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Acute Toxicity and Respiratory Responses in Freshwater Fish, Labeo Rohita exposed to An Agrochemical Indoxacarb

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ABSTRACT/RESUME

Abstract: *In the present study, an attempt has been made to analyze the toxicity of the Indoxacarb on the freshwater fish Labeo rohita. The toxicity tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static system. Finney's probit analysis, (Finney, 1971) was followed to calculate the LC50 values. Experimental fish were exposed to different concentrations of Indoxacarb for different hours, percent mortality was recorded. The 96 h LC50 value of toxicant to the fish were found to be 0.0521 mg/L. Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased secretion of mucus higher ventilation volume, decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth Behavioral patterns were observed in during exposure period, test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening and closing of mouth for gasping, losing scales, hyperactivity were observed experimental group. The results suggest that indoxacarb is considered as hazardous pesticide in common carp with 96 hrs LC50 value. Also, the mortality rate increased with increase in the concentration of pesticide. All the studies mentioned above indicate a considerable effect of insecticides on oxygen consumption in different species of fish in lethal as well as sub lethal concentrations.*

I. Introduction

Food production has been mainly dependent on the synthetic chemicals to combat the natural pests in the agricultural areas. Due to continuous increase in population, the need for chemical pesticides is valuable in more than one way. Poisoning by pesticides results party through ignorance and party by negligence. It seems probable are to aquatic environment, its fauna from escaping the unwanted pollution, the organisms that include fish, frog, and other useful species are forced to face hazards of pesticides. However, these pesticides account much for the toxicological studies, which are potential health hazards to livestock, wildlife, fishes, birds, mammals and human beings [1]. The undue persistence, high mammalian toxicity and developing resistance of the organochlorine,

organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. To increase productivity, modern methods are practiced in agriculture. One integral part of this is to resort to increased use of pesticides to curb agricultural losses due to pests [2].Unfortunately, after use pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment. Since pesticides are poisons and are meant to kill, during their sojourn through the different compartments of the globe, they kill a host of other non-target organisms. Pesticides are regularly utilized in agricultural to destroy or repel pests, these pesticides as well as some of their active substances; their presence in the environment is of concern. Pesticides from agriculture runoff and other sources into aquatic systems have been increasing their contamination by these products in several areas around the world with potential adverse effects [23, 26]. Unfortunately, after use pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment through surface runoff. Since pesticides are poisons and are meant to kill, repel or destroy during their sojourn through the different compartments of the globe, they kill a host of other non-target organisms [3]. Uptake of the pesticides by different organisms can take place in two ways viz., direct absorption from the medium and biomagnifications through the food chain. While the later plays a major role in the deposition of pesticides in the body tissues of terrestrial organisms, both processes seem to be equally significant in the aquatic organisms. However, during short-term exposure of aquatic organisms to pesticides, absorption from the medium assumes greater importance than uptake through food. Besides accumulating the pesticides in their tissues, many species of fish have been reported to metabolize the pesticides. In fact, an evaluation of the effects of a chemical cannot be said to be complete without an evaluation of the effects of its metabolites [4, 28].

The work of [5,6] and several such other workers has clearly established that the pesticide residues are transported to the aquatic environment either through surface runoff or through precipitation into which they get in by evaporation from cropland. The increasing awareness of the environmental hazards of pesticides necessitated the testing of toxicity of different pesticides to different aquatic organisms. Different types of toxicity tests serve different purposes. The 96 h toxicity test or the short term or acute toxicity test is one of the most commonly employed tests in the evaluation of toxicity. The modern aquatic toxicity protocols in use are the results of a series of attempts at the standardization of the test methodology. The earliest and one of the most useful of these test methods is that of [7]. This forms the basis for all other attempts. In the standard methods of the American Public Health Association, [8] bioassay and toxicity test procedures are described in detail. Indoxacarb is an insecticide with a new class of chemistry and with a new mode of action. It is a reduced risk pesticide with a low mammalian toxicity and a benign profile for avian and aquatic toxicity. Indoxacarb is a broad spectrum insecticide with activity on codling moth, white apple leafhopper, Panda leaf roller, and Lacanobia fruit worm. Hence in the present study, acute toxicity and the effect of sub lethal and lethal concentrations of indoxacarb on the oxygen consumption of fish.

II. Materials and methods

The freshwater fish, Labeo rohita is an edible and commercially valuable fish. Live fish of size 6± 7 cm and 6± 8 g weight were brought from a local fish farm Nandivelugu, India and acclimatized at 28 ± 20C in the laboratory for 15 days. During acclimatization period, if 5% mortality is observed the total batch was discarded. Indoxacarb (14.5% S.C= Suspension concentrate) was supplied by Rallies India Ltd. Hyderabad. The water used for acclimatization and conducting experiments was clear unchlorinated ground water.

II.1. Physico-chemical analysis of water

Temperature- 28±2oC, pH-7.05± 0.10, Dissolved oxygen-8.92± 0.39 mg/L, Carbon dioxide-2.21±0.29 mg/L, Hardness-22.5±2.1 mg/L, Total alkalinity-25.2±3.5 mg as CaCO3/L, Conductivity-< 10 µS/cm, Specific gravity-1.005, Particular matter-< 29 mg/L, Total organic carbon-< 2 mg/L, Unionised ammonia-< 0.75 µg/L, Residual chlorine-< 0.71 µg/L and Electrical conductivity at 28± 2oC -816 micro ohms/cm. All the precautions laid by committee on toxicity tests to aquatic organisms [8, 29] were followed.

Duration of the test:

The concentration of pesticide, which may normally be sub lethal during short-term exposures (24 or 48 h), may prove to be lethal, if the exposure time is extended (up to 96 to 120 h). Since, the toxicity of the poison is a function of time; it is customary to expose the test organisms over a fixed period of time to the toxicant usually for 24, 48, 72 and 96 h. The containers of the test media are of 10 liters capacity; wherein for each test five containers were used and in each container 10 fishes were introduced. Experiments were conducted to determine the toxicity of indoxacarb in various concentrations in static system. The data on the mortality rate of the fish was recorded. The dead fish were removed. The toxicity tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static system. Finney's probit analysis, [24] was followed to calculate the LC50 values. The fish were exposed to lethal (48 h LC50

of 0.05649 mg/L) and sub lethal were (1/10 of 48 h LC50 of 0.005649 mg/L).

II.2.Assay of respiratory rate

Respiratory rate (oxygen consumption) of Indoxacarb exposed fish was measured besides control by following the method of (Welsh and Smith, 1953) as described by [9] and the apparatus setup was the same as described by[10].

Description of respiratory chamber

The apparatus used for the measurement of whole animal oxygen consumption is a wide mouthed bottle, which is called a respiratory chamber (RC). Its mouth was fitted with a four-holed stopper (S) and through one of the holes a thermometer (T) was passed to know the temperature of the medium in the respiratory chamber.

From the remaining three holes three glass tubes were passed whose outer ends were fitted with rubber tubes. These three tubes served as delivery tubes and designated as T1, T2 and T3 respectively. They were fitted with pinch locks P1, P2and P3. T1 was connected with the reservoir (R) and though this water could be drawn (inlet) into the respiratory chamber. T2 was atmospheric tube; useful for testing the air tightness of the respiratory chamber. Through the T3 tube (outlet) water samples from the respiratory chamber were collected for estimation of dissolved oxygen. The respiratory chamber was coated black to avoid photochemical reactions and to keep the animal activity at normal during the experiment.

Only one fish was introduced into each respiratory chamber and was filled with water drawn through T1 from the reservoir. After checking the air tightness pinch lock P2 was closed and pinch lock P3 was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a spate of normalcy from the state of excitement, if any, due to the handling and also to allow the animal to adjust to the darkness in the chamber (acclimatization).

II.3.Collection of the initial and final samples

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through T3. After the collection of initial sample, the respiratory chamber was closed by closing P3 first and then P1 after one hour. The next sample was collected from the respiratory chamber. Likewise, other samples were also collected at the end of each hour for total 22 hours period of the experiment. Along with three experimental fish chambers, one respiratory chamber without fish (control) was maintained. The control serves to estimate the initial amount of oxygen. After experiment, the fish were individually weighed and their unit metabolism was calculated and expressed as ml oxygen consumed/g wet weight/h.

$$O_2 \text{ consumed by fish/ Gram body weight/ hour} = \frac{\alpha-\beta \times N \text{ of hypo} \times 8 \times 1000}{\text{Vol. of the sample taken} \times \text{Correction factor} \times \text{Wt. of the fish} \times \text{Time interval for each sample}}$$

α = hypo rundown before exposure
β = hypo rundown after exposure
N= Normality of Hypo

II.4. Statistical analysis

Student's t-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant.

III. Results and discussion

The results of the present work with reference to observed percent mortality for Avaunt (Indoxacarb 14.5% S.C) for 24, 48, 72 and 96 h to the fish Labeo rohita in static system are given in tables 1,2,3,4& 5.

The results of the present work with reference to the observed percent mortality for indoxacarb were compound with the other animals and different pesticides LC50 values with the indoxacarb. The percent mortality and probit mortality increased with the increasing concentration of indoxacarb.

The percent mortality plotted against log concentration of indoxacarb gave sigmoid curves. The 24, 48, 72 and 96 h. LC50 of indoxacarb was obtained by taking the mean LC50 derived from the percent and probit mortality curves (Figures 1&2).

Table 1. *Determination of static LC50 – 24 h*

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.054	20	4.1584	0.740362	0.548136	17.29229	3.078724
2	0.056	30	4.4756	0.755874	0.571346	20.03099	3.382993
3	0.058	50	5	0.770852	0.594212	25	3.854260
4	0.060	70	5.5244	0.785329	0.61674	30.51899	4.338476
5	0.062	80	5.8416	0.799340	0.638945	34.12429	4.669427

- X 0.770352
- Y 5
- SXX 0.0021739
- SYY 1.9665718
- SXY 0.065082
- Slope B 29.938321
- Variance B 46.000953
- Variance A 27.398906
- M 0.770352
- LC50 = 0.0569 mg/L
- 95% confidence limits upper bound and lower bound = 0.056-0.060 mg/L

Table 2. *Determination of static LC50 – 48 h*

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.052	20	4.1584	0.72427	0.524575	17.29229	3.011828
2	0.054	30	4.4756	0.740362	0.548136	20.03099	3.313567
3	0.056	50	5	0.755874	0.571346	25	3.779374
4	0.058	70	5.5244	0.770852	0.594212	30.51899	4.258494
5	0.060	80	5.8416	0.785329	0.61674	34.12429	4.587582

X 0.7553391
Y 5
SXX 0.0023296
SYY 1.9665718
SXY 0.0673716
Slope B 28.919837
Variance B 42.925846
VarianceA 24.590787
M 0.7553391
LC50 = 0.05649 mg/L
95% confidence limits upper bound and lower bound= 0.054-0.058 mg/L

Table 3. *Determination of static LC50 – 72 h*

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.050	20	4.1584	0.707570	0.500655	17.29229	2.94235
2	0.052	30	4.4756	0.72427	0.524575	20.03099	3.241569
3	0.054	60	5.2533	0.740362	0.548136	27.59716	3.889347
4	0.056	70	5.5244	0.755874	0.571346	30.51899	4.175755
5	0.058	90	6.2816	0.770852	0.594212	39.45849	4.842183

X 0.7397871
Y 5.13866
SXX 0.0025027
SYY 2.8688078
SXY 0.0836428
Slope B 33.420985
Variance B 39.956777
Variance A 21.967744
M 0.7432173
LC50 = 0.05422 mg/L
95% confidence limits upper bound and lower bound= 0.052-0.055 mg/L

Table 4. *Determination of static LC50 – 96 h*

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.048	20	4.1584	0.69019	0.476370	17.29229	2.870111
2	0.050	40	4.7467	0.707570	0.500655	22.53116	3.358623
3	0.052	60	5.2533	0.72427	0.524575	27.59716	3.804838
4	0.054	70	5.5244	0.740362	0.548136	30.51899	4.090059
5	0.056	90	6.2816	0.755874	0.571346	39.45849	4.748103

X	0.7236559	Variance B	37.093744
Y	5.19288	Variance A	19.525175
SXX	0.0026959	M	0.7256316
SYY	2.5680928	LC50 =	0.0521 mg/L
SXY	0.0824442	95% confidence limits upper bound and lower bound= 0.049-0.053 mg/L	
Slope B	30.581625		

Table 5. *Regression values for pesticide indoxacarb*

S.No	Exposure periods in hours	LC50 in % concentration(mg/L)	No.of experimental fish exposed	% of mortality	Regression equation Y=(y-bx)+bx
1	24	0.0569	10	50	$y = 0.18x, R^2 = 0.970$
2	48	0.0564	10	50	$y = 0.2x - 0.04, R^2 = 0.972$
3	72	0.0542	10	50	$y = 0.13x + 0.09, R^2 = 0.986$
4	96	0.0521	10	50	$y = 0.13x + 0.16, R^2 = 0.992$

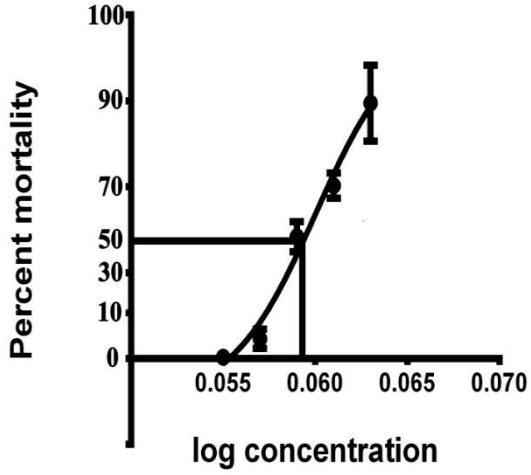

Figure 1. *The graph showing sigmoid curve between percent mortality of fish against log concentration in fish, Labeo rohita on exposure to indoxacarb*

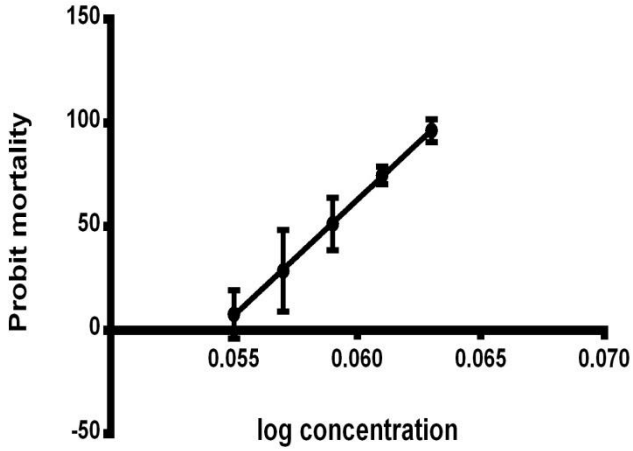

Figure 2. *The graph showing linear curve between percent mortality of fish against log concentration in fish, Labeo rohita on exposure to indoxacarb*

The results revealed that the relentless of the behavioral changes was dependent to the concentration of indoxacarb and exposure time. When the present LC50 values of indoxacarb to the test organisms are compared to the degree of harmfulness shown in the above tables 1-5, it was observed that the pesticide indoxacarb is extremely toxic to the fish, *Labeo rohita*. The toxicity of indoxacarb, the DPX-KN 127 isomer and associated degradates are moderately to very highly toxic to freshwater and estuarine/marine fish on an acute basis with LC_{50}s ranging from 0.024 to >1.3 mg/L [11, 12]. Chronic toxicities range from 0.0006 to 0.0184 ml/L for estuarine fish and invertebrates and from 0.004 to 0.15 mg/L for freshwater fish and invertebrates. They are also moderate to very highly toxic to freshwater and estuarine/marine invertebrates on an acute basis with EC 50 ranging from 0.029 to 2.9 mg/L.[13] reported the LC_{50} values of DPX-MP 062 to blue gill as 900 ppb, for rainbow trout as 650 ppb and for sheep head minnow 96 h-LC_{50} as 374 ppb. These authors also reported the LD_{50} values for rat and bobwhite quails as 1730 ppm and 808 ppm respectively. In the present study also the LC_{50} values for 24, 48, 72 and 96 h for indoxacarb to the fish *Labeo rohita* are in agreement with the earlier reports. The results showed that LC50 of indoxacarb in the present study was lower than other studies on same fish species, which reported 96 hrs LC50 of 0.0531-16.85ppm [13, 14]. Such variances may be species specific. Also, physico-chemical properties of water are the most important factors involved in the different results of indoxacarb toxicity. Some authors have reported the toxicity assessment of different pesticides in the same freshwater fish, *Labeo rohita*.

The alterations can be attributed either to potency of pesticide or variance in the test conditions. [15].Reported that median lethal concentrations (LC_{50}) of monocrotophos for 24, 48, 72 and 96 h were 0.0041, 0.0039, 0.0037 and 0.0036 ppm respectively, whereas the LC_{50} values of synthetic pyrethroid lambda cyhalothrin for 24, 48, 72 and 96 h were 0.0026, 0.0024, 0.0022 and 0.0021 ppm, to fish *Labeo rohita*. The LC_{50} value of malathion an organophosphate pesticide to freshwater fish was found to be 9.0 µl/l, reported by[16]. The 96 h LC_{50} values of botanical pesticide, Kethrin and an organophosphate pesticide (Bhat *et al.*, 2012) Dichlorvos was found to be 21.68ppm and 16.71ppm to freshwater fish *Labeo rohita*, respectively. (Singh, 2013) reported that acute toxicity of dimethoate to freshwater fish *Colisa fasciatus* (Bl. & Schn.) for 24, 48, 72 and 96 h were found to be 22.15 mg l^{-1}, 21.99 mg l^{-1}, 21.74 mg l^{-1} and 21.65 mg l^{-1}, respectively. Carbamates are moderately toxic to fish. Carbaryl has a 96 h LC50 of 2 to 39 mg/L and carbofuran 150 to 87g/L for many freshwater American fish [17] organophosphates has negligible chronic toxicity, but some of them have moderate to high acute toxic. Temperature, hardness, pH, alkalinity and biological factors such as sex, age, weight and physiological status are reported to have profound effects on the acute toxicity of dimethoate. The estimated LC_{50} values of dimethoate to freshwater fish *Labeo rohita* were found to be 17.532 mg l^{-1}, 17.321 mg l^{-1}, 16.721 mg l^{-1}, and 16.350 mg l^{-1} for 24, 48, 72, and 96 h, respectively [5]. Variation in lethal concentration (LC) values of dimethoate in different species occurs probably due to differences in susceptibility and tolerance related to differences in rates of bio-accumulation, biotransformation and excretion of toxicant. It is evident form the above stated LC_{50} values that organochlorines, organophosphates, carbamates and synthetic pyrethroids are more toxic. Due to their persistence and high toxicity on fish and mammals, a restriction on their use is imposed.

III.1.Behavioral variations

The morphological and behavioral changes exhibited by the test fish can be taken as a useful parameter in assessing the toxicity caused by pesticides to some extent [18]. In the present investigation, throughout exposure period of fish to lethal and sub lethal concentration of pesticide indoxacarb for 96 h, numerous behavioral changes were observed which includes erratic swimming, opercular movements and the fish surfaced more often gasping for oxygen. Hyperactive excitation, loss of equilibrium, widening of gills, increase in production of mucus from the gills, darting movements and hitting against the walls of test tanks were noticed in all the species tested. A film of mucus was observed all over the body. Physiological stress has occurred in the form of neuronal excitation, which seemingly has resulted in the incessant synthesis and demolition of neuro transmitters and enzymes [19].

Thus studies on symptomology need much emphasis in understanding the changes in animals[20] in the present study, the behavioral changes observed in the test fish were, swimming near the water surface, hyper-excitability, muscular incoordination, hyperactivity, erratic movement, loss of buoyancy, increased gill mucus secretion and restlessness before death. These activities may be due to the increased metabolic rate and interference of the pesticide with neural transmission.

III.2. Oxygen consumption

The comparative data on the whole animal oxygen consumption of control and experimental fish calculated and expressed as ml oxygen consumed/g wet weight/h, under sublethal and lethal concentrations of indoxacarb on the fish Labeo rohita are given in the Table 6 and represented in figure.3. In sublethal concentrations of indoxacarb, it was observed that fish showed similar tendency of

increase in oxygen consumption during the initial time of exposures i.e.1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The presence of sub lethal concentration of toxicants is inevitable. The toxicant stress in oxygen consumption along with depletion in oxygen in aquaculture practices makes them less fit and reduces growth due to lack of proper metabolism.

Table 6. *The amount of oxygen consumed in mg/g body weight/h of the fish, Labeo rohita*

Hours	Control	Sub lethal 24 h Oxygen consumption ± SD	Sub lethal 8 days Oxygen consumption ± SD	Lethal 24 h Oxygen consumption ± SD
2	0.0662	0.07795^{a} ± 0.002	0.084^{a} ± 0.029	0.09444^{b}± 0.004
4	0.0611	0.0730^{c}± 0.002	0.0461^{a} ± 0.004	0.0444^{b}± 0.029
6	0.0620	0.0584^{a} ± 0.004	0.0410^{b}± 0.003	0.0388^{b}± 0.002
8	0.0477	0.0487^{b}± 0.002	0.0358^{c}± 0.002	0.0277^{a}± 0.001
10	0.0381	0.0341^{a} ± 0.001	0.0256^{b}± 0.004	0.0166^{c}± 0.003
12	0.0286	0.0243^{c} ± 0.004	0.0153^{a} ± 0.001	0.0111^{a}± 0.004
24	0.0243	0.0097^{b} ± 0.001	0.0051^{c} ± 0.042	0.0055^{b}± 0.043

Values are means ± SD (n=3) for oxygen consumption in a row followed by the same letters and are not significantly different (P< 0.05) from each other

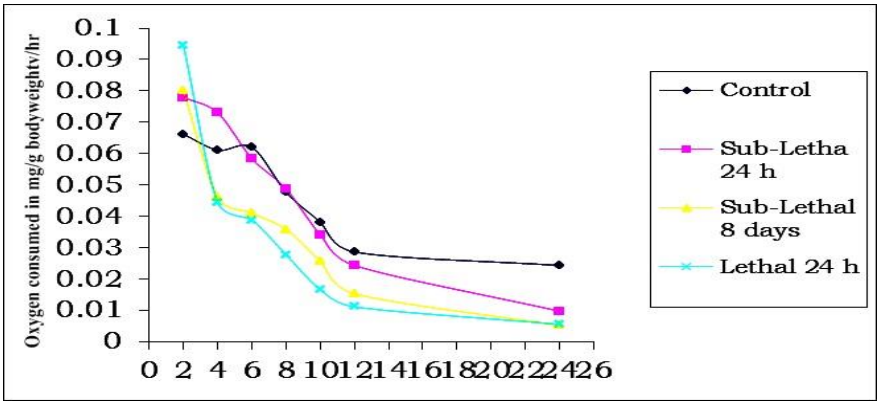

Figure 3. *The amount of oxygen consumed /g wet weight/h of the fish*

In control also, the rate of oxygen consumption gradually decreased and this can be attributed to the reduced metabolic rates in starved conditions. In exposed fish, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium. Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased secretion of mucus higher ventilation volume, decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth. The increased oxygen consumption in the present study is in agreement with [21, 22, 25] in which an raise in oxygen uptake is observed during initial stages of exposure i.e. 1-4 hours followed by decrease in subsequent hours.

IV. Conclusion

The symptoms induced by the indoxacarb insecticide in fish can also be attributed to an increase in physiological stress. Physiological stress may have occurred in the form of neural excitation,

which apparently might have resulted in the continuous synthesis and destruction of neurotransmitting enzymes. All the studies mentioned above indicate a considerable effect of insecticides on oxygen consumption in different species of fish in lethal as well as sub lethal concentrations. The present study revealed alterations in the oxygen consumption of the fish Labeo rohita, exposed to sub lethal and lethal concentrations of Indoxacarb.

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