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Research Paper

Estimation of Pesticide Residue in Surface Water and Acute Toxicity of DDT and Chlorpyrifos on Common Carp in Coffee Plantations of the Western Ghats Region, Chikkamagalur, Karnataka

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A B S T R A C T

The study estimated the pesticide residues in 40 surface water samples from four locations of arabica coffee plantations in the Western Ghats. Residual analysis of water samples confirmed the contamination of both organochlorine (OC) and organophosphate (OP) pesticides in surface water. OC pesticide residues present in the range 0.01 - 1.10 µg/L, with the maximum presence of HCH, heptachlor, DDT, and DDT isomers. Chlorpyrifos present in the range of 0.02-.069 µg/L among the targeted OP pesticides. Acute toxicity estimated by using common carp fish for both p', p' DDT, and chlorpyrifos. LC_{50} value of p', p' DDT, and chlorpyrifos was 0.023 and 0.403 mg/L, respectively. This study confirmed the presence of excessive pesticide contamination in surface water and its toxic effect on common carp.

***Key words*:** Contamination, Fish, Residue, LC_{50}, Western Ghats, Agriculture, Environment

All over the world, agriculture practices dependent on several hundred pesticides [1]. The majority of them are organochlorine and organophosphates insecticides [2]. The usage of these non-degradable hazardous chemicals in pest management leads to pollution of water and soil in ecosystems [3]. Contaminations by legally banned pesticides in the agriculture environment are maybe due to their long persistence or their continued usage in agriculture [1]. Usage of these pesticides reaches the nearby water source by runoff during the rain and washing of pesticide spray equipment and disposing of pesticide containers in the surface water [5]. The Western Ghats region of Karnataka state is one of the important coffee-growing areas in India [6]. Like other agricultural practices, coffee growers also use several kinds of pesticides for their crop protection against insect pests of coffee [7]. Coffee agroforests are more fragile because of the presence of several native shade trees in the hilly region, which naturally includes many water bodies, i.e. ponds, streams, and rivulets [8]. These water bodies are a sink for the agricultural toxic chemicals in the coffee agronomy. The pesticide residues in the coffee agroecosystems have a deleterious effect on non-target organisms, soil, water, atmosphere, and food [9]. The effect of hazardous chemicals on the ecology, especially in water bodies, is directly dependent on the residual concentration in them. Rampant use of pesticides in agriculture leads to the contamination of nearby water bodies with pesticide residues [10], [11]. Accumulation of the pesticide residues majorly affects the life around the environment, including man [12].

Toxicity analysis is the best tool for estimating the impact of pesticide pollution on aquatic organisms [13]. Fishes are one of the best tools to check the toxicity in aquatic media as the absorption rate of pesticides through their body surface, and gill is very high, and they are also involved in the food chain [14], [15]. Acute toxicity in fishes can determine the contaminant pesticide potency to harm the aquatic environment as well as man. There was no studies on the estimation of pesticide residues in surface water and their toxic effects in the coffee plantations area of Western Ghats. Farmers are using water sources in the coffee plantations for drinking and domestic purposes. Moreover, they also use naturally grown or cultivated common carp (*Cyprinus carpio* L.) (Pisces: Cyprinidae) in these water sources as food [16]. Therefore, this study aimed to estimate the level of pesticide residues in surface water of coffee plantations and their toxic effect (LC_{50}) on the common carp.

MATERIALS AND METHODS

The arabica coffee plantations were selected within 50 km^2 from Chikkamagaluru town (Karnataka) as a study area. The climate of the Chikkamagluru region falls under the category of the tropical wet area with an annual average temperature range of 15° to 25°C, relative humidity 70-80%, and annual rainfall range 1600-2500 mm. About 80% of the rainfall is between May and September. Study areas have a moderate slope with an elevation range of 1000-1500m from sea level. Sampling areas represent the northern, southern, and western hilly regions of Chikkamagaluru town. The Eastern part of the Chikkamaglauru town is a low land area. In each

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direction; the study area represents one sampling location, i.e. a) L1(13°19'25.06"N 075°39'51.7"E) b) L2 (13°21'10.4"N 075°41'45.0"E) c) L3 (13°14'47.5"N 075°44'42.7"E) and d) L4 (13°26'14.6"N 075°36'48.4"E) (Fig 1).

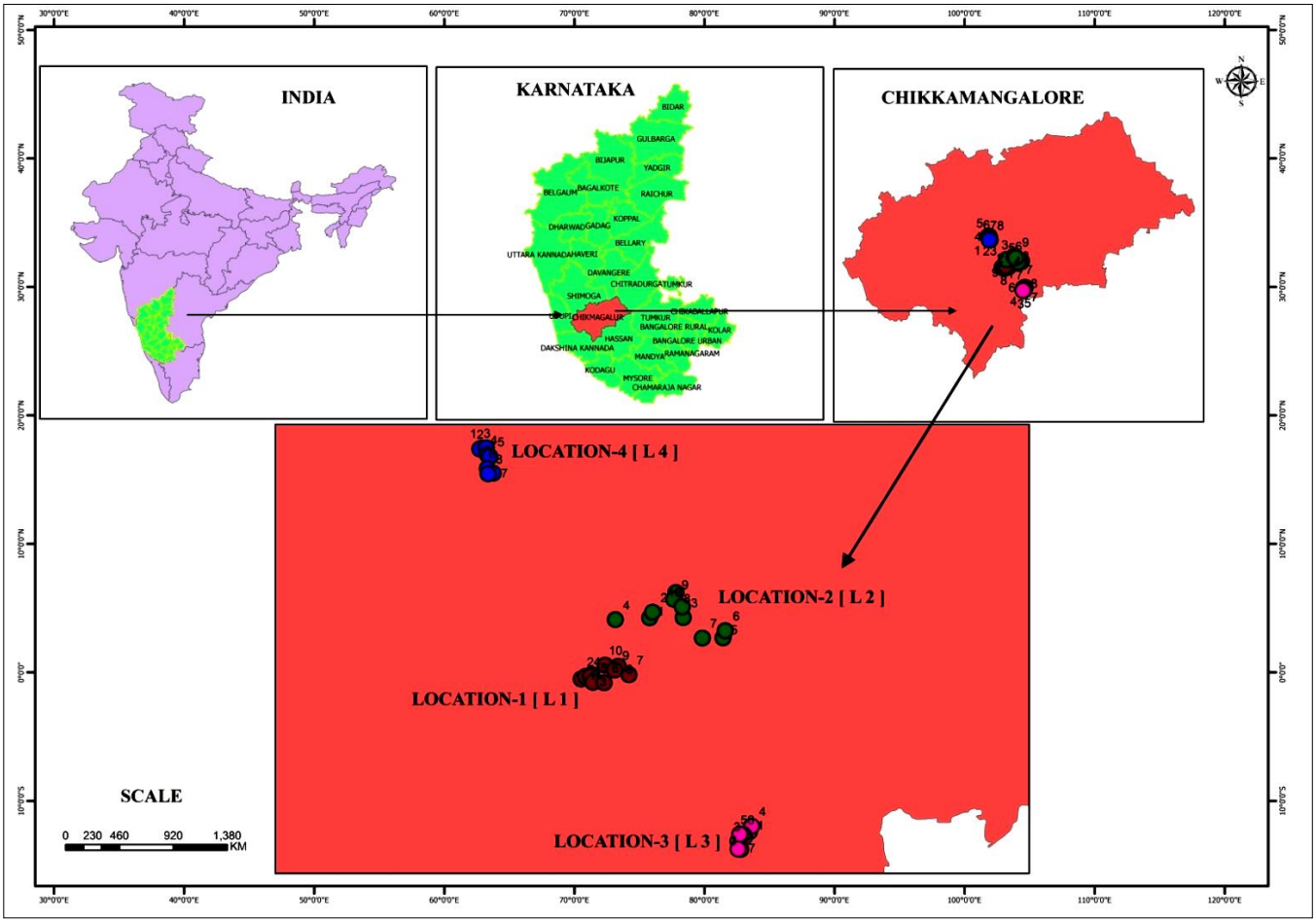

Fig 1 Map showing study areas of coffee plantations in Chikkamaglaur district with Sampling locations

Sample preparation for analysis

Reference OC pesticides (16 components), OP pesticides (4 components), solvents, and chemicals were obtained from Supelco, Sigma Aldrich, and Merck (Merck Darmstadt, Germany). Solvents, i.e. ethyl acetate, n-hexane, and diethyl ether used for sample processing and analyses were of HPLC grade. Pesticides residue extraction and clean-up were performed according to the method developed by [17] with small modifications. From the stored water samples, 250 ml was taken in a 500ml amber colour conical flask and added 5-6 g magnesium sulphate, 1-2 g sodium chloride, and 50 ml 15% diethyl ether in n-hexane. The mouth of the flask was covered with aluminum foil and gently shaken on a rotatory shaker (Orbitek Mumbai, India) for one hour. From this mixture, the organic phase was collected. The same extraction process was repeated once by adding 50ml of n-hexane to the sample mixture. The twice extracted solution was collected in a 500 ml separating funnel and passed through a glass wool bed containing sodium sulphate anhydrous crystals, and the filtrate concentrated up to 10 ml by the rotatory evaporator (Buchi Switzerland) and divided into two equal parts. One part was used for the estimation of OC pesticides and the other part for the estimation of OP pesticides. Residues from the evaporated extraction for both pesticides dissolved in n-hexane (approximately 1 ml) and transferred to GC vail for estimation.

Operating conditions

(1) *OC insecticide residues*: Detector- micro ECD (63Ni), column- J&W122-5032 fused silica capillary column (30 m length, 0.32 mm dia, 0.25 µm film thicknesses) with the pulsed split less system. Temperatures; 60°C (1 min) at 25°C min^{-1} to 170°C (0 min) at 10°C min^{-1} to 280°C (8 min), the total run time was 24.4 minutes. The injection port 280°C, detector 290°C; carrier gas- N_2, flow rate 1.2 ml min^{-1}, pulsed split less injection mode with pulse pressure 25psi and the injection volume was 0.1 µL.

(2) *OP insecticide residues*: Detector- Gas chromatography-mass spectrometry (GC–MS) 7890A gas chromatography coupled to a 5975C mass spectrometer and operated using MSD Chemstation software. Column- Agilent 19091S-433 (30 m length, 0.25 mm inner dia, 0.25 µm film thickness) coated with biphenyl and dimethylsiloxane stationary phase. Temperature 80°C (0.5 min) at 12°C min^{-1} up to 180°C (4 min) at 8°C min^{-1} to 220°C (0 min) and finally at 45°C min^{-1} up to 300°C (2 min). The total run time was 22 min. The temperature of the injector and interface was 280°C and 300°C, respectively. Helium was used as the carrier gas at a flow rate of 1.2 ml per min with pulsed splitless injection with 2 µL volume. The mass spectrometer was operated in the electron impact (70 eV) selected ion monitoring (SIM) mode.

Limits of detection (LOD) and Limit of quantification (LOQ) were calculated for water samples, as followed by [18]. Recoveries in water samples were in the range of 64–136 and 85–108 at the fortification levels of LOQ for OC and OP pesticides, respectively.

Bioassay test for toxicity

Test chemicals

DDT (purity 98.0%) and chlorpyrifos (purity 99.5%) were procured from Hindustan Insecticide Limited, Cochin (India). Stock solutions of DDT and chlorpyrifos prepared in analytical grade acetone (purity 99%), and all working solutions prepared from this stock solution. The concentration of acetone was kept at <0.02% in all pesticide solutions used for testing.

Test organisms

The common carp (mean body length, 3.2 ± 0.01 cm; mean body weight, 0.95 ± 0.05 g) juvenile life stages were collected from Karnataka State Fisheries Research and Information Centre, Hesaraghatta, Bengaluru, India for an acute toxicity test. The fishes acclimated to experimental conditions for 15 days using dechlorinated tap water to maintain the mortality <10% at dissolved oxygen concentration remained above 7 mg/L, and pH 7.4 ± 0.2. The water temperature adjusted to 22 ± 1°C, and the photoperiod was 12 h of light and 12 h of dark. Fishes were fed once a day with commercial food according to the manufacturer's guidelines during the acclimation period.

Acute toxicity test

Five different concentrations of DDT (0.01, 0.02, 0.03, 0.04 and 0.05 mg/L) and chlorpyrifos (0.1, 0.3, 0.5, 0.7 and 0.8 mg/L) were used by adding respective stock solution for common carp bioassay. For each DDT and chlorpyrifos, 15 aquaria containing 10 L dechlorinated water was used for the test. The control group kept in dechlorinated water mixed with a solvent (acetone <0.02%), each of the test and control group was set up in triplicate.

Following the range-finding method, toxic doses were calculated. The values of the water quality variables were determined, according to [19]. The range for water quality were as follows: temperature (20-23°C), Hardness (205-306 mg/L), dissolved oxygen (7.0-4.48 mg/L), conductivity (396-418 µs/cm) and pH (7.3-74). For the evaluation of toxicity, in 15 aquaria (triplicate of each concentration) of 75 fishes (each aquaria 5 fishes) was maintained for DDT and chlorpyrifos separately. The tests were exposed to the fishes for 96 h under a 12:12 h light: dark day and night condition. Mortality was recorded every 12 hours. The dead fish were removed from the aquaria immediately. Feeding of fishes stopped during the bioassay experiment. Acute toxicity of the DDT and chlorpyrifos in common carp was determined by Probit analysis on log10-transformed values by using the SPSS window software 25 version.

RESULTS AND DISCUSSION

The result of surface water samples analysis from four locations of the coffee plantations (Table 1) affirmed the presence of ∑HCH (alpha, beta, gamma, delta hexachlorocyclohexane), ∑aldrin (aldrin+dieldrin), ∑heptachlor (heptachlor + heptachlorepoxide), ∑endosulfan, (endosulfan 1 + endosufan 2), ∑DDT (p',p'DDD + p',p'DDE + p', p'DDT), ∑endrin (endrin + endrin aldehyde), methoxychlor and chlorpyrifos pesticide residues. In four sampling locations, L1 and L2 were more contaminated than L3 and L4. Among various pesticides, ∑HCH, ∑aldrin, ∑heptachlor, ∑endosulfan, ∑DDT, ∑endrin, methoxychlor, and chlorpyrifos were consistently present. OP pesticides, i.e., dimethoate, malathion, M-parathion, were present in below detection level (BDL) in all locations. The highest concentration of ∑HCH was 0.13µg/L, and in 13 samples of L1 showed residue concentration more than the quantification level. The concentration of ∑aldrin was in the range of 0.01-0.11 µg/L and ∑heptachlor in the range 0.01-1.19 µg/L in L1 and L2 locations. Samples of Location 1 and 2 showed the range of 0.02-0.12 µg/L and 0.01-0.04 µg/L of ∑endosulfan and ∑ DDT, respectively. ∑Endrin and methoxychlor were in the range of 0.01-0.12and µg/L in L1and L2 locations. Among OP residues, only chlorpyrifos was in the range 0.02-0.69 µg/L in L1 location.

Table 1 Organochlorine and organophosphate pesticide residues (µg/L) in surface water from four sampling locations of coffee plantations in Chikkamaglauru

Location (No. of samples)	L1 (13)	L2 (11)	L3 (06)	L4 (10)
OC-Insecticides	n (mean, SE, range)	n (mean, SE, range)	n (mean, SE, range)	n (mean, SE, range)
∑ HCH	13 (0.03, 0.008, 0.013-0.13)	04 (0.004, 0.002, 0.01-0.016)	BDL	BDL
∑ Aldrin	12 (0.03,0.01,0.01-0.11)	02 (0.01, 0.007, 0.01-0.03)	BDL	BDL
∑ Heptachlor	12 (0.59,0.17, 0.01-1.19)	08 (0.004 ,0.001, 0.01-0.019)	BDL	BDL
∑ Endosulfan	2 (0.07,0.05, 0.02-0.12)	02 (,0.02,0.01, 0.03-0.05)	BDL	BDL
∑ DDT	06 (0.02,0.007, 0.01-0.04)	2 (0.008, 0.006, 0.01-0.02)	BDL	BDL
∑ Endrin	04 (0.04,0.02, 0.01-0.12)	2 (0.006,0.004, 0.01-0.02)	BDL	BDL
Methoxychlor	11 (0.01, 0.005, 0.01-0.06)	2 (0.02, 0.01, 0.01-0.04)	BDL	BDL
OP-Insecticides				
Dimethoate	BDL	BDL	BDL	BDL
Malathion	BDL	BDL	BDL	BDL
M-Parathion	BDL	BDL	BDL	BDL
Chlorpyrifos	2 (0.47, 0.33,0.02-0.069)	BDL	BDL	BDL

∑HCH = Alpha HCH + gama HCH + beta HCH + delta HCH
∑Aldrin = Aldrin + Dieldrin
∑Heptachlor = Heptachlor + Heptachlorepoxide
∑Endosufon = Endosulfan1 + Endosufan2
∑DDT = p',p'DDD + p',p'DDE + p',p'DDT
∑Endrin = Endrin + Endrin aldehyde
n = Above LOQ of OC and OP residues in total number of samples
BDL = below detection limit

As per European Commission for drinking water standard, the total pesticide level should not exceed 0.5 µg/L and individual pesticide should not be more than 0.1 µg/L [20]. Samples of Location 1 and 2 were exceeding the standard indicated by European Commission for drinking water requirements. However, they were within the guideline value of individual pesticides assigned by the World Health Organization [21]. In India, the surface water contamination by the effect of agricultural usage of the pesticides has been reported [22], [23], [24]. The contamination of these pesticide residues is disturbing the aquatic life in the water bodies [25] and also noticed their bioaccumulation [26]. Measuring the toxicity of the contamination and adverse effects of pollutants need assessment of water quality and toxicity [27].

Table 2 Relation between the concentration and the mortality rate of common carp in p,' p' DDT, and chlorpyrifos

Concentration (mg/L)	Log. Conc.	Number of Fish	Number of fish dead	Morality (%)
		p',p' DDT		
Control	0.00	15	0	0
0.01	-2.00	15	1	6.7
0.02	-1.70	15	6	40
0.03	-1.52	15	9	60
0.04	-1.40	15	12	80
0.05	-1.30	15	15	100
		Chlorpyrifos		
Control		15	0	0
0.1	-1.00	15	1	6.7
0.3	-0.52	15	6	40
0.5	-0.30	15	7	46.7
0.7	-0.15	15	9	60
0.8	-0.10	15	15	100

The (Table 2) shows the relation between pesticides (p',p' DDT and chlorpyrifos) and the mortality rate of Common carp. The mean LC_{50} of p',p' DDT, and chlorpyrifos on common carp was 0.023 mg/L and 0.403 mg/L, respectively. Fishes were trying to avoid the test solution by an immediate behaviour response like fast swimming, jumping, darting, hanging vertically, and random movements in p',p' DDT, and chlorpyrifos test tanks. Behavioural changes of the fishes were the evidence of toxic effects of the chemicals during the test period [28]. At higher concentration, fishes showed erratic swimming with jerky movements with more secretion of mucus. Finally, they settled down at the base of the test tank and died.

Table 3 Estimated LC values and confidence limits

P', p, DDT		95% concentration limits	
Point	Concentration	Lower	Upper
LC 1	0.007	0.003	0.01
LC 5	0.01	0.005	0.014
LC 10	0.012	0.007	0.016
LC 15	0.014	0.008	0.017
LC 20	0.015	0.01	0.019
LC 50	0.023	0.019	0.028
LC 80	0.036	0.03	0.049
LC 85	0.04	0.033	0.057
LC 90	0.046	0.037	0.069
LC 95	0.055	0.043	0.093
LC 99	0.079	0.056	0.164
Chlorpyrifos		95% concentration limits	
LC 1	0.056	0.01	0.111
LC 5	0.1	0.028	0.168
LC 10	0.136	0.048	0.21
LC 15	0.168	0.069	0.245
LC 20	0.198	0.092	0.279
LC 50	0.403	0.288	0.535
LC 80	0.821	0.605	1.528
LC 85	0.968	0.691	2.025
LC 90	1.191	0.811	2.907
LC 95	1.619	1.019	5.013
LC 99	2.881	1.542	14.12

These behaviours were not observed in control. The results obtained from acute static 96-h toxicity experiments of p', p'DDT, and chlorpyrifos on common carp, LC_{50} values with confidence limits were presented in (Table 3). Several studies confirmed the toxicity effect of both p',p' DDT, and chlorpyrifos on fishes [29], [30], [31], [32], [33]. The difference in LC_{50} values of toxicants depends on the withstanding capacity of test animals to toxicants [34]. Acute toxicity results in an adverse effect on the survival ability of test animals as the concentration of test doses increased as observed in the present study (Table 2). This study revels LC_{50} value of the p', p' DDT (0.023 mg/L), and Chlorpyrifos (0.403 mg/L) on common carp fish observed less than the mean contamination (DDT 0.024 mg/L and chlorpyrifos 0.47 mg/L) level of these pesticides in the surface water of the study locations. Nevertheless, the toxic effects of the theses pesticide contamination not only depending on the quantity, but influenced by several other environmental and biological factors [35]. The present study estimated the contamination of excessively used run-off pesticide residues in surrounding water bodies and their toxic effects on aquatic organisms in coffee plantations.

CONCLUSIONS

The widespread uses of synthetic pesticides contaminate the environment and cause adverse effects to organisms and human. Especially agricultural chemicals contaminate the abiotic components especially water and soil. This study indicated the contamination of OC and OP pesticide residues in the surface water bodies in coffee plantations. Affirmative acts of the farmers on the safe use of these chemicals are well needed. Fish is the last chain of the aquatic ecosystem usually affected by these toxicants, and the same may affect other animals, which feed on them. The pesticide p', p' DDT, and chlorpyrifos tested in this study confirmed the high toxicity to fish. This study revealed the pesticide contamination of surface water in coffee plantations and a major threat to aquatic life. Therefore, there is urgent need to protect the environment of coffee plantations in the fragile Western Ghats region.

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