# EFFECTS OF DICHLORVOS ON FRESHWATER FISH ROHU (*Labeo rohita* Hamilton, 1822)




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A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture.

**SUBMITTED TO**

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April 2021

## DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere of the award for any degree. All sources of information have been specifically acknowledged by reference to author(s) or institution(s).

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## RECOMMENDATION

This is to recommend that the thesis entitled **"EFFECTS OF DICHLORVOS ON FRESHWATER FISH ROHU (*Labeo rohita* Hamilton, 1822)"** has been carried out by Ms. Bhawani Sapkota for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture. This is her original work and has been carried out under our supervision. To the best of our knowledge, thesis work has not been submitted for any other degree in any institutions.

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## LETTER OF APPROVAL

On the recommendation of supervisors "Prof. Dr. Kumar Sapkota and Mr. Prabesh Singh Kunwar" this thesis submitted by Ms. Bhawani Sapkota entitled **"EFFECTS OF DICHLORVOS ON FRESHWATER FISH ROHU (*Labeo rohita* Hamilton, 1822)"** is approved for the examination in partial fulfillment of the requirements for Master's degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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## CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. Bhawani Sapkota entitled **"EFFECTS OF DICHLORVOS ON FRESHWATER FISH ROHU (*Labeo rohita* Hamilton, 1822)"** has been accepted as partial fulfillment for the requirements Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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# LIST OF ABBREVIATIONS

| Abbreviated form | Details of abbreviations |
|---|---|
| AChE | Acetylcholinesterase |
| ANOVA | Analysis of variance |
| BCG | Bromocerol reagent |
| BW | Body weight |
| CBS | Central Bureau of Statistics |
| CFPCC | Central Fisheries Promotion and Conservation Center |
| DDT | Dichlorodiphenyltrichloroethane |
| DDVP | 2, 2-Dichlorovinyl dimethyl phosphate |
| DO | Dissolved Oxygen |
| EC | Emulsifiable Concentrate |
| EDTA | Ethylene Diamine Tetra Acetic acid |
| EPA | Environmental Protection Agency |
| FAO | Food and Agriculture Organization |
| Hb | Haemoglobin |
| IPM | Integrated Pest Management |
| LC | Lethal Concentration |
| MCH | Mean Corpuscular Haemoglobin |
| MCHC | Mean Corpuscular Haemoglobin Concentration |
| MCV | Mean Corpuscular volume |
| PCV | Packed cells volume |
| ppm | Parts per million |
| RBC | Red Blood Cells |
| SPSS | Statistical Package for Social Science |
| WBCC | White Blood Cells Count |

# ABSTRACT

Pesticides are applied to control the pests indoor and outdoor; however, their remarkable amount reaches the aquatic system through various routes like run-off, leaching, spray-drift, and effluent from factories. These pesticides are reported to have a negative metabolic impact on different non-target aquatic organisms like fishes. Thus, the present study was aimed to evaluate the acute toxicity of commonly used organophosphate pesticide dichlorvos to the freshwater fish Rohu (*Labeo rohita*). The experimental setup was designed to test the acute toxicity, advanced behaviour, and some haematological as well as biochemical analysis for a period of 96h. The $LC_{50}$ values for dichlorvos after 96hr treatment was found to be 11.36 mg/L. In treated fish, alterations in various behavioural patterns respiratory metabolism, opercular beat rate, and blood parameters were examined for sub-lethal end-points following 1h, 24 hr, 48 hr, 72 hr, and 96 hr exposure. Accelerated opercular beat rate ($P < 0.05$) was recorded in pesticide-exposed groups in comparison to control. Though the respiratory metabolism was not significantly affected, an increment in oxygen consumption rate was recorded. The pesticide stress caused a significant elevation in haemoglobin ($P < 0.01$) whereas total protein content was significantly dropped ($P < 0.05$). Glucose content was found to be increased ($P > 0.05$) in the pesticide group, compared to the control. Some general behavioural changes such as erratic movement, gulping, schooling, mucus secretion, equilibrium, aggregating behavior, and paleness in the body were observed in pesticide-exposed fish. The acute toxicity data reported in this study can be used to assess the tolerance level of Rohu to insecticide dichlorvos.

# 1. INTRODUCTION

## 1.1 General background

Pesticides are a group of toxic compounds used to kill animals, plants, insects, and pests in agricultural, domestic, and institutional settings. Pesticides are of two types: chemical-based and biological products-based. The extensive use of the chemical has been started after the identification of DDT as a potential pesticide by Paul Herman Muller in 1939. Pesticides include herbicide, insecticides, nematocides, molluscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial, and fungicide. Pesticides are used in agriculture to maintain high production efficiency to support the growing human population. However, pesticides are predominantly used in the agrarian sector, these days they are frequently practiced in the public health sector too (Dhital et al. 2015). Therefore, the use of pesticides is expected to increase soon (Patnaik and Patra 2006). The indiscriminate and intensive use of pesticides in agriculture and post-harvest technology is a threat to the natural water system, public health, and welfare of humankind (Tilak et al. 2007). In 2007, about 2363 million kg of pesticides were used in the planet with herbicides constituting the very best share of 950.7 million kg followed by 404.6 million kg of insecticides and 262.17 million kg of fungicides (EPA 2011). The appliance of pesticides is increasing worldwide and from 1996 to 2016, global pesticide use has increased by 46% (WHO and FAO 2019).

The pesticide was introduced in Nepal for malaria eradication and is now mostly being used in vegetables and crops (Sharma 2015). According to World Health Organization (WHO), the application of pesticides in commercial farming is exceptionally high in Nepal as pesticide consumption is increasing 10-20% per year. Total active ingredients used in the pesticides during 2011/2012 were about 345 thousand kg or liters (CBS 2014). According to World Health Organization, most of the pesticides banned in Nepal are at a moderately hazardous level. The average domestic use of pesticides in Nepal is far below as compared to developed countries and also below the global average but the pesticide use in certain crops and vegetables is higher than the national average (Gyawali 2018). Not all pesticides reach their target (Cessna and Allah 2009). Accounts of field application of pesticides in developed countries revealed that less than 0.1% of pesticides applied to crops reach the target pest, thus over 99% moves into an environment to pollute the land, water, and air (Pimental 2005). The runoff from treated areas enters the river and aquaculture ponds fed by rivers. Therefore, rainfall and runoff

water play a vital role to mix up the pesticides in the aquatic ecosystem (Tang et al. 2012). Most of the adjacent aquaculture ponds and rivers to the agricultural fields, are contaminated by pesticides (Begum 2004). The biotransformation and bioaccumulation of pesticides residues in water threaten the human and aquatic ecosystems.

### 1.1.1 Biology of Rohu (*Labeo rohita*)

*Labeo rohita* (Hamilton, 1822) commonly known as Rohu is a freshwater fish of the carp family. It's an outsized omnivore and extensively utilized in aquaculture. The Rohu is a crucial aquaculture freshwater species in South Asia (FAO 2018) and it doesn't breed in cultured ponds so, induced spawning is required to breed (De Graaf and Latif 2002, Nandeesha et al. 1990).

### 1.1.2 Organophosphate pesticides

Organophosphate pesticides (OP) are synthetic pesticides, which are frequently used in developing countries to control different agricultural pests (Banni et al. 2005). Organophosphorus pesticides function to inhibit cholinesterase. They bind with acetylcholinesterase (AChE), inhibition of AChE results in repeated, the uncontrolled firing of neurons leading to death, usually by asphyxiation as respiratory control is lost (Sparling and Fellers 2007). The most advantage of the OP pesticides includes their less persistence and low cumulative ability. Although, OP pesticides have been replaced by pyrethroid-based pesticides within the last 10-15 years but still intensively used today (Svoboda et al. 2001). Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an organophosphate pesticide commonly used to eradicate crustacean ectoparasites and treat sea lice on commercial fish farms (Murison et al. 1997, Varo et al. 2003) and equally effective against mushroom flies, aphids, spider mites, caterpillars, and whiteflies in the greenhouse, outdoor fruits, and vegetable crops (Lotti 2001). It is also commonly used to control household pests, in public health, and protecting stored products from insects (Das 2013). It had been first introduced in 1961 and has formula C4H7Cl2O4P and relative molecular mass to be 220.98 (CASRN 62-73-7, 1993). It is extensively used in most of the world including Asian countries (Sun et al. 2015).

DDVP is volatile, highly toxic by inhalation, dermal absorption, and ingestion, and readily absorbed through the skin. Rath and Mishra (1981) had reported the inhibitory effect of dichlorvos on Acetylcholinesterase (AChE) activity in Tilapia. DDVP was first banned by the United States Environmental Protection Agency in 1981 but is still

available in many countries including Nepal. This pesticide often finishes up leading to both lethal and sub-lethal effects on the fish and zooplankton whereas, at only 1.00 ppm, dichlorvos has the potential to point out both acute and chronic toxicity in fish (Gupta et al. 2008). This chemical had been commercially available since 1961 and has become controversial because its prevalence extends well beyond insects (Das 2013).

### 1.1.3 Acute toxicity and sub-lethal effects

Environmental pollution by toxicants has become one among the foremost important problems within the world (Chandran et al. 2005). The unmanaged and intensive use of pesticides in agriculture, animal husbandry, and post-harvest technology is creating a threat to the natural water system as well as public health (Tilak et al. 2007). Among the various reasons behind the water pollution such as untreated home and industrial sewage disposal, mining activities, and burning of fossil fuels, etc., overuse of pesticides is also equally responsible (Zhang 2018). Acute toxicity of a pesticide is known as the chemical's ability to cause the immediate effects (0-7 days) of a particular dose of the pesticide on a particular species (OECD 1992). The 96-h $LC_{50}$ tests are conducted to assess the vulnerability of organisms to particular toxic chemical substances. An $LC_{50}$ is a measure of how much chemical is required to kill 50% of the test population over some time. Chemical agents with lower $LC_{50}$ values are more toxic because their lower concentrations result in 50% of mortality in organisms. Over 98% of sprayed insecticides and 95% of herbicides have been reported to reach the non-target species because they are sprayed and spread across entire agriculture fields (George 2004) which finally end up in the aquatic environment making it polluted (Firat et al. 2011). Aquatic pollution by pesticides has detrimental effects on biological life including human beings (Langston 1990). Pesticide poisoning symptoms according to Bouman et al. (1990) may be acute or may be chronic. Sub-lethal concentrations of pesticides, which are present in the aquatic environment, are too low to cause rapid death but have the potential to cause structural and functional changes in aquatic organisms and this is more common than mortality (Sancho et al. 2003). Fishes are the most important inhabitants of the aquatic ecosystems, which are more frequently exposed to the water surface and affected by these toxic pesticides (Little et al. 1993, Scott and Sloman 2004). The toxicity of pesticides to fish can vary with each pesticide group according to the properties of pesticides (Sabra and Mehena 2015). Pesticide doses that are not lethal for fish may have sub-lethal effects on physiology and

behaviour (Kegley et al. 1999). Fishes are usually very sensitive animal's i.e. they show the corresponding reaction against any hormones or enzymatic disrupters. Behaviour is considered a promising tool in ecotoxicology (Drummond and Russom 1990, Cohn and MacPhail 1996) and these studies are important in toxicity assessments (Tadehl and Häder 2001) of insects (Jensen et al. 1997) and fish (Little and Finger 1990). The most common behavioural changes in fish include erratic swimming, impatience, paleness, jerky movement, irregular swimming, sinking, etc. Toxicants can also influence the outcome of predator-prey interactions through altering schooling (Sullivan et al. 1978) swimming patterns and general activates which helps predators to identify the fish (Buskey et al. 1993). Behaviour change is a symptom of stress, which is highly related to biochemical physiological disturbances (Peakall et al. 2002, Weis et al. 2009). One of the early symptoms of acute pesticide poisoning is the alteration of respiratory metabolism (Holden 1973) which is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate. Changes in oxygen uptake of fishes in response to pesticide exposure are varying in different fishes exposed to a variety of pesticides (Karuppiah 1996). Blood is the most essential and abundant body fluid and is a vehicle for quickly mobilizing defense against trauma and ill health (Adewumi et al. 2018). Hematological parameters immediately reflect the poor condition of fish than other commonly measured parameters.

## 1.2 Objectives of the study

### 1.2.1 General objective

To assess the effects of dichlorvos on freshwater fish Rohu (*Labeo rohita* Hamilton, 1822) was the general objective of this study.

### 1.2.2 Specific objectives

- To determine the $LC_{50}$ value of dichlorvos on *Labeo rohita*
- To observe the general behaviours, respiratory metabolism, and opercular beat rate of fish after dichlorvos exposure
- To examine the blood parameters (haemoglobin, glucose, albumin, globulin and total protein) of dichlorvos exposed fish

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## 1.3 Rationale of the study

Pesticides used for various purposes finally end up in the aquatic ecosystem, which deteriorates the water quality. Exposure to the pesticide causes acute and chronic effects on the aquatic flora and fauna, especially the fish as they have direct contact with contaminated water. Hence, pollutants such as insecticides may cause serious impairment to the health status of fish (Banaee 2013). Poisoning/toxicity is categorized as either acute or chronic and the determination of the median lethal concentration ($LC_{50}$) is considered as the preliminary step for studies into the extent of acute or chronic toxicity. Being the major source of protein, the fish consumption rate is increasing worldwide. Therefore fish health and quality matter in human health too. Though different pesticide toxicity to fish has been assessed, the information on the effects of pesticides on major carps is still scarce. However, some studies have been conducted to assess the sub-lethal effects on behavioural patterns of Rohu (Shivakumar and David 2004, Bhat et al. 2012) acute toxicity and histopathical changes (Bhat and Bhat 2016) haematological alteration (Das and Mukherjee 2001) biochemical changes (Rani et al. 2008).

Different pesticides, however, have different $LC_{50}$ values in different organisms (Mathur et al. 2006). This study has been designed to evaluate the acute toxicity of frequently used dichlorvos as well as its sub-lethal effects on fish behaviour, aerobic energy metabolism, opercula movements, and blood parameters of Rohu in laboratory conditions. Results obtained from this research may be useful for management and monitoring dichlorvos (organophosphate) contamination in the environment. This study will be helpful for environment protection authorities to assess the risk of dichlorvos on the aquatic ecosystem and threats to the freshwater Rohu. The information will play a role to formulate the specific water quality guidelines of the pesticide for the welfare of fauna.

# 2. LITERATURE REVIEW

## 2.1 Pesticide toxicity in fishes

Pesticides are employed frequently in the integrated farming system to protect crops and animals from insects, weeds, and diseases. These chemicals end up mixing in the aquatic system entering the body either directly or through the food chain and attack them causing a serious problem in most aquatic fauna and flora and to a considerable extent (Avoaja et al. 1997). Contamination of fish products by pollutants is becoming an unavoidable problem these days. Because of the environmental longevity and toxic effects of organochlorines, the agriculture industry has increasingly relied upon organophosphate pesticides (Jenyo-Oni et al. 2011, Ragnarsdottir 2000). In fish, pesticides may cause hematological, biochemical, renal, reproductive, behavioural, neurological deleterious effects (*Tilapia mossambica*; Rath and Mishra 1981) by crossing the blood-brain barrier, which can eventually lead to death. Among the wide majority of pesticides, dichlorvos (2, 2-dichlorovinyl dimethyl phosphate), an organophosphate compound, is commonly used as an agricultural insecticide. Dichlorvos has elicited worldwide concern for many reasons as it is one of the few organophosphates still registered for use (Das 2013). It is extremely toxic to non-target organisms like fish (Das 2013) and hampers fish health through impairment of metabolism (*Denio rerio*; Bui-Nguyen et al. 2015), sometimes leading to death. The use of plant-based pesticides is less disastrous and more eco-friendly. The study by Mesnage et al. (2014) who compared the toxicity of active ingredients of three classes of pesticides reported that insecticides have been more toxic than herbicides. Plant-based pesticides are biodegradable and are more target-specific than the highly persistent broad-spectrum synthetic chemicals. Bhat et al. (2012) recommended the use of plant-based pesticides as they are less toxic to fish compared to dichlorvos after conducting the 96h-LC50 of *Labeo rohita* to dichlorvos and neem-based pesticide. Their study reported $LC_{50}$ value to be 16.71mg/L and 42.66mg/L respectively. Samprath et al. (1993) observed that the relevance of hematological studies in fish lies in the possibility that the blood will reveal anomalies within the body of the fish long before there is an outward manifestation of symptoms of diseases or effects of unfavorable environmental factors. Fish exposed to pesticides may also exhibit stress responses, which is defined as a state of re-established homeostatic (Chrousus 1998). Sub-lethal concentration increase the stress, but cannot lead to death (Roudraouda et al.

2009, Bhat et al. 2016). Cohn and MacPhail (1996) reported behavioural responses like erratic movements, convulsions, imbalanced swimming which ended in a collapse to the bottom of the aquarium, and an increase in respiratory motions during the entire period. Before death of the fish, some clinical signs like fading of body color, gulping onto air-water interface in aquaria, and in extreme cases, the hemorrhagic patches on the skin were observed with the termination of the experiments.

### 2.2 96h-$LC_{50}$ values of different pesticides on different fishes

Acute toxicity of a pesticide is known as the ability of a chemical to cause the effects on a particular species, generally in a short exposure period. The 96-h $LC_{50}$ tests are conducted to assess the vulnerability of organisms to particular toxic chemical substances. Chemical agents with lower $LC_{50}$ values are considered more toxic because their lower concentrations result 50% of mortality in organisms. Acute toxicity caused by the pesticide is positively correlated with dosage and the duration of exposure (Ishi and Patil 2017, Koprucu et al. 2006, Hussain et al. 2015). Sweilum (2006) recorded a low survival rate of Nile tilapia with increasing concentrations of pesticides. Boateng et al. (2006) recommended the adverse effect of deltamethrin on *Orechhromis niloticus* as its $LC_{50}$ value was found to be very low, which reveals the high toxicity of the pesticide. Works of literature on $LC_{50}$ values of different pesticides to *labeo rohita* have also highlighted the mortality of fish depends on the concentration of pesticides (Koul et al. 2007). The $LC_{50}$ values obtained at 96 hours exposures (95% confidence) limits for the two pesticides revealed that lambda cyhalothrin showed higher toxicity than monocrotophos on Rohu. The 96 h- $LC_{50}$ values of monocrotophos and lambda cyhalothrin were 0.0036 mg/L and 0.0021 mg/L, respectively (Muthukumaravel, Sukumaran and Sathick 2013). With increasing diazinon concentrations, the European catfish exposed duration 1– 96 hr significantly increased the number of dead fishes for each case (Koprucu et al. 2006). The acute toxicity of malathion and cypermethrin to Rohu was found to be 9.0 µl/ and 4.0 µg/L respectively (Patil and David 2008). The 96-h $LC_{50}$ value of dichlorvos for *Cyprinus carpio* var. communis was found 2.56 mg/L by probit analysis (Cohn and MacPhail 1996). Amaeze et al. (2020) reported 96h-$LC_{50}$ value of pesticides abamectin, carbo-furan, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, dimethoate, fipronil, lambda-cyhalothrin, and paraquat varied widely from 2.043 µg/L (Lambda-cyhalothrin) to 10284.288 µ/L (Paraquat). Omoniyi et al. (2013)

in their investigation found out that the dichlorvos is 1.79 times more toxic to the fingerlings than the juvenile *Clarius gariepinus* where they indicated the $LC_{50}$ value for fingerlings and juveniles as 275.2 and 492.0 µg/L respectively under static renewable bioassay. Saha et al. (2016) reported 96 h-$LC_{50}$ value of dichlorvos to *Oreochromis mossambicus* as 2.90 mg/L. The significant variation ($P < 0.05$) in the mortality rate of the fish was seen at all the exposure times (24, 48, and 72 and 96h) at 2.6 mg/l of toxicant and above. Lethal Concentration ($LC_{50}$- 96hours) of cypermethrin-based pesticide towards Nile tilapia (*Oreochromis niloticus*) was found to be 0.082 mg/L (Yuniari et al. 2016).

### 2.3 Behavioural study of fishes after pesticide exposure

Sukirtha and Usharani (2013) examined the acute effect of dichlorvos on adult *Danio rerio*. Neural degeneration oedema in the stroma of the brain tissue and collections of inflammatory cells predominantly lymphocytes were reported at 5mg/L. The decrement of operculum movement, hyperactivity, mucus secretion, widely opened mouth, and operculum sinking, to the bottom and darting swimming movements, are generally known as organophosphorus compounds that induce neurotoxicity (Mishra and Poddar 2014).

Mallum et al. (2016) reported some behavioural alterations in *Oreochromis niloticus* exposed to dichlorvos (0.5, 1, 1.5, and 2µg/L for 96h). Lethal Concentration ($LC_{50}$-96h) of cypermethrin-based pesticide towards Nile tilapia (*Oreochromis niloticus*) was found to be 0.082mg/L (Yuniari et al. 2016). Hyperactive activities, loss of equilibrium, and mucus secretion around the gills were the most common responses on *O. niloticus* and were dose-dependent which was also accepted by State and State (2016). Some existing works of the literature show sub-lethal toxicity of dichlorvos, resulting in abnormal behavioural changes in rohu (Kesharwani et al. 2018) and guppy fish (*Poecilia reticulate*; Gunde and Yerli 2012). Abnormal behavioural changes such as irregular, erratic, and darting swimming movements, hyperexcitability, loss of equilibrium, and sinking to the bottom might be due to inactivation or decrease in acetylcholinesterase, which was already documented (Balint et al. 1995). Loss of coordination was also exhibited by common carps in Chlorpyrifos (20% EC) contaminated water (Halappa and David 2008). At the beginning of dichlorvos exposure, fishes don't show any serious behavior changes but at high concentration, fingerlings became agitated and restless, swam to the surface for air and assumed vertical position before death (Ashade et al. 2011).

## 2.4 Oxygen uptake and opercular beat rate

The respiratory potential and the oxygen consumption of an animal are the important physiological parameters to assess toxic stress because it is a valuable indicator of energy expenditure during metabolism (Proser and Brown 1973). Both lethal and sublethal concentrations bring variation in the oxygen consumption rate of fish (Patil and David 2008). It is also evident from the study that exposure to dichlorvos can alter oxygen consumption rate in *Ctenopharyngodon idella* (Tilak and Kumari 2009) and also cause histopathical changes in Rohu (Kesharwani et al. 2018). Alterations in oxygen consumption may be due to respiratory distress because of impairment in oxidative metabolism. Sub-lethal concentration increases the stress, but cannot lead to death (Bhat et al. 2016, Roudraouda et al. 2009). The effect of three pesticides namely Metacid-50 (Organophosphate), Dithane M-45 (Carbamate), and Kelthane (Organochlorine) in dual mode of oxygen consumption of an air-breathing murrel fish, *Channa gachua*, the pesticides brought a significant decrease in total aquatic oxygen uptake, while it increases oxygen consumption rate through the aerial route as compared to control. It is due to the action of pesticides on acetylcholinesterase enzyme, respiratory muscles paralysis, and respiratory failure causing finally death. (Rahman and Sadhu 2009). Hyper-excitability of *Oreochromis mossambicus* fish was also recorded at higher concentrations during 24h of dichlorvos exposure. The opercular movement of treated fish was increased significantly over the control with the increasing concentrations of toxicant (Peter et al. 2018).

## 2.5 Alteration on haematological and biochemical parameters

Kesharwani et al. (2018) reported the adverse effect of dichlorvos on haematological changes in Rohu (*Labeo rohita*). The sub-lethal exposure studies for up to 45 days at 1/10 and 1/50 of 96h-LC of cypermethrin (0.139mg/L) to Rohu resulted in decrement of serum protein level, haemoglobin percentage, and total erythrocytes over control at both concentrations of the pyrethroid whereas, blood glucose level and total leucocytes were elevated with compared to control (Das and Mukherjee 2003). According to their study, extracts of the herb *Datura stramonium* were effective in countering the toxicity of this pesticide. Rohu exposed to sub-lethal levels of cypermethrin and carbofuran elicited significant ($P < 0:05$) depletion in RBC during the exposure period. There was a positive correlation between RBC count and recovery period at all concentrations

tested (Adhikari et al. 2004). Similarly, significant depletion of erythrocyte, leukocyte, haemoglobin, hematocrit, MCV, MCH, and MCHC values was seen in Indian carp *Cirrhinus mrigala* too (Raif and Arain 2013) with compared to control. In addition, the result showed a significantly negative correlation between these haematological parameters and exposure times of diazinon (Koprucu et al. 2006). Koul et al. (2007) also highlighted the concentration-dependent relationship between pesticide dichlorvos and various biochemical parameters, which summarizes that the high concentration of DDVP results in a significant alteration in glucose, protein, cholesterol, and lipid content. Sub-lethal dose (1.6 mg/L) of DDVP on haematological parameters of rainbow trout (*Oncorhynchus mykiss*) increased the red and white blood cell counts, haemoglobin, erythrocyte sedimentation rate, mean corpuscular volume, and mean corpuscular haemoglobin concentration (MCHC) followed by decrement of thrombocyte, hematocrit and mean corpuscular volume (Aksakel et al. 2008).

Tamizhazhagan (2015) suggested that exposure of monocrotophos 36% EC on Rohu caused the depletion of total Red Blood Corpuscle and haemoglobin content with the increasing hours of exposure. It indicates count leading to anemia, because of inhibition of erythropoiesis, haemosynthesis, and increase in the rate of erythrocyte destruction in haemopoietic organs. Dichlorvos at acute levels to *Oreochromis niloticus* and *Clarius gariepinus* elicited response, which involved a decrease in the percentages of Packed Cells Volume (PCV), haemaglobin, Red Blood Cells (RBC), neutrophils, monocytes, and lymphocytes, indicating severe anemia in the exposed fish (Ezike 2017, State and State 2016). White Blood Cells Count (WBCC) in fish blood exhibits a direct correlation with feeding status and the reduction of WBCC might be due to poor feeding by fish. The investigation of acute toxicity of organophosphate pesticide (chlorpyrifos and malathion), synthetic pyrethroid (lambda-cyhalothrin), and herbicide (buctril) on the total protein content of fish *Oreochromis mossambicus*, showed that the total protein content is inhibited in fish after exposure (Naqvi et al. 2017). Amaeze et al. (2020) observed a significant difference between mean hematological parameters such as WBC, RBC, and MCH in exposure to pesticides abamectin, carbo-furan, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, dimethoate, fipronil, lambda-cyhalothrin, and paraquat These pesticides have a potential effect on blood profile of *Clarius gariepinus.*

# 3. MATERIALS AND METHODS

## 3.1 Laboratory work

The study was completely laboratory-based and was carried out at Central Fisheries Promotion and Conservation Center (CFPCC) Balaju, Kathmandu.

## 3.2 Materials

### 3.2.1 Materials used for experimental setup

a) Aquaria (35 L×12 and 350 L×2)
b) Aquarium cover
c) Aquarium heater
d) Aquarium filter
e) Aquarium pump
f) Air stones
g) Beakers
h) Buckets
i) Sponges
j) Siphoning tubes
k) Scissors and tapes
l) Scoop net
m) Volumetric flasks and other laboratory essentials

### 3.2.2 Instrumental apparatus

a) Cuvette
b) Refrigerator
c) Weighing balance
d) Multiparameter water quality analyzer (Hanna, Hl98194)
e) Spectrophotometer (UV/VIS Spectrophotometer, 1000 series, Shanghai Yoke Instrument Co., Ltd, China).

### 3.2.3 Analytical apparatus

a) Beakers
b) Bullet tubes
c) Dropper
d) Eppendorf tube
e) Ethylene Diamine Tetra Acetic acid (EDTA ) vials
f) Gloves
g) Icebox
h) Manual counter
i) Measuring cylinder
j) Micro Pipette (1000µl, 100 µl)
k) Stopwatch
l) Paper towel and soft towel
m) Syringe heparinized (1ml, 3ml)

## 3.3 Chemicals used

**For acute toxicity**

For this experiment, Commercial grade Dichlorvos **(**2, 2-dichlorovinyl dimethyl phosphate) with the tag G-VAN (80% EC) supplied by Greenriver Industry co., Ltd., China was purchased from an authorized shop. The stock solution of 20mg/L was prepared in distilled water in a volumetric flask and covered with help of aluminum foil to protect the solution from light exposure.

**For haematological and biochemical parameters**

- Eco-pak glucose reagent (glucose test) and standard
- Biuret reagent (protein test) and standard
- Bromocerol Green reagent (albumin test) and standard
- HEMOCHORD-D (haemoglobin reagent) and standard

## 3.4 Methodology

The study was conducted from September to November 2019. The experiment was designed in such a way as to estimate the lethal concentration (24, 48, 72, and 96hr) of the organophosphate pesticide dichlorvos to the fingerlings of Rohu, as well as to observe the changes in behavioural change, opercular beat rate, respiratory metabolism and blood parameters of the pesticide exposed fish.

### 3.4.1 Fish collection and acclimatization

Firstly, fish were transported safely from Bhairahawa and stocked in hapas at CFPCC Balaju for two weeks. Fishes were screened for any pathogenic infections. Glass aquaria were washed with one % KMnO4 to avoid fungal contamination and then sun-dried. Healthy fishes were then transferred from hapas to the laboratory and housed in the glass water tank (350 L) which was continuously aerated containing de-chlorinated tap water for acclimatization. Water was renewed on alternate days to avoid any pollution. The process was continued for 15 days. The freshwater healthy fingerlings of Rohu of the bodyweight (9 ± 2g) and length (6 ± 0.5 cm) were selected for the experiment. They were regularly fed with commercial pellet feed (32% protein). The aquarium water was changed daily to remove feces and food remnants.

**Table 1:** Physicochemical properties of water.

| Water quality parameters | Value |
|---|---|
| Temperature (℃) | 24.96±0.25 |
| Ammonia (mg/L) | 0.18-0.23 |
| Hardness of water (mg $CaCO_3$/L ) | 53-59 |
| Dissolve oxygen (ppm) | 5±0.45 |
| pH | 7±0.2 |

### 3.4.2 Acute toxicity test

The acute toxicity test, to know the lethal concentrations of dichlorvos pesticides was accompanied in semi-static condition (water was renewed by pesticide containing water) in the laboratory, following the standard method (OECD 203 testing guidelines, 1992).

**Table 2:** Experimental design for acute toxicity test.

| Pesticide = dichlorvos | | | | General fish Behaviour | Water quality parameters |
|---|---|---|---|---|---|
| **Concentration (mg/L)** | **Total Replications** | **No of fish** | **Mortality** | | |
| Control= 0 | 4 | 28 | | -Erratic | -DO |
| C1= 2 | 4 | 28 | | -swimming | -Ammonia |
| C2=4 | 4 | 28 | | -Color change | -Hardness of |
| C3=8 | 4 | 28 | | -Mucus | water |
| C4=16 | 4 | 28 | | secretion | -pH |
| C5=32 | 4 | 28 | | -Avoiding | -Temperature |
| C6=64 | 4 | 28 | | schooling | |
| C7=128 | 4 | 28 | | -Aggregating | |
| | | Total =224 | | at corner | |

For the acute toxicity test, a total of thirty-two glass aquaria with a capacity of 35L were filled with well-filtered water. Fishes with 9±2 g body weight were sorted out and distributed in each aquarium. Altogether 224 fish were used for this experiment. Feeding was stopped a day (24 hours) before exposure and no feed was administrated

to fish throughout the experiment period to avoid any interference in the toxicity of pesticides by excretory products. Stock solution with a concentration of 20 mg/L was prepared freshly. Fishes were exposed to seven different concentrations of pesticide and a control without any pesticide on it. To determine $LC_{50}$ values for 24, 48, 72, 96 hours, four replicates for each concentration group along with control were run simultaneously. During the experiment, the water in each aquarium was aerated and the temperature was maintained to 24.96±0.25℃. Hardness and total ammonia were ranged between 53-59 and 0.18-0.23 mg/L respectively and the pH value was always near 7. The pH value, temperature, DO and mortality were recorded at 1, 24, 48, 72, and 96 hours, and dead fish were removed immediately to avoid water contamination. Lethal toxicity of pesticides was calculated and analyzed by the log probit analysis method using the statistical package SPSS version 20. The analyzed result was then presented as 24 h to 96 h- LC10-LC90 with a 95% confidence limit, where LC10 and LC90 represent the 10% and 99% fish mortality at given concentrations.

#### 3.4.2.1 General behaviour changes

During the acute toxicity test, general physical changes in fish of control and pesticide groups were compared, which generally comprises the difference in body color, accumulation of mucus around gill and body surface, condition of fins, observation of red patches over body head, size of the operculum, position in aquarium, body posture and maintenance of body equilibrium. Other behaviours such as hyperactivity, hypoactivity, swimming pattern, and schooling behaviour were also carefully observed in a particular time and results were recorded sequencing in the record sheet. Unnecessary human flow around the aquarium was avoided to ensure no disturbances for fishes, which consequently minimizes any potential error.

### 3.4.3 Experimental design for advanced behaviour

The experimental setup was designed to investigate the opercular beat rate and respiratory metabolism of Rohu to dichlorvos pesticide. Fishes of similar sizes and body weight (11±2gm) were distributed in each aquarium having an equal level of fresh filtered water. The experiment set up had four replications for sub-lethal concentration also keeping the same no of replications for the control group. Feeding was stopped a day (24 hours) before exposure and no feed was administrated during the experiment. Fishes were exposed to only one sub-lethal concentration of the test chemical i.e. 50%

of the 96h $LC_{50}$ of DDVP (5.6 mg/L). The stock solution was prepared and released in test fish with the help of a micropipette leaving the control normal and left for one hour to mix up with water. The study on advanced fish behaviour was mainly focused on opercular beat rate and oxygen consumption rate.

### 3.4.3.1 Opercular beat rate

The fishes were left overnight suspending their feeding for acclimatization. Operculum beat rate per 5 min for each fish was counted at a different time interval (1- 96 hr) with the help of a hand tally counter. The process was continued once every 24 hours and the opercular beat rate was counted three times for each fish to calculate the accurate average. The data obtained from the experiment are presented as the average opercular beat rate per minute.

### 3.4.3.2 Respiratory metabolism

The level of dissolved oxygen in water alters with the consumption of DO by aquatic animals. The increase and decrease in DO level relate to respiratory metabolism. The media was left aerated before exposure so that the oxygen level reaches up to 5-6 ppm and the pH and temperature were constant as mention above. Once, the aerator was switched off, the initial oxygen level was measured quickly using a Milwa ukee MW600 PRO portable dissolved oxygen meter, then aquaria were sealed tightly with a glass lid and duct tape to avoid the mixing of external air in water. After 24 hours, the aquaria were unsealed without disturbing fish, and at the same time, the final DO, pH, and temperature were recorded. In addition, the process was repeated in the same way every 24 hours for four days. Based on the difference between the initial and the final DO, the oxygen consumption rate (mg/g/h) of fish was measured using the following formulae,

$$(\Delta O_2 i - O_2 f) \times V \times (1/BW) \times (1/T)$$

Where $O_2 i$ is initial oxygen concentration (mg/L) and $O_2 f$ is the final oxygen concentration

(mg/L); V is total water volume (L)

BW is body weight (gm) and T is the time interval (hr).

### 3.4.4 Blood parameters

Blood sampling for important blood tests was also attempted in the semi-static condition in six replicates. The experiment mainly focused on the comparative study of blood parameters of pesticides exposed to fish and control fishes. Fingerlings with similar body sizes were taken out from the acclimatization tank with a scoop net and weighed. Two concentrations of pesticide were designed for exposure,

1) Dichlorvos low dose: 1 mg/L (1/10 of 96 h- $LC_{50}$)

2) Dichlorvos high dose: 5.6 mg/L (1/2 of 96h-$LC_{50}$)

The experiment was run parallel in three groups (control, DDVP low dose, and DDVP high dose). Each group had three replication. Glass aquariums were filled with water up to the appropriate level (30 L), aeration was regulated and the temperature was maintained. Altogether 18 fishes have experimented. After the 96h of exposure, fishes from each concentrated group were taken out and anesthetized in clove oil. Fresh blood was collected from the caudal vein puncture utilizing a 1ml heparinized plastic syringe and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA) vials to assess haemoglobin and in Eppendorf tubes to determine blood glucose, albumin, and total protein.

#### I. Haemoglobin test

To evaluate the haemoglobin, the Cyanmethaemoglobin method described by Baker and Silverton (1976) was applied using the HEMOCHORD-D haemoglobin reagent. For this procedure, three test tubes were labeled as (B) for blank, (S) for standard, and (T) for the test. Five ml of HEMOCHORD-D reagent was pipetted into two test tubes marked as ‘B’ and ‘T’. Twenty µl of distilled water was added to the ‘Blank’. The collected blood in the EDTA tube was mixed thoroughly by the gentle inversion and 20µl of blood sample was taken out with a micro-pipette and expelled to the test tube marked as ‘T’. The Standard test tube was filled only with 20µl of haemoglobin standard (60 mg/dl) as shown in table 3. After mixing thoroughly, the samples were left undisturbed for 15 minutes at room temperature. The absorbance (Abs.T) of the ‘Standard’ and the ‘Test’ were then measured at 540 nm against blank using UV/VIS Spectrophotometer, 1000 series, Shanghai Yoke Instrument Co. Ltd, China.

**Table 3**: Quantity of reagents and solutions required for haemoglobin test.

| Addition sequence | B (ml) | S(ml) | T(ml) |
|---|---|---|---|
| HEMOCHORD-D Reagent | 5.0 | …….. | 5.0 |
| Distilled water | 0.02 | ….. | …… |
| Haemoglobin Standard | …… | 0.02 | ….. |
| Sample (serum) | …… | ….. | 0.02 |

Haemoglobin (Hb) concentration was calculated using the following formula,

$$\text{Haemoglobin in g/dl} = \frac{Abs.Test}{Abs.std} . \times \frac{251}{1000} \times 60$$

Where,

251 the dilution factor i.e. total reagent volume (5.02 ml)/ sample volume (0.02 ml).

1000 is the Multiplication Factor to convert mg to grams.

60 is the concentration of the Haemoglobin Standard in mg/dl

## II. Glucose

The glucose test was done by Eco-Pak Glucose method using the Glucose test kit (Accurex Biomedical Pvt. Ltd.) Mumbai, India, which involves an enzymatic action i.e. oxidase and peroxidase. The Eppendorf tube containing the blood sample was subjected to the centrifugation process at 3000 rpm to obtain the serum. One ml Eco-Pak glucose reagent (working solution) was taken into three test tubes labeled as blank (B) standard (S) and test (T). After that 10 µl of distilled water, 10 µl of known concentration of glucose (100 mg/dl), and 10-µl serum were added to the test tube marked as blank (B), standard (S), and test (T) respectively. Then the assay solutions were incubated at 37℃ for 15 minutes till the color complex was formed. After then the absorbance of test and standard solutions against blank solution was measured at 505 nm (UV/VIS Spectrophotometer, 1000 series, Shanghai Yoke Instrument Co., Ltd, China).

**Table 4:** Quantity of reagents required for glucose content test.

| Addition sequence | B (ml) | S(ml) | T(ml) |
|---|---|---|---|
| Eco-Pak Reagent | 1.0 | 1.0 | 1.0 |
| Distilled water | 0.01 | ….. | …… |
| Glucose Standard | …… | 0.01 | ….. |
| Sample( serum) | …… | ….. | 0.01 |

The glucose content was then calculated by using the following formula,

$$\text{Glucose in mg/dl} = \frac{\textit{Absorbance of sample}}{\textit{Absorbance of standard}} \times \mathbf{100}.$$

### III. Total protein

The total plasma seral protein was measured by the standard Biuret method using total protein kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India) as described by Lawrence (1986), which is based on the principle that the reaction between peptide bond of protein and Cupric ions (from the copper Sulfate solution) that produces a blue-violet color complex in alkaline solution. In this method, 1 ml of Burette reagent was pipetted into clean and dry test tubes labeled as blank (B), standard (S), and Test (T). After then, 20 µl distilled water was added to the blank (B), 20 µl of protein standard (8g/dl) was added

to the standard (S) and 20 µl of serum was added to the test tube labeled as 'T'(Table 5). The solution was mixed well and incubate at 37 ℃ for 10 minutes until slight color changes were seen. Thereafter, the absorbance of standard and test solution were measured against the blank in the spectrophotometer at 540 nm (UV/VIS Spectrophotometer, 1000 series, Shanghai Yoke Instrument Co., Ltd, China).

**Table 5:** Quantity of reagents and solution required for total protein test.

| Addition sequence | B (ml) | S(ml) | T(ml) |
|---|---|---|---|
| Biuret Reagent | 1.0 | 1.0 | 1.0 |
| Distilled water | 0.02 | ….. | …… |
| Protein Standard | …… | 0.02 | ….. |
| Sample | …… | ….. | 0.02 |

Total protein was calculated using the following formula,

$$\text{Total protein (g/dl)} = \frac{\textit{Absorbance of Test}}{\textit{Absorbance of standard}} \times \mathbf{8}$$

### IV. Albumin

Albumin was assessed by the BCG (Bromocresol Green) method using an albumin kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., and India). In this procedure, 1 ml of BCG reagent was taken into test tubes marked as blank (B), standard (S), and test (T) as before. Ten µl of distilled water was added to the 'B' test tube, 10 µl of a standard solution of known concentration (4g/dl) was added to the 'S' and 10 µl of blood serum was added to the test tube labeled as 'T.' The assay mixtures were mixed well and

incubated at room temperature for 5 min. Then the absorbance of the test and standard solution were measured against the blank in the spectrophotometer at 630 nm (UV/VIS Spectrophotometer, 1000 series, Shanghai Yoke Instrument Co., Ltd, China).

**Table 6:** Quantity of reagents and solution required for albumin test.

| Addition sequence | B (ml) | S(ml) | T(ml) |
|---|---|---|---|
| BCG Reagent | 1.0 | 1.0 | 1.0 |
| Distilled water | 0.01 | ….. | …… |
| Albumin Standard | …… | 0.01 | ….. |
| Sample( serum) | …… | ….. | 0.01 |

Albumin was calculated using the following formula

$$\text{Albumin (g/dl)} = \frac{\boldsymbol{Absorbance\ of\ Test}}{\boldsymbol{Absorbance\ of\ Standard}} \times \mathbf{4}$$

### V. Globulin

Globulin was estimated by subtracting an albumin value from the total protein as mentioned by Busher (1990).

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

### 3.5 Data Analysis

The lethal concentration (LC10-90) of dichlorvos pesticide to fish *Labeo rohita* was determined by the Probit analysis. All the data were normally distributed. Opercular movement and oxygen consumption data were analyzed by t-test whereas the hematological and biochemical parameters were analyzed by one-way ANOVA followed by TUKEY-HSD post hoc test. For this analysis, a statistical software package (SPSS-version 20) was used.

# 4. RESULTS

## 4.1 Acute toxicity

To ensure the accuracy of the outcomes, fishes were exposed to seven different concentrations of DDVP (from low to high). During the experiment, no fish mortalities were recorded in control groups. By the 24 hr of exposure, mortality started to begin from 32 mg/L. During the entire 96 hr experimental period, 100 % of fishes were found to be dead at 64 and 128 mg/L concentration within a very short exposure period (24 hr). No fish were able to survive up to 96 h at higher concentrations from 16 to 128 mg/L (Table 7). The result shows mortality depends on the concentration of pesticide i.e. fishes cannot tolerate the high toxicity of the pesticide.

**Table 7**: Cumulative mortalities of fish in different concentrations and time.

| **Concentrations (mg/L)** | **Dose (ml)** | **No of Fish exposed** | **Mortality ( number)** | | | |
|---|---|---|---|---|---|---|
| | | | **24 hr** | **48hr** | **72hr** | **96hr** |
| 0 | 0 | 28 | 0 | 0 | 0 | 0 |
| 2 | 2.5 | 28 | 0 | 0 | 0 | 0 |
| 4 | 5 | 28 | 0 | 0 | 0 | 0 |
| 8 | 10 | 28 | 0 | 0 | 0 | 0 |
| 16 | 20 | 28 | 0 | 0 | 24 | 28 |
| 32 | 40 | 28 | 4 | 28 | 28 | 28 |
| 64 | 80 | 28 | 28 | 28 | 28 | 28 |
| 128 | 160 | 28 | 28 | 28 | 28 | 28 |

From the experiment, it came to know that that the concentration of DDVP below 8 mg/L is not lethal to the Rohu fish but it caused serious sub-lethal effects.

The $LC_{50}$ value of dichlorvos to Rohu in 24, 48, 72, and 96 hours with a 95% confidence limit was recorded as 40.08 mg/L, 22.49 mg/L, 12.62 mg/L, and 11.35 mg/L respectively. The inverse relation between lethal concentration values and exposure period was seen i.e. the lethal pesticide concentration decreases with an increase in exposure period (Table 8). The lethal concentrations of dichlorvos to Rohu (Table 8).

**Table 8:** LC (10-99)% of dichlorvos to Rohu in different exposure hours.

| Species | Pesticide | LC (10-90%) | Hours | | | |
|---|---|---|---|---|---|---|
| | | | 24 | 48 | 72 | 96 |
| *Labeo rohita* | dichlorvos | 10 | 26.148 | 15.399 | 8.968 | 8.124 |
| | | 20 | 30.279 | 17.537 | 10.086 | 9.115 |
| | | 30 | 33.657 | 19.262 | 10.977 | 9.903 |
| | | 40 | 36.84 | 20.869 | 11.801 | 10.63 |
| | | 50 | 40.087 | 22.492 | 12.627 | 11.358 |
| | | 60 | 43.621 | 24.242 | 13.511 | 12.136 |
| | | 70 | 47.747 | 26.264 | 14.525 | 13.028 |
| | | 80 | 53.074 | 28.847 | 15.808 | 14.155 |
| | | 90 | 61.459 | 32.853 | 17.778 | 15.88 |
| | | 99 | 87.071 | 44.744 | 23.498 | 20.87 |

## 4.2 General behavioural responses of fish

The observation was done every three hours from the first hour of pesticide exposure. The alterations in general behaviour exhibited by Rohu (when they were alive) have been provided in detail in table 9. The fish in the control group and low concentration (2 and 4 mg/L) group exhibited normal behaviour i.e. no notable changes were witnessed. Initial changes in behaviour were recorded after three hours of exposure in medium concentration (8 mg/L), where some fishes exhibited hyper-exciting movements such as water surfacing and jerky movements.

**Table 9:** General behaviour changes in fish (alive) in different concentrations. (-: absent, +: mild, ++: moderate, +++: high).

| Fish behaviours | Concentration of DDVP (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
| Mucus Secretion | - | - | - | - | - | - | - | ++ |
| Hyper excitement | - | - | - | + | + | ++ | ++ | +++ |
| Equilibrium loss | - | - | - | - | + | ++ | + | ++ |
| Change in body-color | - | - | - | + | + | + | ++ | ++ |
| Avoiding schooling behaviour | - | - | - | + | - | ++ | + | +++ |
| Aggregating at the corner | - | - | - | + | + | + | ++ | +++ |

Fishes were unable to balance their body from 48 hours at 16 mg/L whereas; it was already noticeable at the high concentrated group (128 mg/L) in 24 hours. Fish exposed to lower concentration showed normal behaviour in the first two days of exposure, after then some abnormal behaviour such as avoiding schooling, and loss of equilibrium was seen in eight mg/L. In low concentrated groups, fishes avoided schooling on the last day of exposure but it was already witnessed in higher concentrated groups by 24-48 hours of exposure. As all the fishes were dead within 24 hours of the exposure at the high concentrated group, no more particular behaviour changes could be recorded but some abnormal behaviour changes such as rapid operculum movements, erratic movements, gulping at water surface with change in body color were observed before death. As the concentration increased, aggregating at the corner and motionless posture in the bottom of the aquarium was frequently observed. Changes in the body coloration and mucus secretion were found in higher concentrations. Mucus was mainly found loaded around the mouth and gill area. Dead fish were seen at the bottom with their mouth opened and mucus-covered in their gills, mouth, and body surface.

### 4.3 Sub-lethal exposure

#### 4.3.1 Respiratory metabolism

At the beginning of the experiment, 24 hr of the exposure, fish consumed less oxygen compared to control ($P < 0.01$). Most of the time, the sub-lethal exposure of DDVP to Rohu revealed a rising oxygen consumption rate though it was not statistically significant in all exposure hours. There was an increasing trend of oxygen consumption with the exposure time and the highest oxygen consumption was observed during 72-96 hr of exposure. There was a negligible fluctuation in oxygen consumption rate in control groups (Figure 1). Furthermore, the compiled statistical analysis of the control and DDVP groups also revealed no significant differences in oxygen consumption rate (Figure 2).

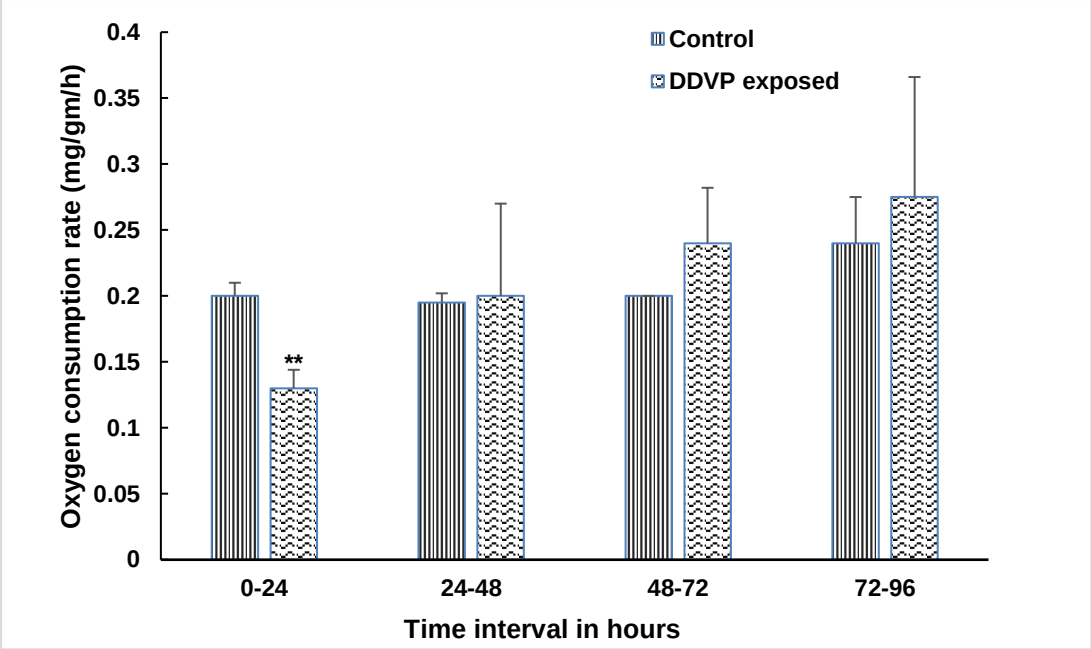


**Figure 1:** Bar represents the mean respiratory metabolism rate of fish Rohu in control and sub-lethal concentration of dichlorvos (5.6mg/L). Data are expressed as mean ± SD (n=4). Asterisks represent the significant difference between the control and pesticides exposed groups (**$P < 0.01$)

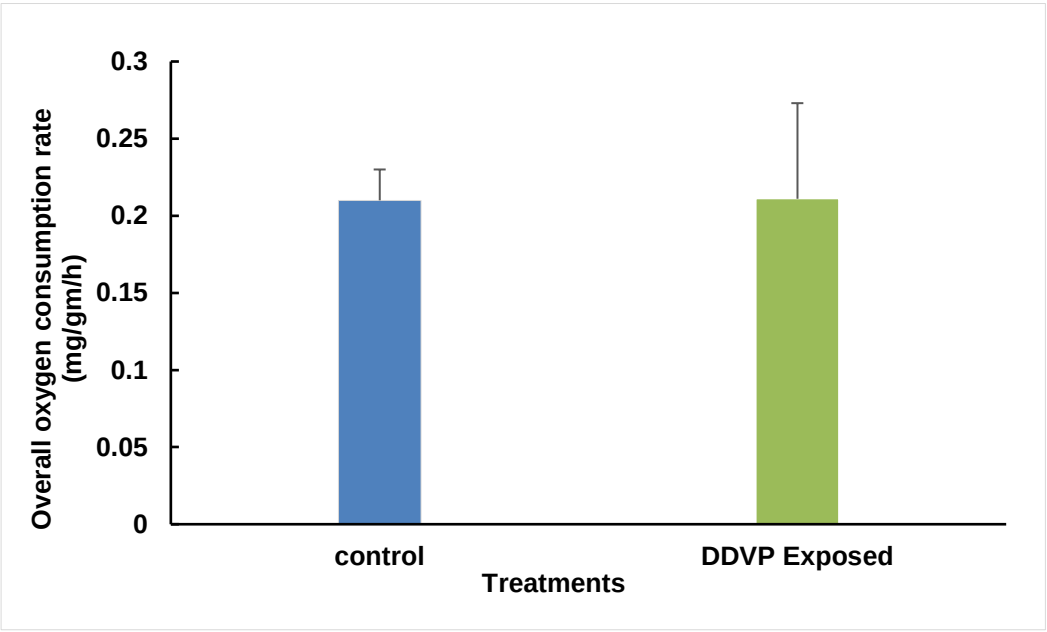


**Figure 2:** Total oxygen consumption rate in control and DDVP. Data are expressed as mean ± SD (n=16)

### 4.3.2 Opercular beat rate

The fish exposed to the DDVP always exhibited a high opercular beat rate in comparison to control ($P < 0.05$ and $P < 0.01$). The highest opercular beat rate was observed during 96 hr where the lowest was observed during 72 hr of exposure but it was still significant compared to its control ($P < 0.01$).

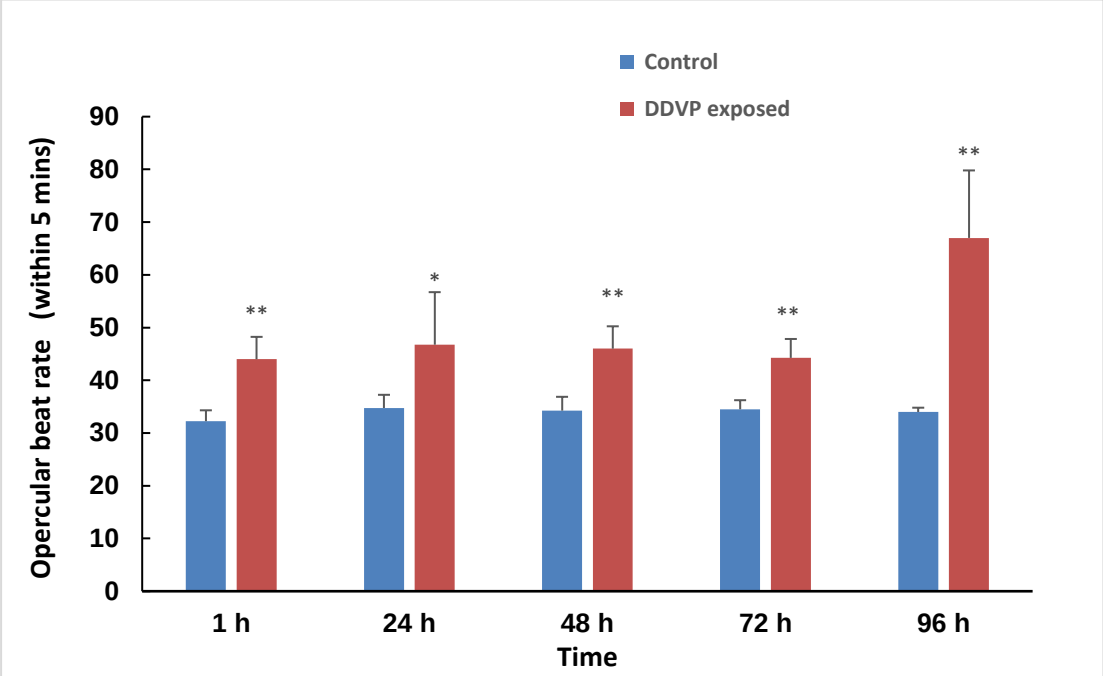


**Figure 3:** Opercular beat rate by Rohu during different exposure periods. Data are expressed as mean ± SD (n=4). Asterisks represent the significant difference between the control and pesticides exposed groups (*$P < 0.05$ and **$P < 0.01$)

### 4.3.3 Blood parameters

Fishes were exposed to the two different sub-lethal concentrations of DDVP as explained in the experimental design. Alteration in the blood contents among control, high dose, and low dose as well as within same pesticide group was seen in the statistical analysis. Simply, the high doses of pesticide exposed fish exhibited the elevation of haemoglobin and glucose content in comparison to control and a low dose of pesticide. However, the total protein, albumin, and globulin were decreased.

#### A. Haemoglobin

After 96 hours of exposure, both low and high pesticide dose exposed fish exhibited higher haemoglobin content compared to control ($P < 0.01$). The haemoglobin of high dose fish was slightly higher than low dose fish but that was not statistically significant indicating no effect of pesticide doses on haemoglobin (Figure 4).

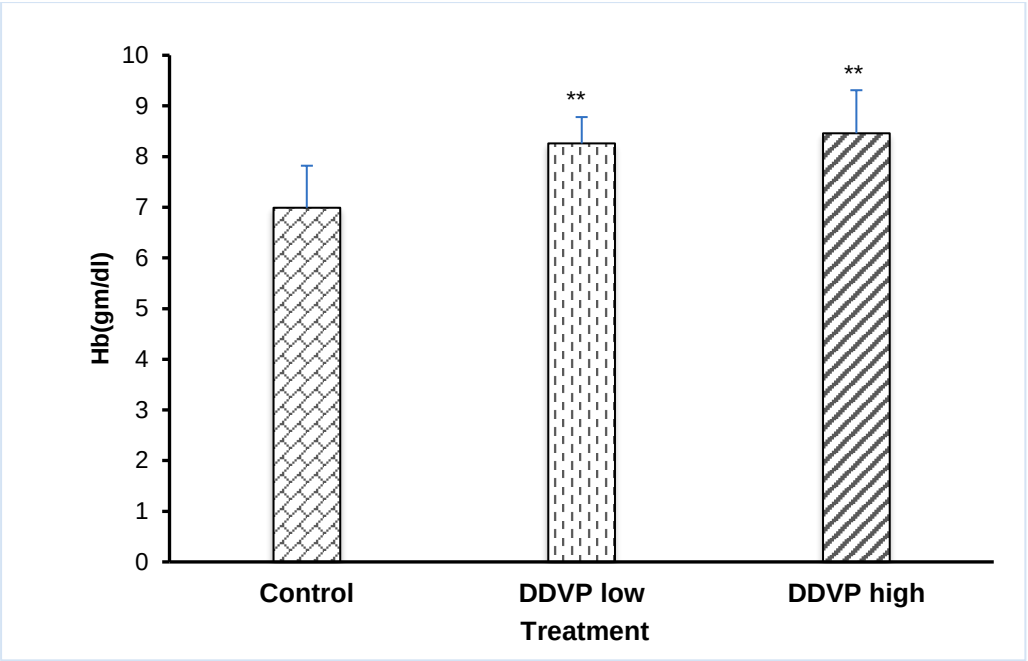


**Figure 4:** Sub-lethal effect of DDVP on haemoglobin content in the blood of Rohu. Data are expressed as mean ± SD (n=6). Asterisks represent the significant difference between the control and pesticides exposed groups (**$P < 0.01$)

## B. Glucose

After 96-hr treatment, the sub-lethal concentration caused the rising of glucose level in blood serum of pesticide-exposed fishes but statistical analysis showed no significant difference between control and pesticide exposed groups. High pesticide dose had a higher glucose level than that of its low dose but the difference was again insignificant (Figure 5).

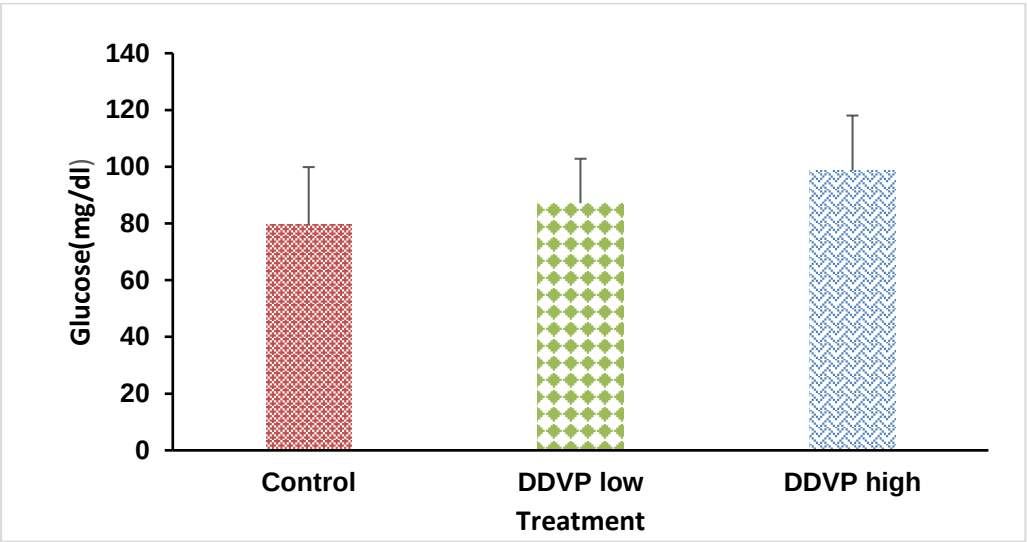


**Figure 5:** Sublethal effect of DDVP (5.6mg/L) in glucose content among three groups in 96-hr exposure duration

## C. Albumin, globulin and total protein

With compared to control, the Albumin content in fish was found to be decreased in pesticide exposed groups and the same result was found in the analysis of globulin content. Reduction in albumin and globulin decreased the total protein content in a similar pattern (Figure 6).

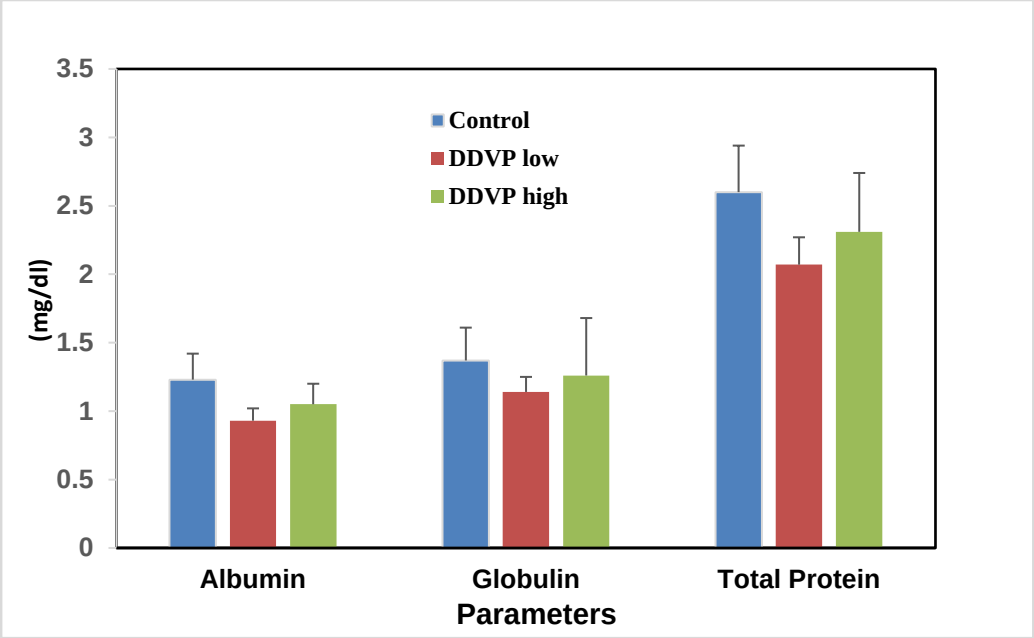


**Figure 6:** Albumin, globulin, and total protein content in three different groups after exposure to sub-lethal concentration of DDVP (5.6mg/L)

## D. Total Protein

The Single-factor ANOVA test showed a significant reduction ($P < 0.05$) in the total protein content among three different treatment groups. Compared to the control, the total protein content in the blood of the fish exposed to the low concentrated group was found to be significantly reduced ($P < 0.01$). Whereas, there was no significant difference between the DDVP high concentrated group and control group.

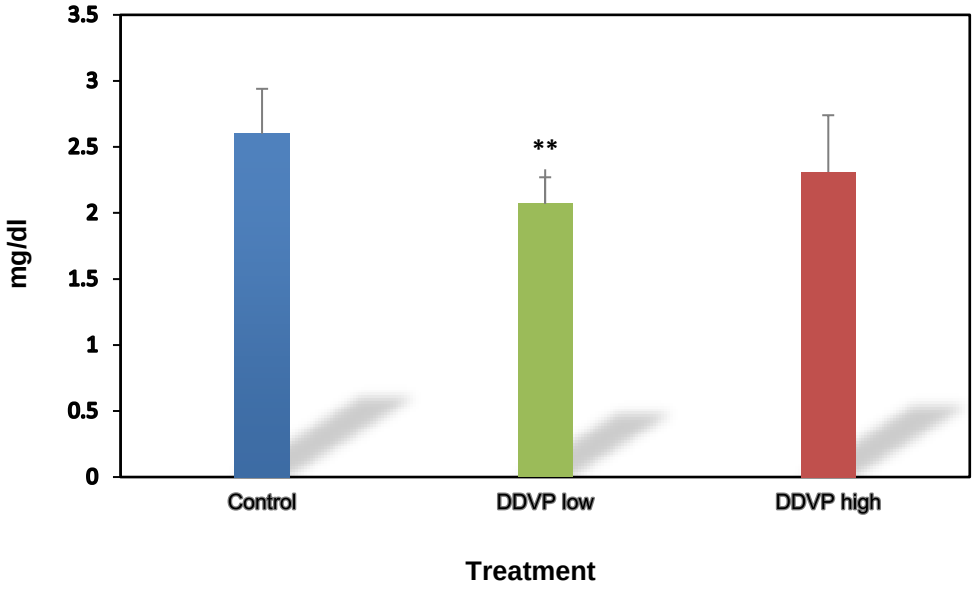


**Figure 7:** Sub-lethal effect of DDVP on total protein content in blood serum of *Labeo rohita*. Data are expressed as mean ± SD (n=6). Asterisks represent the significant difference between the control and pesticides exposed group (**$P < 0.01$)

# 5. DISCUSSION

Pesticides are biocides that are applied broadly within the open environment. Thus, they have a relatively large potential for effects on non-target organisms like fish, as they are very sensitive to the environment (Das 2013). Acute toxicity of the pesticide refers to the ability of a pesticide to cause damage to an animal for single exposure generally in a short period (OECD 1992). Acute and chronic toxicity tests are performed to explicitly quantify the effect of toxic chemicals in non-target organisms (Omoregie et al. 2009, Santos et al. 2010).

A survey of acute toxicity of different pesticides to the fish for different exposure periods reveals wide differences in LC50 value. It varies with duration of exposure and types of fish (Mathivanan 2004, Ramasamy et al. 2007). The 96 h-LC50 value of dichlorvos has been reported in *Cirrhinus mrigala* to be 9.1mg/L (Velmurugan et al. 2009). Saha et al. 2016 stated 24, 48, 72, and 96 h-$LC_{50}$ values of dichlorvos to *Oreochromis mossambicus* as 3.84, 3.5, 3.12, and 2.9mg/L respectively using a static-renewal bioassay. In this study, the $LC_{50}$ value of dichlorvos in 24, 48, 72, and 96 hours in *Labeo rohita* were recorded as 40 mg/L, 22.49 mg/L, 12.62 mg/L, and 11.36 mg/L respectively. The findings of the acute toxicity assessment suggest that DDVP was slightly toxic at 24 and 48hr that shows the similarity in the result obtained from the experiment of fingerling mirror carp to DDVP (Ural and Çalta 2005). Zhang et al. (2010) recorded 96 h-$LC_{50}$ value of DDVP for *Danio rerio* as 13mg/L. The present study reported the 96h-$LC_{50}$ value of DDVP on Rohu as 11.36mg/L wherein, the previous study reported a range between 6.5 mg/L to 9.41 mg/L (Ural and Calta 2005, Tilak and Kumari 2009, Velmurugan et al. 2009, Satyavani et al. 2011).

The $LC_{50}$ value of a pesticide to a particular fish species in a laboratory condition also depends on the laboratory setup, fish size, and weight, physicochemical parameters, fish handling method, etc. That may be the reason behind the variation in the $LC_{50}$ values of the same pesticide to the same fish species. The mortality rate of fishes increased with increasing concentration of pesticide in water, which shows the dose-dependent mortality relationship and the same finding has been reported by different authors in different fish (Agbon et al 2002, Sweilum 2006, Omoregie et al. 2009, Musa et al. 2010, Omoniyi et al. 2013). In the experiment, mortality of exposed fish was first observed in 24 h at 16mg/L and no mortality was seen in the lower concentration of DDVP. The pesticide toxicity on fishes depends on the tolerance capacity of individuals

which differs depending on the concentration of pollutants in the water/environment, temperature, state of the test animals, and physiological activity (Hendri et al. 2010).

The respiratory potential and the oxygen consumption of an animal are the important physiological parameters to assess toxic stress because it is a valuable indicator of energy expenditure during metabolism (Proser and Brown 1973) and used worldwide in toxicity studies to evaluate the changes in the metabolism under stressful environmental conditions (Patil and David 2008). The result shows the elevation of the oxygen consumption rate by Rohu fish when exposed to the pesticide. The elevation of oxygen consumption rate in comparison to control may be due to the increase in metabolic activities of fish and might have been helpful to reduce the toxic effect. It was noted that the sub-lethal concentration on day five depicted the high respiratory metabolism rate. A similar result was found in the investigation of Malathion toxicity in Rohu (Patil and David 2008). According to the existing works of literature, the high value recorded on the 5th day of sub-lethal exposure may be attributed to the initiation of specific protein synthesis or increased to detoxify the toxicant (Connell et al. 1999). In contrast, Tilak and Swarna Kumari (2009) reported the depletion of Oxygen consumption in *Ctenopharyngodon idella* when the time of exposure to Nuvan toxicant is increased. The reason behind depletion of the oxygen consumption is the disorganization of the respiratory action caused by a rupture in the respiratory epithelium of the gill tissue.

In the present study, all sub-lethal exposure periods increased the opercular beat rate, which resembles the finding made by Patil and David (2008). Prasanth et al. (2005) also made a similar observation in *C. mrigala* exposed to cypermethrin. Increased gill opercular movements observed may compensate for the increased physiological activities under stressful conditions (Shiva Kumar and David 2004).

The behavioural changes are directly related to complex physiological responses and have often been used as a sensitive indicator of stress (Little and Finger 1990). Many researchers have observed erratic swimming, equilibrium loss, and surfacing phenomenon in the fish following pesticide exposure. During this study, general behaviour changes and some clinical signs were also observed with increasing concentration as well as exposure time. The lower concentrations (2mg/L, 4 mg/L) had similar behaviour with the control group but fishes in the highest concentrated group showed loss of equilibrium, spinal movement, rapid operculum beat rate, and motionless lying at the bottom of the aquarium. Such changes were also documented

by Bhat et al. (2012) in the same fish to DDVP. Several studies have shown that decreased AChE activity due to organophosphate exposure can disturb several behaviours in fish such as swimming (Brewer et al. 2001, Sandahl et al. 2005), feeding (Sandahl et al. 2005), and predator avoidance (Scholz et al. 2000). In this study behavioural changes such as erratic swimming loss of equilibrium, mucus secretion, avoiding schooling behaviour and imbalance posture were frequently observed during the experiment. The dead fish were seen with mouth and operculum wide opened and body slime covered. A similar observation was recorded by Mishra and Poddar (2014) in *Channa punctatus*. It may be the response of the fish body to avoid the contact of pesticide. Mucus secretion in fish probably reduces the contact of toxicants and forms a barrier between the body and toxic media to minimize its toxic effect (Bhat et al. 2012). The avoidance of schooling is the consequence of the stress that is the symbolic behaviour to know about the toxic effect in fish. Change in the skin coloration, loss of scales, and some hemorrhagic patches were seen on the body surfaces in high concentration in the last two days. Surfacing phenomenon and gulping of air was commonly observed in sub-lethal concentrations. Gulping performed by fishes at high concentrations of dichlorvos might be to intake maximum possible air to ease the tension, which resembles the observation made by Rao and Rao (1987) and Ural and Simsek (2006). The surfacing phenomenon in the exposed group might be due to the high oxygen demand during the experimental period (Kalavathy et al. 2001).

Contamination of the aquatic environment by chemicals may affect at cellular and molecular level results which results in the biochemical changes of the organisms (Kavitha et al. 2010). Blood parameters analysis revealed the changes in the hematological content as well as biochemical contents. Generally, fishes exposed to the pesticides had shown a decrease in Hb level in the blood (Adhikari et al. 2004, Kumar et al. 2016, Sharmin et al. 2016, and Ismail et al. 2018) however, in this study, the hematological response of haemoglobin (Hb) seen increased under sublethal toxicity of dichlorvos. Similarly, Sarvanan et al. (2012) also reported the elevation of Hb in fish blood during the toxic treatment where the authors explained that the increment in Hb content is to supply enough oxygen to the tissue. The increase in Hb value in pesticide-exposed fish also might have resulted from the replacement of oxidized denatured Hb by toxicant and to supply more oxygen to tissues (Nussey et al.1995). An increase in Hb concentration in the fish blood also relates to a high metabolic rate. The rate of elimination of the toxic substances off the body also increases with the increase of

metabolic rate (Wang et.al. 2017). The haemoglobin plays the role of the carrier which transfers the oxygen in the blood from the lungs to the tissues and facilitates aerobic metabolism, therefore, an increased level of Hb in the present study may be due to the high demand for the oxygen inside the body for the cellular detoxification.

Protein is the alternative source of energy to meet the increased energy demand. It has been observed that exposure to sub-lethal concentrations of dichlorvos led to an increase in the level of plasma glucose and total protein. In this study, the total protein content in the blood of exposed fish was declined compared to non-treated fishes. A similar result was given by Das and Mukherjee (2003) in the same fish but a rising level of protein was found in the study of Koul et al. (2007) in *Channa gaucha* . The study by Rani et al. (2008) also reported a very significant decrement in protein and lipids in *Labeo rohita* due to nuvan toxicity. Lakshmanan et al. (2013), Sarvanan et al. (2012), El-Sayed et al. (2007) also observed depleted levels of total protein, albumin content in blood serum of different fishes and to different pesticides in their experiment. A similar result was reported in freshwater fish *Colisa fasciatus* exposed to malathion (Singh et al. 2004). Baskaran and Palanichamy (1990) also stated that under a stressed condition, the protein consumed by fish is not stored in the body tissue. It may be due to the mobilization of protein to produce glucose by the process of gluconeogenesis to meet the extra energy requirement (Vasanthi et al. 1990). Brodbury et al. (1987) stated the depletion of protein content due to the destruction of cells and consequent impairment in protein synthesis machinery. The reduction of protein level also may be due to toxicant stress of pesticide and the disorder of liver and kidney (Lavanya et al. 2011).

This study shows the increasing pattern of glucose level in plasma after exposure to a pesticide that has been accepted in the study of *Channa gaucha* to DDVP (Koul et al. 2007) and *Oreochromis niloticus* to cypermethrin (Yuniari et al. 2016). An increase in blood glucose level may be due to the high utilization of glucose to meet the metabolic demands caused by the toxicants (Sarvanan et al. 2012). Jyothi and Narayan (1999) studied the sub-lethal effect of two pesticides on freshwater fish *Clarius batrachus* suggesting the increase of serum glucose levels significantly over the control throughout the experiment with both carbamate and organophosphate pesticides. The conversion of protein into glucose as a source of energy may be the reason behind the elevation of glucose and the depletion of protein.

# 6. CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

The major finding of this study is the acute toxicity of the pesticide dichlorvos and its sub-lethal effects on freshwater fish Rohu (*Labeo rohita*). The study confirms that dichlorvos is toxic to the fish. To conclude, dichlorvos enhanced operculum beat rate and oxygen consumption rate. The research shows the increment in haemoglobin, glucose level but depletion of albumin globulin and total protein content under sub-lethal toxicity of dichlorvos. After the overall analysis, it is concluded that pesticide dichlorvos shows a serious effect on the physiological metabolical, and biochemical aspects of Rohu. It is expected that the use of this pesticide, eventually becoming an environmental hazard to non-target organisms at different biological scale levels unless protective measures are taken.

## 6.2 Recommendations

Because of the significant deleterious effects of pesticides on aquatic life, their use for any purpose must be judicious. Therefore, I recommend the future researchers,

- To perform more research to test whether these effects are detrimental to other fish species in the field.
- The alternatives to synthetic chemical pesticides (mostly biological means) should be encouraged for pest suppression in agriculture.
- The governments should emphasize research and extension activities related to IPM and continuity of IPM programme for minimizing the use of chemical pesticides.

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# APPENDICES

Photo plates 1:  Experimental set up for acute toxicity test.


Photo plates 2: Recording  water quality parameters using multiparameter analyzer

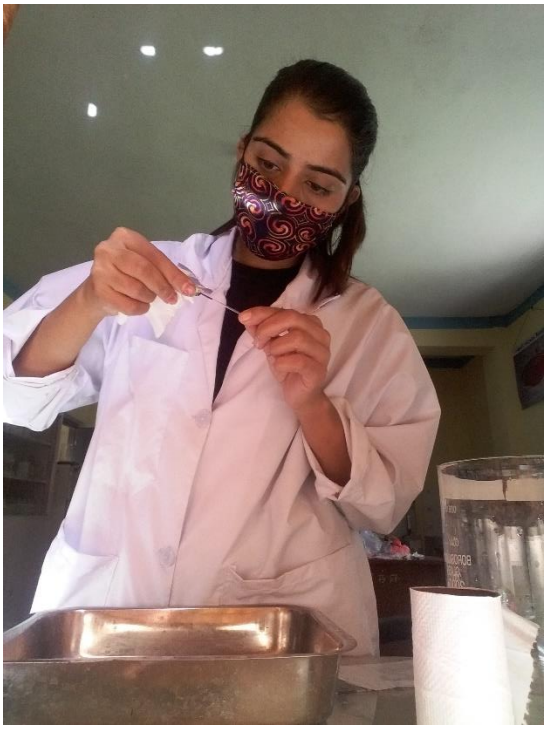

Photo plates 3: Collection of blood using syringe and capillary tubes.