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DETERMINATION OF RESIDUES OF TWO ORGANOPHOSPHATES AND THEIR DISSIPATION PATTERN BY QuEChERS METHOD

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Abstract

An experiment was carried out at the main farm of Bangladesh Tea Research Institute (BTRI) to find the residue levels of commonly used two organophosphate insecticides, namely chlorpyrifos and quinalphos in green leaf and black tea, at various plucking intervals after spraying during 2017-2018. The safe harvest interval was also calculated based on the degradation of pesticide residue from green leaf to processed tea. Pesticides were applied in the experimental plots following BTRI recommended dose (Chlorpyrifos 48 EC @ 1.0 L ha^{-1} and Quinalphos 25 EC @ 0.5 L ha^{-1}). Green leaf and black tea samples were analyzed for pesticide residue of the above insecticides at the pesticide analytical laboratory (ISO/IEC 17025 accredited lab) of Bangladesh Agricultural Research Institute (BARI). The samples were extracted and cleaned up using the QuEChERS extraction method. Flame Thermionic Detector (FTD) with ATTM-1 capillary column (30 m x 0.25 mm x 0.25 µm) was used to determine the pesticide residue. The initial (0 days) pesticide residue of chlorpyrifos was 7.822 mg kg^{-1} in green leaf and 0.893 mg kg^{-1} in black tea, respectively. These were reduced to an undetectable level in the green leaf on the 10th day and 0.013 mg kg^{-1} in the black tea on the 7th day. On the other hand, quinalphos residue levels were initially (0 day) detected in green leaf and black tea at 4.973 and 0.193 mg kg^{-1}, respectively. It decreased to undetectable levels on the 5 and 10 days in both cases. The result showed that pesticide residue decreased considerably in the process stage. Chlorpyrifos and quinalphos had pesticide residue degradation rates ranges from 70.70-92.07% and 94.21-96.12%, respectively.

Keywords: Chlorpyrifos, Quinalphos, Residue, Tea, QuEChERS, MRL

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Introduction

Tea is the most consumed non-alcoholic beverage after water worldwide. It is made from the tender shoots of *Camellia sinensis* (L) O. Kuntze. About 6,455.19 million kg of tea is produced annually in over 50 countries worldwide (ITC, 2022). Tea provides Bangladesh's entire domestic demand, which is of immense economic significance. Bangladesh is ranked 9th in terms of production (ITC, 2022).

The tea plant is subjected to the attack of pests and diseases. If appropriate management measures are not done in time, numerous pests, especially insects, mites, and nematodes, could cause the loss of about 15% of its crop (Ahmed, 2005; Mamun, 2022). A variety of pests can damage any part of the tea plant. Pests, including termites, thrips, jassids, aphids, mites, and tea mosquito bugs, thrive in the prevailing warm climate (Sana, 1989; Ahmed, 2005). To keep these insect populations below the economic threshold level, it is crucial to implement an Integrated Pest Management strategy that includes pesticide application (Mamun & Ahmed, 2011; Mamun, 2022). It is well known that the chemical management of pests benefits tea-producing countries around the world. Hence, chemical pest management is a key aspect of Bangladesh tea production (Alam, 1999). Since 1960, many groups of pesticides, including Organophosphate, Pyrethroids, Carbamates, Avermectin, Neonicotinoid, and other unidentified groups, have been applied in tea fields to tackle these pest problems (Ahmed et al., 2009a; Ahmed et al., 2009b; Mamun, 2019; Ali et al., 2021). It is impossible to completely avoid using pesticides in tea cultivation because of the complex pest condition. Therefore, the fixation of Maximum Residue Level (MRL) of pesticides is essential. However, the use of pesticides on food crops could leave toxic residues in those finished products. Pesticides should be used correctly following Pre Harvest Interval (PHI) to avoid harmful residues.

Food safety, i.e., pesticide residue, is a burning issue worldwide. Pesticide residue in processed tea refers to what is left over or still present in the processed tea after a pesticide has been applied to the tea bushes in the field before harvesting. The pesticide residue in made tea harms human health as tea is a beverage (Dharmadi, 2007; Zhongmao, 2013). Bangladesh exports tea to several countries, primarily West and East European as well as Middle Eastern countries. The awareness of pesticide residue among consumers in these countries has increased. Hence, the EPA, the Codex Commission, the FAO, the WHO, the EU, German law, etc., have imposed restrictions on the production and purchase of tea that contains pesticide residue (Ahmed, 2007; Ahmed et al., 2010; Ahmed & Mamun, 2010; Ahmed & Mamun, 2012). Bangladesh is one of the signatories to the FAO code of conduct on the distribution and use of pesticides. Bangladesh is working to implement the role of pesticides in IPM, particularly as defined in the FAO code, for ensuring usage as low-volume crop protection agents to ensure a friendly environment. In addition, the government has already banned some highly dangerous chemicals.

Chlorpyrifos (O, O-diethyl O-[3,5,6-trichloro-2-pyridyl] phosphorothioate) is a chlorinated organophosphate insecticide with a broad spectrum of activity against different foliar and soil-borne insects. Plants may take up chlorpyrifos through leaf surfaces; however, most applied chlorpyrifos is often lost through volatilization, and very little is translocated within the plant. Chlorpyrifos has documented half-lives of between 7 and 120 days and is stable in soils. A higher pH of the soil makes chlorpyrifos less persistent. Chlorpyrifos is most likely to be lost through volatilization from water, with half-lives of between 3.5 and 20 days predicted for pond water. Temperature and alkalinity both speed up the process of hydrolysis.

On the other hand, quinalphos (O, O-diethyl-O-[2-quinoxalinyl phosphorothioate]) is one of the most widely used organophosphorus insecticides in the world (Hu et al., 2010). It is categorized as a 'moderately hazardous' insecticide by the World Health Organization (WHO), and hence, most countries either ban or limit the use of quinalphos (Gangireddygari et al., 2017). It is the most widely used pesticide for combating rice pests in India. Due to its widespread usage in agriculture, ease of availability, and farmers' lack of awareness of its negative consequences, the danger of quinalphos exposure to humans is particularly high in developing countries (Kumar et al., 2010; Singh & Khurana, 2009). Two-thirds of the applied insecticides evaporate after 14 days, while the leaf surface absorbs one-third and penetrates the plants. It breaks down quickly in the soil in aerobic conditions.

Chlorpyrifos and quinalphos work against insect pests by direct contact and ingestion. These pesticides are used to control leaf-sucking (tea mosquito bug, aphid, Jassid, and thrips), leaf-eating (looper caterpillar), and root feeder (termite) pests in Bangladesh tea. Judicious use of insecticides has the least harmful impact on biocontrol agents, ensuring the eco-friendly management of insect pests in tea plantations. Considering the above facts, a study was undertaken to determine the residues with PHI of widely used two organophosphate insecticides (Chlorpyrifos 48 EC and Quinalphos 25 EC) in Bangladesh tea.

Materials and Methods

The experiment on chlorpyrifos (Gola 48 EC) and quinalphos (Quicklac 25 EC) was conducted on the mature tea at BTRI main farm during 2017–18 to determine residue and fix the safe harvest interval (PHI). The experiment was laid out in RCBD with three replications. Chlorpyrifos and quinalphos were applied in all the experimental plots at the BTRI-approved dose of 1.0 L and 0.5 L per ha in 500 L of water.

Sampling

Green leaf samples weighing about 3 kg were taken from plots treated with chlorpyrifos and quinalphos at 0, 1, 3, 5, 7, and 10 days following pesticide application (FAO, 2009). After applying the insecticide, leaf samples for the first day (0 days) were taken 3 hours later, and samples for the remaining days were taken at the respective intervals. Each sample had at least 70% of two leaves and a bud. For leaf analysis, 250 g of green leaves were taken from each plucking and preserved at -20^{0}C in the laboratory. In addition, black tea was made in the Miniature Tea Factory (MTF) of the Institute following CTC (Crush, Tear &

Curl) process using 2.0 kg green leaves. Withering, rolling, oxidation (fermentation), and drying were followed in the manufacturing process. Before spraying, leaves from the experimental plots were collected for the blank sample, then processed in the MTF using the same procedure.

Extraction and Clean up

The pesticide residue from green leaf and black tea samples was determined at the pesticide analytical laboratory (ISO/IEC 17025 accredited lab) of BARI, Gazipur. The QuEChERS extraction method was used to extract and clean up samples, modified by Prodhan et al. (2015).

The blender was used to grind the samples finely. Ten-gram sample was taken in a 50 mL polypropylene centrifuge tube. Acetonitrile (MeCN) of 10 ml was then added to the centrifuge tube. A vortex mixer was used to agitate the centrifuge tube for 30 seconds after it had been correctly closed. The centrifuge tube was filled with 1 g of NaCl and 4 g of anhydrous $MgSO_4$. The vortex mixer rapidly shook it for 1 minute to avoid the development of magnesium sulfate aggregates.

The extract was then centrifuged at 5000 rpm for 5 minutes. A 15 mL microcentrifuge tube containing 600 mg anhydrous $MgSO_4$, 150 mg charcoal, and 120 mg Primary Secondary Amine (PSA) was filled with an aliquot of 3 mL of the MeCN layer. The mixture was then vigorously mixed for 30 seconds in a vortex and centrifuged for 5 minutes at 4000 rpm (Laboratory Centrifuges, Sigma-3K30, Germany). A clean GC vial was used to transfer a 1 ml supernatant that had been centrifuged and filtered through a 0.2 m PTFE filter.

Detection and quantification residues of chlorpyrifos and quinalphos

GC-2010 (Shimadzu) machine was selected to analyze the concentrated extracts using an ATTM-1 column (30 m x 0.25 mm x 0.25 µm) with a Flame Thermionic Detector (FTD). Both the carrier gas and the make-up gas were helium. Sigma-Aldrich Laborchemikalien, Seelze, Germany, provided the standard for chlorpyrifos and quinalphos via S. F. Scientific Pvt. Ltd., Dhaka, Bangladesh. The purity of the standards of these insecticides was 99.6 percent. The purity of formulated insecticide was also examined and determined to be 100% pure. Standard solutions of various concentrations of chlorpyrifos and quinalphos were prepared before the injection of the sample extract, and each was injected with the aforementioned instrument parameters individually. The samples' retention duration, peak area, and other measurements were calibrated using a five-point calibration curve of the standard insecticide solution. The retention time of each peak served as an identification of that insecticide. The GC program automatically expressed sample results in mg kg-1, which indicated the concentration of the final volume injected. This value was used to calculate the actual amount of chlorpyrifos and quinalphos residue in the sample using the formula below:

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

Results and Discussion

Pesticide residue of chlorpyrifos and quinalphos in green leaf and made tea

Tables 1 and 2 provide a summary of the analytical findings for the residues of chlorpyrifos and quinalphos detected in green tea leaf and made tea samples. Chlorpyrifos was found in green tea leaves up to 7 DAS, and the amounts were above MRL. At 0, 1, 3, and 5 DAS, the quantities of chlorpyrifos residue were 7.822 mg kg^{-1}, 1.975 mg kg^{-1}, 0.645 mg kg^{-1}, 0.446 mg kg^{-1}, and 0.164 mg kg^{-1}, respectively (Table 1). On the other hand, chlorpyrifos was found in made tea up to 7 DAS, and the amounts were above MRL up to 5 DAS. At 0, 1, 3, and 5 DAS, the quantities of chlorpyrifos residue were 0.893 mg kg^{-1}, 0.531 mg kg^{-1}, 0.189 mg kg^{-1}, and 0.107 mg kg^{-1}, respectively (Table 1). In addition, the sample of 7 DAS had a residue concentration of 0.013 mg kg^{-1}, which was below the MRL established by the EU.

Table 1. The residue level of chlorpyrifos (mg kg^{-1}) in green leaf and made tea.

Days after spraying (DAS)	Sample weight (g)	Injected volume (µL)	Amount of residue in green leaf (mg kg^{-1})	Amount of residue in made tea (mg kg^{-1})	EU MRL (mg kg^{-1})
0	10	1	7.822	0.893	0.1
1	10	1	1.975	0.531	
3	10	1	0.645	0.189	
5	10	1	0.446	0.107	
7	10	1	0.164	0.013	
10	10	1	ND	ND	

ND=Not Detectable

The result showed that quinalphos residue might be found in green tea leaves up to 7 DAS. At 0, 1, 3, and 5 DAS, the residue amounts were 4.973 mg kg^{-1}, 1.364 mg kg^{-1}, 0.64 mg kg^{-1}, and 0.058 mg kg^{-1}, respectively and all these amounts were over the MRL up to 5 DAS (Table 2). The amount of residue 0.022 mg kg^{-1} in the sample of 7 DAS was less than the MRL (0.05) established by the EU. At 10 DAS, no residue was detected. Therefore, 7 DAS, might be chosen as the PHI of quinalphos for green tea leaves. The findings showed that made tea samples contained quinalphos residue up to 3 DAS. The residue amounts were 0.193 mg kg^{-1} and 0.079 mg kg^{-1} at 0 and 1 DAS, respectively, and were above MRL up to 1 DAS (Table 2). The amount of residue 0.025 mg kg^{-1} in the sample from the 3 DAS was less than the MRL (0.05) established by the EU. At 5 and 7 DAS, no residue was found.

Table 2. The residue level of quinalphos (mg kg^{-1}) in green leaf and made tea.

Days after spraying (DAS)	Sample weight (g)	Injected volume (µL)	Amount of residue (mg kg^{-1}) in green leaf	Amount of residue (mg kg^{-1}) in made tea	EU MRL (mg kg^{-1})
0	10	1	4.973	0.193	0.05
1	10	1	1.364	0.079	
3	10	1	0.64	0.025	
5	10	1	0.058	ND	
7	10	1	0.022	ND	
10	10	1	ND	ND	

ND=Not Detectable

Degradation of pesticide residue from green leaf to black tea

In both green and black tea, the pesticide residue assessed degraded over repeated picking intervals (Fig. 1-2). Even seven days after spraying, it was observed that the pesticide residue on green leaves was higher than the MRL. However, compared to green leaf, the residue is substantially lower in black tea. Thus, manufacturing plays a significant role in lowering the pesticide content of black tea produced from green leaves. The levels of selected pesticide residues in black tea dropped below MRL within 7 days. The degradation rate of chlorpyrifos and quinalphos residues varies from 70.70-92.07% and 94.21-96.12%, respectively (Table 3.).

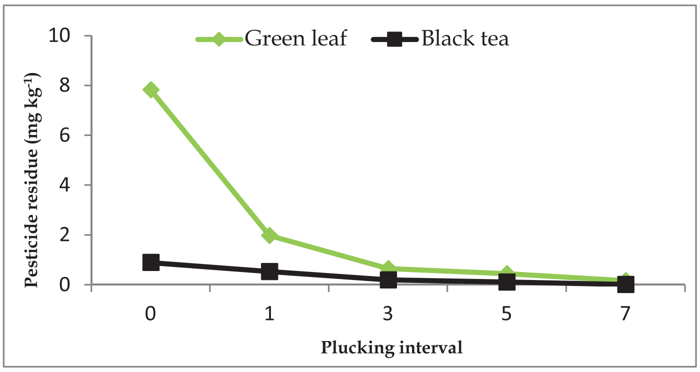

Fig. 1. Degradation of chlorpyrifos residue in green leaf and black tea at different plucking intervals.

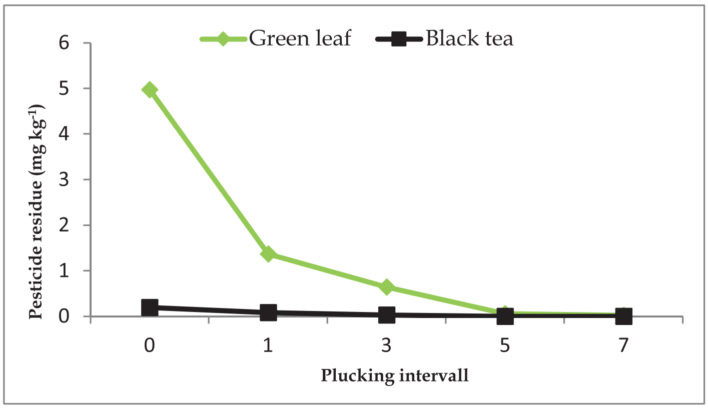

Fig. 2. Degradation of quinalphos residue in green leaf and black tea at different plucking intervals.

Table 3. Percent degradation of pesticide residue from green leaf to black tea at different plucking intervals.

Days after spraying (DAS)	Chlorpyrifos			Quinalphos		
	Green leaf	Black tea	% degradation	Green leaf	Black tea	% degradation
0	7.822	0.893	88.58	4.973	0.193	96.12
1	1.975	0.531	73.11	1.364	0.079	94.21
3	0.645	0.189	70.70	0.640	0.025	96.09
5	0.446	0.107	76.01	0.058	ND	-
7	0.164	0.013	92.07	0.022	ND	-
10	ND	ND	-	ND	ND	-

Safe harvest interval

It is normal to harvest tea leaves at various intervals following regrowth from earlier plucking. According to the aforementioned studies, the residue content increases with closer harvest intervals, while residue levels decrease with wider harvest intervals (Table 4).

Table 4. Safe harvest intervals for the tested pesticides in Bangladesh tea.

Chemical	Dose ha1	Residue level* (mg kg-1)	Safe harvest interval (days)
Chlorpyrifos	1.0 lit.	0.013	7
Quinalphos	0.5 lit.	ND	7

** Black tea at 7 PHI (Pre Harvest Interval)*

Table 5 compares the residue levels of the selected pesticides in Bangladeshi tea to those of several international organizations. Seven days after picking, the residue levels of the aforementioned pesticides are below the MRL established by the EPA, Codex Commission/FAO, EU, Japan, and German Law.

Table 5. Residue level of two commonly used organophosphate pesticides in Bangladesh tea compared to the international organizations.

Pesticides	MRL (mg kg-1) fixed by different organizations					
	EU	FAO/WHO	EPA	German Law	Japan	Bangladesh
Chlorpyriphos	0.1	0.1	-	0.1	3.0	0.013
Quinalphos	0.05	-	-	0.1	0.1	ND

Discussion

According to the results of Seenivasan and Muraleedharan (2011), Bishnu et al. (2009), Amaraweera & Wickramasinghe (2019), and Amirahmadi et al. (2013), none of the pesticides tested in the current study do not exceed the maximum residue levels specified by the EU, FAO/WHO, EPA, German Law, and Japan. In a three-year, extensive survey conducted by Seenivasan & Muraleedharan (2011), 912 samples of tea produced in south Indian factories were examined for pesticide residues from various pesticide classes, including organochlorine, organophosphate, and synthetic pyrethroid. Only 0.5 percent of the samples had traces of the targeted insecticides, according to the laboratory results. The residues in the remaining samples were within the upper limits for tea established by the EU, FAO/WHO, and the Prevention of Food Adulteration Act of the Indian government. Chlorpyrifos, cypermethrin, deltamethrin, ethion, heptachlor, dicofol, and endosulfan residue levels in tea plantations of the Dooars regions of West Bengal, India, were quantified by Bishnu et al. (2009). They reported that the consumption of tea that contains the residues of banned pesticides, such as heptachlor, may be harmful to the consumer's health.

Amaraweera & Wickramasinghe (2019) analyzed 15 tea samples from the leading tea exporters in Sri Lanka using five black tea grades (OP, BOP, FBOP, BOPF, and PEKOE). Some of the samples that were examined contained detectable pesticide residues. However, no samples went beyond the upper limits for residue set by Codex, the EU, and Japan. Amirahmadi et al. (2013) observed pesticide residue of tea in a market in Tehran. They examined 53 samples from several pesticide classes including synthetic pyrethroid, organochlorine, and organophosphate. Out of 53 samples, 15 (28.3%) were found to be contaminated with pesticides. None of the samples showed contamination over the maximum residue limit established by the EU and India.

In the current study, it was observed that the pesticide residues of chlorpyrifos and quinalfos gradually declined in following plucking intervals after spraying. Black tea made from the corresponding green leaf had far less pesticide residue. Therefore, tea production has a higher impact on lowering pesticide residues. Sood et al. (2004) reported a similar result. They also mentioned that the natural processes of rain, dew, evaporation, photolysis, biodegradation, growth dilution, and the interval between the application of pesticides and the harvesting of the tea leaves caused the pesticides sprayed on tea bushes to deteriorate. During manufacture, insecticides in the green leaves deteriorate as a result of evaporation and heat degradation. When making tea, mostly drying, Chen & Haibin (1988) observed a 30–60% reduction in pesticide residue in tea during manufacturing process.

The amount of pesticide residue, quality, and tea yield are all affected by the time between the last application and harvest of the crop (PHI). According to GAP (Good Agricultural Practice), PHI is 7 days (Chaudhuri, 2008). Following the approved PHI, the maximum residue level (MRL) in made tea is taken into consideration.

Conclusion and Recommendations

To circumvent non-tariff trade obstacles under World Trade Organization (WTO) regulations, all necessary steps must be taken for this export-oriented item to maintain residue levels below the MRL. According to FAO recommendations, other criteria of residue-level such as the No-observed-Adverse Effect Level (NOAEL), Acceptable Daily Intake (ADI), Food Factor, and Maximum Permissible Intake (MPI) should be taken into account. These requirements make it possible to strictly adhere to the MRL and PHI in order to gain access to the global market. Therefore, the scientific method should be consistent in its practical and regular application for the sake of the tea sector. The GAP states that the PHI is 7 days. The approved PHI is used to fix the MRL in made tea. The study revealed that 7 day PHI may be suggested for the aforementioned pesticides in Bangladeshi tea. When necessary, BTRI-approved insecticides with prescribed dosage should be used.

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