Estimation of Lethal Concentrations of Chlorpyrifos and Its Effect on Behavioral and Hematological Parameters in Exposed *Cyprinus Carpio*

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Pesticides are harmful to humans, birds, fish, and other organisms. Chlorpyrifos is an insecticide (organophosphate) used universally in farming and home settings around the world to control pests. Assessing damage caused by pesticides is very crucial to find out their harmful effects on non-targeted fauna. The current research was designed to identify the toxicity and harmful impact of Chlorpyrifos on the mortality, behaviour, and haematology of Cyprinus carpio. The lethal concentrations were observed for 24, 48, 72, and 96 hrs. The behavioral and hematological alterations were also observed in the groups exposed to Chlorpyrifos. The LC0 was determined as 0.08, 0.10, 0.15, and 0.19 mg/L for 24, 48, 72, and 96 hrs. The LC50 was found to be 0.160, 0.18, 0.21, and 0.48 mg/L for 24, 48, 72, and 96 hrs, while the LC100 values were found to be 0.32, 0.64, 0.96, and 1.28 mg/L for 24, 48, 72, and 96 hrs. Changes in behaviour, such as loss of body balance, irregular swimming, etc, have been observed. A significant change in haematological parameters has been determined in experimental groups as compared to the control ($p < 0.05$). It has been concluded that chlorpyrifos has a lethal effect on Cyprinus carpio.

Keywords: Chlorpyrifos; *Cyprinus Carpio*; Lethal Concentration; Pesticide; Swat and Toxicity.

Introduction:

Pesticides like Chlorpyrifos (CPF) and Cypermethrin (CYP) are extensively used in Pakistan for the large-scale control of pests and insects in crops, vegetables, and fruits [1]. However, these pesticides belong to a class of environmental toxins known to cause significant harm to non-target organisms. Numerous studies have reported both acute and chronic toxic effects of these chemicals on aquatic life, particularly fish, with residues commonly detected in various fish tissues [2]. Fish may exhibit modifications in various haematological parameters when exposed to different chemical substances, which are commonly used to assess fish health. Haematology has long been used to detect physiopathological changes in response to a diversity of stressors. Consequently, the most frequent way to find out the sublethal impacts of contaminants is to use haematological tests [3].

Chlorpyrifos (CPF) is a broad-spectrum organophosphate insecticide extensively used in agricultural and domestic applications worldwide [4]. It is the second most widely used organophosphate (OP) [5]. Organophosphates (OPs) have gotten a lot of attention in Pakistan for agricultural operations since they are more effective, selective, inexpensive, and less persistent than other groups of pesticides like organochlorines (OCs), pyrethroids, and carbamates [6]. It has the potential to cause oxidative stress as well as impair physiological and antioxidative functions [7]. Chlorpyrifos is an OP insecticide that suppresses acetylcholinesterase activity, which is essential for modulating nerve impulses in cholinergic synapses [8].

The common carp (*Cyprinus carpio* Linnaeus, 1758) is a freshwater fish that is frequently used as an experimental model for aquatic ecotoxicology [9]. These species are simple to raise in the lab and are suggested for evaluating the special effects of contaminants in aquatic habitats. The common carp is a widely distributed species that is widely bred in aquaculture. Furthermore, past investigations relating to laboratory tests, field training, or biomonitoring programs have demonstrated the effectiveness of Cyprinus carpio as a sentry species [10].

Chlorpyrifos is mostly used in agriculture to defend corn, fiber, and fruit crops in contradiction of insects [11]. Pesticides are man-made chemicals that are widely utilized as plant protection products around the world, mainly to protect harvests from harm and increase crops [12].

Pesticides are responsible for around 220,000 fatalities worldwide. Pesticide exposure can have serious adverse effects, particularly on human health. It has been associated with an increased risk of several cancers, including leukemia, non-Hodgkin lymphoma, Hodgkin's disease, and skin cancer. Pesticide coverage has also been related to a variety of neurological problems, respiratory complaints, hormone imbalances, and reproductive abnormalities [13]. Fish can absorb insecticides through three primary routes: (i) direct absorption through the skin, (ii) uptake via the gills, and (iii) ingestion through contaminated food or water. They can result in acute and chronic poisoning, as well as anomalies like changes in fish haematological responses, antioxidant activities, and histopathological changes [14]. Pesticides can impact aquatic organisms through both direct and indirect pathways. One direct route is surface runoff from agricultural fields, while indirect exposure can occur through bioaccumulation within the food chain [15]. Chlorpyrifos' sublethal toxicity in aquatic habitats can cause morphological, neurobehavioral, oxidative, biochemical, histological, haematological, developmental, and other changes in non-target creatures in general, and fish in particular, die in large numbers at fatal doses [1].

The current study was aimed at finding the harmful effects of Chlorpyrifos on *Cyprinus carpio* at District Swat.

Objectives of the study:

The objectives of the current study included;

1. To determine the lethal concentrations of Chlorpyrifos for *Cyprinus carpio*.
2. To find out behavioral changes in *Cyprinus carpio* as a result of exposure to Chlorpyrifos.
3. To find out whether hematological changes in *Cyprinus carpio* occurred due to the exposure to chlorpyrifos.

The harmful effects of Chlorpyrifos on the behavioral and hematological parameters of *Cyprinus carpio* in the District. The major concernof the current study was to identify the toxicity and harmful impact of Chlorpyrifos on the mortality, behaviour, and haematology of *Cyprinus carpio*.

Novelty statement:

Biodiversity is decreasing day by day due to environmental changes and an increase in the human population. The use of toxic materials such as pesticides by farmers is very harmful to animal fauna generally and aquatic fauna specifically. Pesticides affect non-target organisms and cause mortality. In the study area, chlorpyrifos is widely used by farmers, which can affect the aquatic fauna. The effects of chlorpyrifos on fish, especially *Cyprinus carpio*, and its lethal concentrations have not been properly studied. This study investigates the acute toxicity caused by chlorpyrifos as well as the effects of chlorpyrifos on haematological and behavioural parameters in exposed *Cyprinus carpio*. *Cyprinus carpio* is used as a biological and ecological marker of environmental stresses, so the study provides conclusive information about the threats to diversity at District Swat, which has not been done before.

Materials and Methods:

Fish sample collection and their acclimatization: The fish samples (*Cyprinus carpio*) (40±3.10g) were taken from a local fish farm and were transferred to the laboratory in oxygenated bags. The acclimatization of fish was done in aquaria with a capacity of 20 liters of water for about 2 weeks. The fish were fed as per body weight twice a day during the acclimatization period with commercial pellet feed, and the water of the aquaria was changed twice daily. The temperature of the water was 25 ± 2°C with 7.0 pH. The fish were exposed to a 12:12 light/dark photoperiod [16][17].

Experimental design & pesticide solution preparation: After acclimatization the fish were divided into four groups labelled A, B, C and D. Group A was marked as a control group while B, C and D groups (experimental) were treated with different concentrations of pesticide for determination of safe concentration (LC0), sub-lethal concentration (LC50) and acute lethal concentration (LC100) for 24, 48, 72 and 96 hours via probit analysis. The different concentrations of Chlorpyrifos i.e., 0.08mg/L, 0.10mg/L, 0.15mg/L, 0.19mg/L, 0.160mg/L, 0.18mg/L, 0.21mg/L, 0.48mg/L, 0.32mg/L, 0.64mg/L, 0.96mg/L and 1.28mg/L were used in the experiments.

Collection of blood and analysis:

Blood was drawn from the control and treated groups to evaluate potential haematological alterations. The fish were anaesthetized with benzocaine (MS-222), and then the blood was collected from the caudal vein of the fish and stored in anticoagulated (K3-EDTA) tubes. The complete blood count (CBC) was performed on the same day from the blood via Hematology Analyzer Sysmex XP-300 [16].

Statistical Analysis:

The data was statistically analyzed through IBM SPSS Version 21 software. A statistical test (one-way ANOVA) was done to compare the data obtained from control and experimental groups [18].

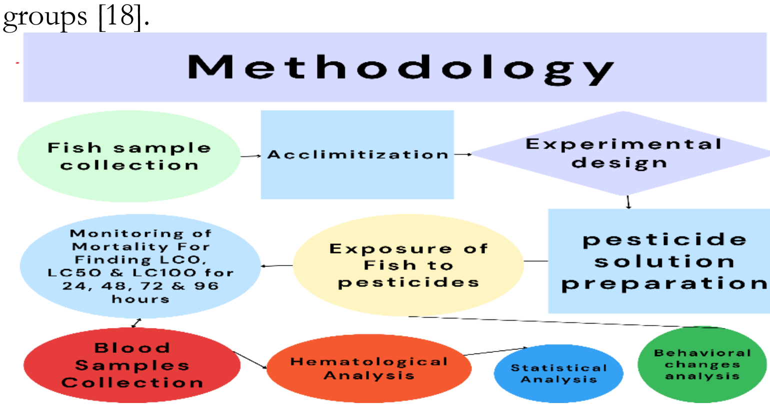

Figure 1. Flow chart of Methodology

Results:

LC100, LC50, and LC0 levels of chlorpyrifos to *Cyprinus carpio*:

For *Cyprinus carpio*, the pesticide's LC values demonstrated a time-dependent reaction. As LC100 means 100% mortality, LC50 means 50% mortality, and LC0 means no mortality of fish in the experiment. The concentration of the test substance representing zero mortality was determined to be 0.19 mg/L at an exposure time of 24 hours, 0.15 mg/L at 48 hours, 0.10 mg/L at 72 hours, and 0.09 mg/L at 96 hours; thus, these values represent the acute LC_0.

Concentrations recorded as having induced 50% mortality were termed as LC_{50}, giving 0.48 mg/L at 24 hours, 0.21 mg/L at 48 hours, 0.18 mg/L at 72 hours, and 0.160 mg/L at 96 hours. Similarly, the respective LC_{100}, inducing 100% mortality, were established at 1.28 mg/L at 24 hours, 0.96 mg/L at 48 hours, 0.64 mg/L at 72 hours, and 0.32 mg/L at 96 hours. Such results indicate that the lethal concentration of the test substance decreased with increasing exposure times (Table 1) (Figure 2).

Behavioral changes in exposed fish:

Normal behavior was observed in the fish belonging to the control group. The fish in the exposed groups showed abnormal behaviors such as increased opercular movements, irregular swimming, over-secretion of mucus from the whole body, loss of body equilibrium, jumping out of the water, becoming motionless at the bottom, opening mouths before death, altered feeding, and social behaviors. The fish died and fell to the bottom or remained at the surface of the water.

Hematological analysis:

Haematological analysis was conducted on fish exposed to a Chlorpyrifos concentration of 0.160 mg/L for 96 hours. The levels of White blood cells (Figure 3), Platelets (Figure 12), mean corpuscular haemoglobin (Figure 8), mean corpuscular hemoglobin concentration (Figure 9), Platelet distribution width (Figure 14), Plateletcrit %(Figure 15) and Large platelet count (Figure 17) increased as compared to the control group ($p < 0.05$). The levels of red blood cells (Figure 4), haemoglobin (Figure 5), hematocrit (Figure 6), MCV (Figure 7), red cell distribution width-coefficient of variation (Figure 10), red cell distribution width-standard deviation (Figure 11), Mean platelet volume (Figure 13), and large platelet percentage (Figure 16) decreased as compared to the control group ($p < 0.05$). There were minor changes in Mean corpuscular volume and Mean platelet volume between the control and exposed groups ($p < 0.05$) (Table 2).

Table 1. Lethal concentrations of Chlorpyrifos to *Cyprinus carpio*

S.No	Exposure time	*LC0 in mg/L	*LC50	*LC100
1	24 hours	0.19	0.48	1.28
2	48 hours	0.15	0.21	0.96
3	72 hours	0.10	0.18	0.64
4	96 hours	0.08	0.160	0.32

*LC0=Lethal concentration 0, *LC50=Lethal concentration 50, *LC100=Lethal concentration 100

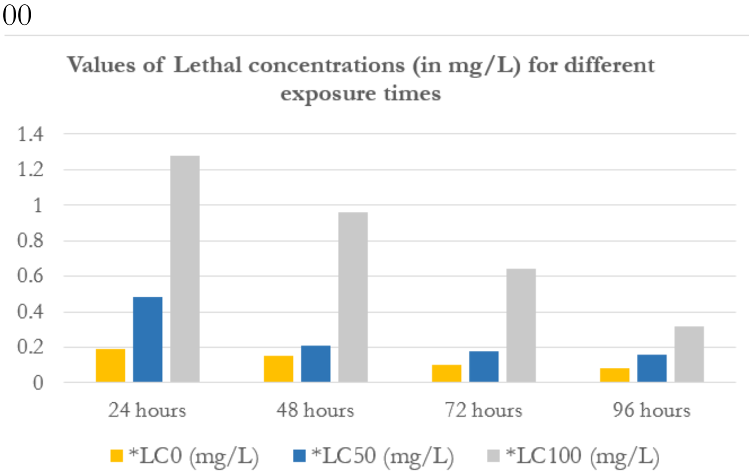

Figure 2. Levels of Lethal Concentrations for different exposure periods

Table 2. Mean levels of haematological parameters of *Cyprinus carpio* exposed to a concentration of 0.160 mg/l Chlorpyrifos for 96 hours

Blood parameter	Control group	Experimental group	p Value
WBC(10^6/uL)	11.32±.16	49.23±1.03	0.001*
RBC(10^6/uL)	3±.00	1.24±.01	0.001*
HGB(g/dl)	8.9±.05	4.7±.01	0.001*
HCT (%)	24.6±.01	10.1±.01	0.001*
MCV(fL)	81.8±.02	81.5±.01	0.001*
MCH(pg)	29.6±.05	37.7±.03	0.001*
MCHC(g/dL)	36.2±.04	46.2±.09	0.001*
RDW-CV (%)	16.8±.03	15.8±.03	0.001*
RDW-SD(fL)	55.8±.03	52.9±.03	0.001*
PLT(10^3/uL)	27±.18	56±.28	0.001*
MPV(fL)	8.5±.03	8.4±.03	0.014*
PDW	17.3±.01	18.5±.03	0.001*
PCT (%)	0.023±.00	0.047±.00	0.006*
P-LCR (%)	20.7±.03	19.5±.03	0.001*
P-LCC(10^9/l)	6±.41	11±.85	0.001*

%= Percentage, uL= microliter, fL=femtoliter, pg=picograms, dL=deciliter.

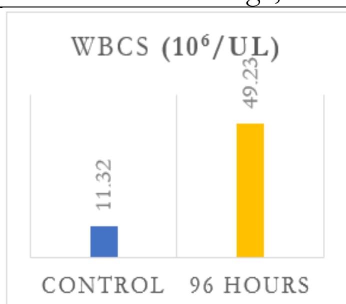

Figure 3. White blood cells

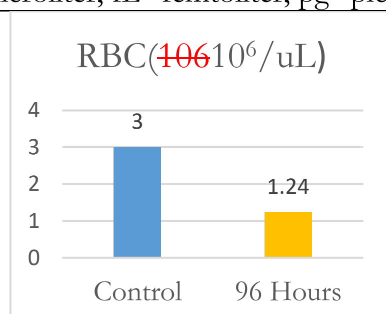

Figure 4. Red blood cells

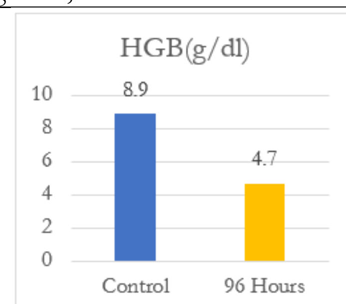

Figure 5. Hemoglobin

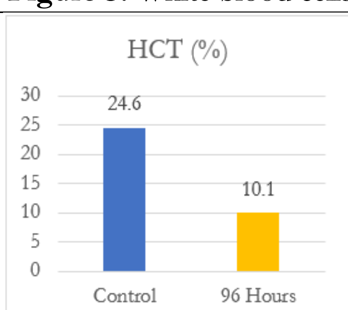

Figure 6. Hematocrit

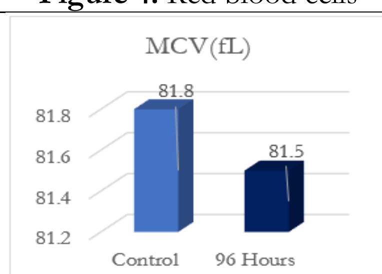

Figure 7. Mean corpuscular volume

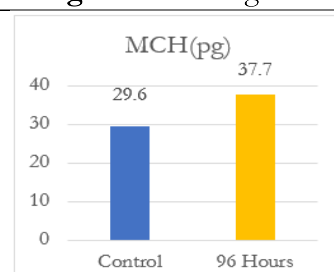

Figure 8. Mean corpuscular hemoglobin

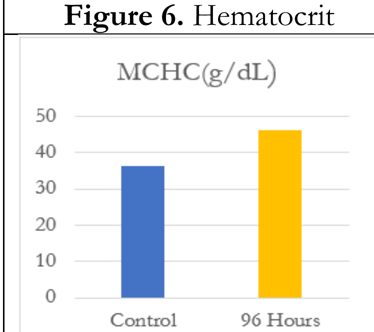

Figure 9. MCHC

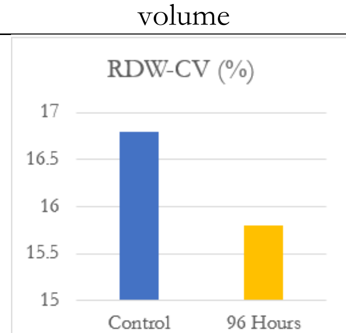

Figure 10. RDW-CV

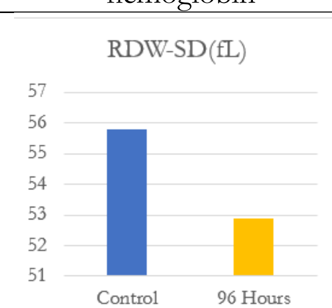

Figure 11. RDW-SD

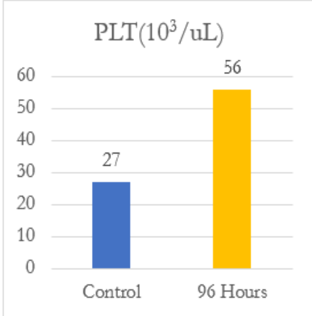

Figure 12. Plateletcrit

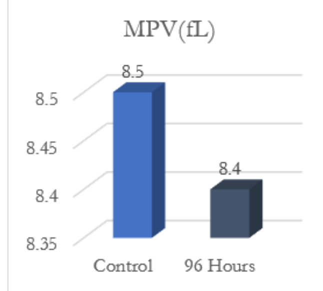

Figure 13. Mean platelet volume

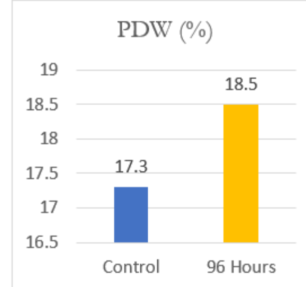

Figure 14. Platelet distribution width

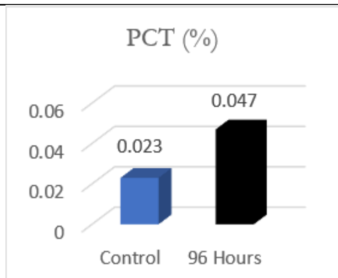

Figure 15. Plateletcrit (%)

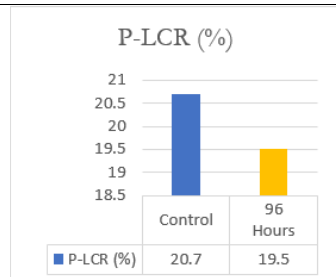

Figure 16. P-LCR

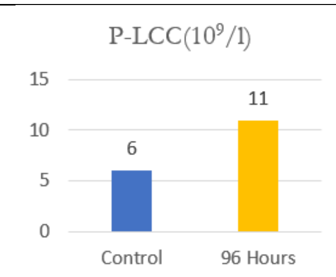

Figure 17. P-LCC

Discussion:

In the present study, various concentrations of chlorpyrifos were applied to determine the adverse impact on *Cyprinus carpio*. Experimental group fish samples showed abnormal behaviours as compared to the control group. In exposed fish samples, the abnormal behaviours were irregular swimming, increased mucus secretion, loss of balance, and efforts to jump out of the water. These behaviours were more apparent as the dosage of chlorpyrifos increased. A significant change was also observed in haematological parameters as compared to the control group ($p < 0.05$).

Author (2019) also conducted a study examining histo-haematological changes in Cnidarian fossils exposed to industrial effluents. Their study showed probable alterations in the haematological parameters and histology of fish. When fish were treated with higher concentrations, they showed behavioural responses, like erratic and jerky swimming, physiological malformation, histological, hematological, and biochemical changes, frequent surfacing and gulping, mucus secretion, an increase in operculum movements, and abundant secretions of mucus from the whole body [19]. Their study closely resembled the present research in terms of observed behavioral changes, as similar patterns were noted in the current investigation.

Authors (2009) studied the impacts of pesticide (chlorpyrifos) on common carp. They found the toxicity of chlorpyrifos to common carp fingerlings for 96 hours as 0.120 to 0.200 mg/L of chlorpyrifos (20% EC), with a lethal concentration (LC50) of 0.160 mg/L. Sublethal doses of one-seventh (0.0224 mg/L) and one-fourteenth (0.0112 mg/L) of the LC50 for exposures of 1, 7, and 14 days were determined. Various behavioral changes were observed, including hyperexcitability, erratic swimming, loss of balance, and tail bending. In addition, a few mortalities were recorded [20]. Their study is similar to the present study, as the LC50 for 96 hours was also 0.160mg/L, which was also reported in their work. Behavioural changes such as irregular swimming, loss of equilibrium, and hyperexcitability were also observed in the present study, similar to their study.

Authors (2008) conducted a study in which they observed significant alterations in the blood parameters of Common carp (*Cyprinus carpio*) exposed to Chlorpyrifos. Red blood cells decreased in the treated groups as compared to the nontreated group. Leucocyte levels increased in the pesticide-exposed groups compared to the control group, ranging from 9.688

to 15.302. Conversely, hemoglobin levels decreased in the experimental group, ranging from 4.450 to 3.633 g/dL. Plasma glucose is increased (91.035-115.029mg/100ml). Plasma protein is also decreased (6.705-5.601mg/L). While in the current study, Erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), mean platelet volume (MPV), and platelet large cell ratio (P-LCR) all decreased, according to the haematological analysis [21]. On the other hand, there was an increase in platelet count (PLT), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell count (P-LCC), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). These modifications show significant changes in blood parameters caused by exposure to chlorpyrifos. In the present work, the levels of Leucocytes, Platelets, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, Platelet distribution width, Plateletcrit, and Large platelet count increased as compared to the control group ($p < 0.05$). The levels of red blood cells, haemoglobin, hematocrit, red cell distribution width-coefficient of variation, red cell distribution width-standard deviation, and large platelet percentage decreased as compared to the control group ($p < 0.05$). While there were minor changes in Mean corpuscular volume and Mean platelet volume between control and exposed groups ($p < 0.05$)

Author (2019) studied the haematological parameters of Heteropneustes fossilis (Asian stinging catfish or fossil cat). Like RBC, HGB, MCH, MCV & MCHC decreased in the treated group as compared to the control, while WBC significantly increased in the treated group as compared to the nontreated group [19][22]. The current research showed major changes in the haematological parameters of fish subjected to 0.160 mg/L of chlorpyrifos for 96 hours. The white blood cell (WBC) count increased dramatically from 1132.5 to 49230, whereas the red blood cell (RBC) count decreased from 3.00 to 1.24. Haematocrit (HCT) also decreased from 24.6% to 10.1%, while haemoglobin (HGB) levels decreased from 8.9 g/dL to 4.7 g/dL. Platelet count (PLT) increased from 27 to 56, associated with an increase in plateletcrit (PCT) and platelet distribution width (PDW), indicating altered blood cell dynamics. Other measures, such as mean corpuscular volume (MCV), remained stable ($p < 0.05$).

Conclusion:

It is concluded that Chlorpyrifos affects behavior, and even lethal concentrations cause mortality in *Cyprinus carpio*. Chlorpyrifos also affected the blood parameters that increased or decreased from the normal range. Chlorpyrifos appeared to be a toxic insecticide because of its harmful effects on blood parameters.

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Author's contributions:

OZ and R collected samples and did experimental work; NU and SI formulated the research and wrote the manuscript; MY and AR formulated the results; I helped in the result analysis.

Conflict of interest:

The authors have no conflict of interest. All authors agree to publish the paper.

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