and at a much lower frequency.

Amirahmadiet *al.* (2013) conducted to monitoring of some pesticide's residue in consumed tea in Tehran market. They analysed 53 samples of different pesticide group such as Organochlorine, Organophosphate and synthetic Pyrethroid. Fifteen (28.3%) of the 53 samples showed contamination with pesticides. None of the samples had contamination higher than maximum residue limit set by EU and India.

It is found that the pesticide residues of Chlorpyrifos, Quinalfos, Cypermethrin and Deltamethrin in present experiment were gradually decreased in subsequent plucking interval after spraying. The pesticide residue decreased drastically in black tea manufactured from the correspondence green leaf. So, manufacturing process of tea has a greater effect on the reduction of pesticides residues. Similar result was found by Soodet *al.* (2004). They reported that pesticides applied on tea bushes degraded as a result of natural factors like rainfall, dew, evaporation, photolysis, biodegradation, growth dilution and the time lapse between the spraying of the pesticides and harvesting of the tea leaves. Degradation of pesticides in the green leaves also take place at the time of manufacturing through evaporation and thermal decomposition. Chen and Wan (1988) reported that 30-60% pesticide residue reduction in tea during manufacturing process mainly drying.

Good Agricultural Practice (GAP)

Tea (*Camellia sinensis*) is an important agricultural plantation crop that is grown and made tea is manufactured in different parts of Bangladesh. The major production of tea is

from organized tea estates having their own manufacturing units. The crop is propagated through seed and clonal material and the bushy frame is maintained for facilitating harvest. The tender shoots along with bud are plucked (harvested) at seven days interval for manufacturing of made tea in the factory. 7 days PHI is widely accepted practice in the Tea Industry of Bangladesh. Therefore, residues from 7-day PHI assume importance for fixation of MRL in tea. The tea manufacturing process involves withering (reduction of moisture in the plucked shoots), rolling, oxidation (fermentation) and drying (95-115°C).

12. Conclusion and recommendations

Tea being an export oriented commodity, all the required measures must be taken to keep the residues well below the MRL to overcome the non-tariff trade barriers under World Trade Organization (WTO) regimes. Other parameters on the level of residues like No-observed-Adverse Effect Level (NOAEL), Acceptable Daily Intake (ADI), Food Factor and Maximum Permissible Intake (MPI) should be taken into consideration according to FAO guidelines. With these parameters, the MRL and PHI can be strictly followed for having access to the world market. As such, scientific approach towards its practical and routine application for the benefit of tea industry should be uniform. According to the GAP, the PHI is 7 days. The MRL in made tea is fixed up following the approved PHI. Only BTRI recommended pesticides with approved dose should be applied when it is absolutely essential. 7 day period is the safe harvest interval after spraying of pesticides. The study indicates that the 7 days PHI may be recommended for above mentioned pesticides in tea plantation of Bangladesh. BTRI approved pesticides with recommended doses should be applied when it is absolutely essential.



Some monitoring & field activities of residue project during 2017-18

13. Research highlight/findings (Bullet point – max 10 nos.):

- Pesticide residues (mg/kg at 7 DAS) in green leaf and black tea of Chlorpyrifos (0.164 & 0.013), Quinalphos (0.022 & ND), Cypermethrin (0.102 & ND) and Deltamethrin(0.138 & ND) in Bangladesh tea are determined.
- The safe harvest interval at 7 days after spraying of these pesticides in tea has been fixed.
- The degradation of pesticide residue at Chlorpyrifos (80.10%), Quinalphos (95.47%), Cypermethrin (91.506%) and Deltamethrin (29.935%) from green leaf to black tea has been understood.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Desktop Computer Laptop Laser Printer Digital Camera UPS Scanner	2,45,000	Desktop Computer Laptop Laser Printer Digital Camera UPS Scanner	2,33,250	
(b) Lab & field equipment	Detector & Column Pesticide standard Reagents Glassware & Chemicals	4,80,000	Detector & Column Pesticide standard Reagents Glassware & Chemicals	3,97,400	
(c) Other capital items	Executive Table Executive Chair File Cabinet Steel Almira Visitor/Front chair Computer Table	1,08,400	Executive Table Executive Chair File Cabinet Steel Almira Visitor/Front chair Computer Table	1,08,360	

	Computer Chair Lab Chair		Computer Chair Lab Chair		
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2. Establishment/renovation facilities:N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized:Not done

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Femal e	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance / unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	122731	122731	122731	0	100%	
B. Field research/lab expenses and supplies	1209625	1094934	1094934	114691	90.51%	
C. Operating expenses	186873	185817	184395	2478	98.67%	
D. Vehicle hire and fuel, oil & maintenance	27900	26075	26075	1825	93.46%	
E. Training/ workshop/ seminar etc.	0	0	0	0	0%	

F. Publications and printing	100000	0	0	100000	0%	
G. Miscellaneous	11261	11113	11113	148	98.96%	
H. Capital expenses	341610	333486	333486	8124	97.62%	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
1. To determine the residue of commonly used pesticides in tea	<ul style="list-style-type: none"> i) Design and layout of supervised fields/plot for sample collection. ii) Application of recommended pesticides with proper dose and method iii) Carry out the fresh sample (green leaf) with proper maintenance and preserved in -20°C for further leaf analysis iv) Residue analysis by GC-2010 (Shimadzu) at pesticide analytical laboratory (ISO/IEC 17025 accredited lab), Entomology Division, BARI. 	Detection of MRL value both in green leaf and made tea of Chlorpyrifos, Quinalphos, Cypermethrin and Deltamethrin with the recommendation of 7 days PHI for above mentioned pesticides in tea plantation of Bangladesh.	Awareness of plucking interval with recommended dose and method of pesticides which cover the food security as well as residue free tea production.
2. To fix the safe harvest interval after spraying of pesticides in tea	Collection of samples under proper supervision at different plucking intervals	Different tea samples obtained at different interval.	Introducing safe harvest interval with judiciously use of pesticides help to reduce the frequency and amount of pesticides application
3. To know the degradation of pesticide residue from	Manufacturing from the collected sample in the MTF of the Institute following CTC black tea	Production of black tea sample from green leaf.	Manufacturing process plays a vital role in reducing pesticide

green leaf to made tea during processing.	manufacturing process.		residue in black tea from green leaf.
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E. Materials Development/Publication made under the Sub-project:None done

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication			
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Fixation of 7 days PHI (Pre-harvest interval) for the production of safe/residue free minimizing risk of health and environment.

ii. Generation of new knowledge that help in developing more technology in future

QuEChERS extraction technique helps in detection new generation of pesticides in future

iii. Technology transferred that help increased agricultural productivity and farmers' income

Judicious use of pesticides help to reduce the frequency and amount of pesticides application with minimum risk as well as increasing agricultural productivity and farmers income.

iv. Policy Support

Production of safe tea and risk free human health and environment through judicious application of pesticides with the fixation of 7 day interval will help in pesticide residue policy.

G. Information regarding Desk and Field Monitoring

i) **Desk Monitoring** [description & output of consultation meeting, monitoring workshops/seminars etc.]:

N/A

ii) **Field Monitoring (Time & No. of visit, Team visit and output):**

Date of visit	Team Member	Remarks
30.01.2018	Dr. Md. Aziz Zilani, Member Director (Crops), BARC Dr. Rina Rani Saha, PSO (Crops), BARC	Satisfactory
19.05.2018	Dr. Mohammad Ali, Director, BTRI Dr. Md. Abdul Aziz, PSO (Botany), BTRI	Good performance

H. Lesson Learned/Challenges (if any)

i) Awareness of Safe harvest interval for tea.

ii) Judicious use of pesticides with proper dose.

iii) Residue free tea production through fixation of PHI and detection of MRL value.

I. Challenges (if any)

Judicious use of pesticides and awareness of Safe harvest interval for residue free tea production are very difficult to implement among the tea planters, owners and growers.

Signature of the Principal Investigator

Date

Seal

Counter signature of the Head of the

organization/ authorized representative

Date

Seal

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- http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/chlorpyrifos08/pdf
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