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RESEARCH ARTICLE

HEMATOLOGICAL CHANGES INDUCED BY IMIDACLOPRID IN CLARIAS BATRACHUS (MAGUR)

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Abstract

Imidacloprid is a Neonicotinoid pesticide extensively utilized in Indian agriculture to manage insect pests.Its water solubility and systemic nature are widely acknowledged to present a risk of contaminating adjacent water bodies.The main objective of this study was to evaluate the effects of Imidacloprid pesticide on the hematological parameters of *Clarias batracus*. In a controlled experimental setting, various sub-lethal concentrations (3, and 4 ppm) of Imidacloprid were administered at different time intervals. The impact of the pesticide was more pronounced in the short-term experiments. Hematological alterations were observed in the fish blood, where the hemoglobin (Hb), Red blood cells (RBCs), Packed cell volume (PCV), Mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin(MCH) declined with increasing concentrations, while the white blood cells (WBCs) increased under the same conditions.The results demonstrate statistical significance at the $P < 0.05$ level. These findings demonstrate that Imidacloprid can lead to significant variations in the hematological characteristics and induce a certain level of stress in fish. Therefore, the hematological factors of fish could serve as a sensitive tool for assessing their health status.

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Introduction:-

Pesticides are agricultural chemicals that are known for their non-biodegradable properties and persistent presence in the environment (Pandeetet al.,2022). Pesticides have found widespread applications, not only in developed nations but also in developing countries, where they play a crucial role in enhancing food production and improving its quality. Consequently, pesticides have become an integral and indelible part of the agricultural progress in our nation (Sarkar et al.,2021). India is the largest producer and consumer of pesticides in Asia, ranking twelfth globally and fourth in pesticide exports (Devi et al., 2017). Pesticides eventually make ways to get into bodies of water, where they may negatively impact aquatic organisms like fish, because water is the ultimate sink. These pollutants can additionally harm aquatic life but also degrade the aquatic environment, making it unsuitable for the growth of different organisms (Pipiletet al.,2011). Imidacloprid is a widely used Neonicotinoid pesticide that primarily targets piercing and sucking insect pests. Due to its systemic properties and high water solubility, it gradually infiltrates nearby water bodies, leading to contamination. This pesticide is a significant environmental contaminant that poses a threat to our ecosystem (Bhardwaj et al., 2020). Neonicotinoids represent the first new class of insecticides introduced in the last 50 years, and among them, Imidacloprid currently holds the distinction of being the most extensively employed insecticide globally (Vijayanetet al.,2017). The walking catfish, also known as *Clarias*

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batracus, is a freshwater, air-breathing catfish found primarily in Southeast Asia. Its unique ability to "walk" across dry land in search of food or suitable habitats gave it its name (Kushwaha et al.,2021). Fish are highly susceptible to aquatic conditions, whether physical, chemical, or biological, and toxic chemicals in aquatic ecosystems pose a significant threat to their survival **(**Sinha et al., 2022). In this study, we examined the hematological changes in *Clarias batracus*that were exposed to sub-lethal levels of Imidacloprid, as the fish's hematological profile is a reliable indicator of fish metabolism and health status under the influence of Imidacloprid stress.

Materials and Method:-

Toxicant Used:
Imidacloprid (17.89% SL), an insecticide commercially produced by Bayer India Private Limited, was chosen for the research based on a survey.

Experiment fish collection:
In aquatic environments, the commonly consumed edible fish, Clarias batracus, is frequently found. Its exceptional adaptability to laboratory settings enhances its market value, making it an excellent choice for toxicological studies (Naiketet al.,2018). The specimens used in the present research ranged in size from 25 to 30 cm and weighed between 100 and 130 grams. These specimens were obtained from the local fish market in Indore, Madhya Pradesh, India.

Acclimatization of fish:
The collected experimental fish were brought into the laboratory and allowed to acclimatize. To prevent skin infection, a 1% $KMnO_4$ solution was used, and they were provided with daily meals to avoid the effects of starvation (Sonietet al.,2018). Before starting the experiment, water analysis was conducted following the guidelines outlined in APHA (2005).

Determination of sub-lethal concentrations:
The most common method to measure a compound toxic effects is to find its LC_{50} value,which is the concentration at which 50% of the test organisms are lethally affected. To determine the LC_{50} values of Imidacloprid accurately, fish mortality was closely monitored, as described by Iqbal et al., in 2005. Exposures were conducted for specific periods (24, 48, 72, and 96 hours).

Collection of Blood Sample:
Following the exposure period, fish from both treated and control groups were captured, and blood samples were aseptically collected from the caudal vein using a sterile syringe without sacrificing the fish. Subsequently, the collected blood was transferred into designated sterile vials for hematological studies.

Fig 1:-*Clarias batrachus*.

Fig2:- Blood collection from the caudal vein of *Clarias batrachus.*

Hematological examination of fish:
After collecting blood, it was used for hematological analysis. Hemoglobin levels were measured using Sahli'sHemoglobinometer, as per Sahli's(1962) methodand we utilized a hemocytometer and Neubauer's counting chamber to assess RBC, WBC, and PCV (%) levels, following the procedures outlined in Dacie and Lewis (1995). Packed Cell Volume (PCV %), Mean Corpuscular Volume (MCV fl), Mean Corpuscular Haemoglobin (MCH pg) were calculated using formula.
MCV (fl) =PCV (%) / Total number of RBCs (million /mm^3) x 10 (fl)
MCH (pg) = Hb concentration / Total number of RBCs (million /mm^3)x 10

Statistical analysis:
In Microsoft Excel, the mean is represented by the standard deviation of the mean for all data. To determine a significant differencein hematological parameters between the treated and control groups, a t-test was performed using Microsoft Excel.

Result and Discussion:-
Alterations in fish hematology reflect their physiological status and can be used to assess their well-being. The blood composition of *Clarias batrachus* showed anomalies after exposure to Imidacloprid.

Haemoglobin (Hb) levels:
In the study, fish exposed to sub-lethal Imidacloprid concentrations displayed a significant decrease in Hemoglobin (Hb) levels compared to the control group, as evident in Table-1 and Figure-3. Notably, the most significant reduction in hemoglobin percentage was observed at 3ppm after 96 hours, measuring 7.85±0.51 (-22.73%). Conversely, the least change was noted at 3ppm after 24 hours, with a value of 8.96±0.26 (-11.63%). Similarly, for 4 ppm, the highest decrease was observed at 96 hours, measuring 7.34±0.07 (-27.75%), while the lowest alteration was seen at 24 hours, with a value of 8.33±0.65 (-15.58%).

Table 1:- Hemoglobin(Hb) levels of *C. batrachus* exposed toImidacloprid over different time period.

Exposure Time	**Treatment of Imidacloprid : Hb (g/dl)**				
	Control	**3 ppm**	**% Decrease**	**4 ppm**	**% Decrease**
24 hrs.	10.14±0.41	8.96±0.26*	-11.63	8.33±0.65*	-15.58
48 hrs.	10.11±0.19	8.59±0.16*	-15.03	8.10±0.55*	-20.11
72 hrs.	10.08±0.58	8.12±0.59*	-19.44	7.67±0.10*	-23.90
96 hrs.	10.16±0.31	7.83±0.51*	-22.73	7.34±0.07*	-27.75

Mean + SD (n=3);*significant at P< 0.05; - Sign represent decreased value, NS represent non-significance.

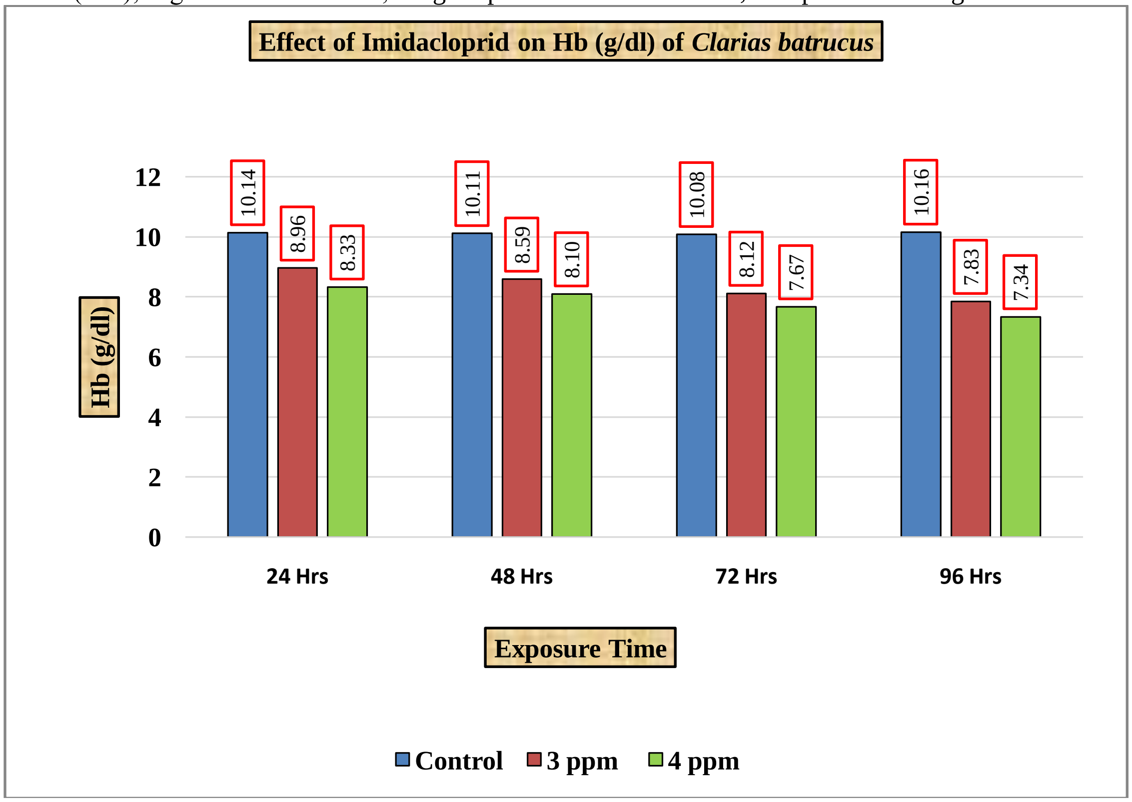

Fig3:- The variations in Hb levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Red blood corpuscles levels (RBCs):

The study revealed a significant decrease in RBC levels in fish exposed to sub-lethal Imidacloprid concentrations for different durations, as shown in Table 2 and Figure 4. The most significant reduction in the percentage of RBCs was observed at 3ppm after 96 hours, measuring 3.61±0.10 (-24.63%). The smallest change was seen at 3ppm after 24 hours, with a value of 4.20±0.33 (-13.58%). Similarly, for 4 ppm, the highest reduction was observed at 96 hours, measuring 3.48±0.51 (-27.72%), while the least change was seen at 24 hours, with a value of 3.93±0.25 (-19.13%).

Table 2:- Red blood cells (RBCs) levels of *C. batrachus* exposed to Imidacloprid over different time period.

Exposure Time	Treatment of Imidacloprid : RBCs (milli./cum.m)				
	Control	**3 ppm**	**% Decrease**	**4 ppm**	**% Decrease**
24 hrs.	4.86±00.7	4.20±0.33*	-13.58	3.93±0.25*	-19.13
48 hrs.	4.80±0.06	3.98±0.61 NS	-17.08	3.68±0.15*	-23.33
72 hrs.	4.84±0.06	3.84±0.48	-20.66	3.56±0.52*	-26.44
96 hrs.	4.79±0.09	3.61±0.10*	-24.63	3.48±0.51*	-27.72

Mean + SD (n=3);*significant at P< 0.05; - Sign represent decreased value, NS represent non-significance.

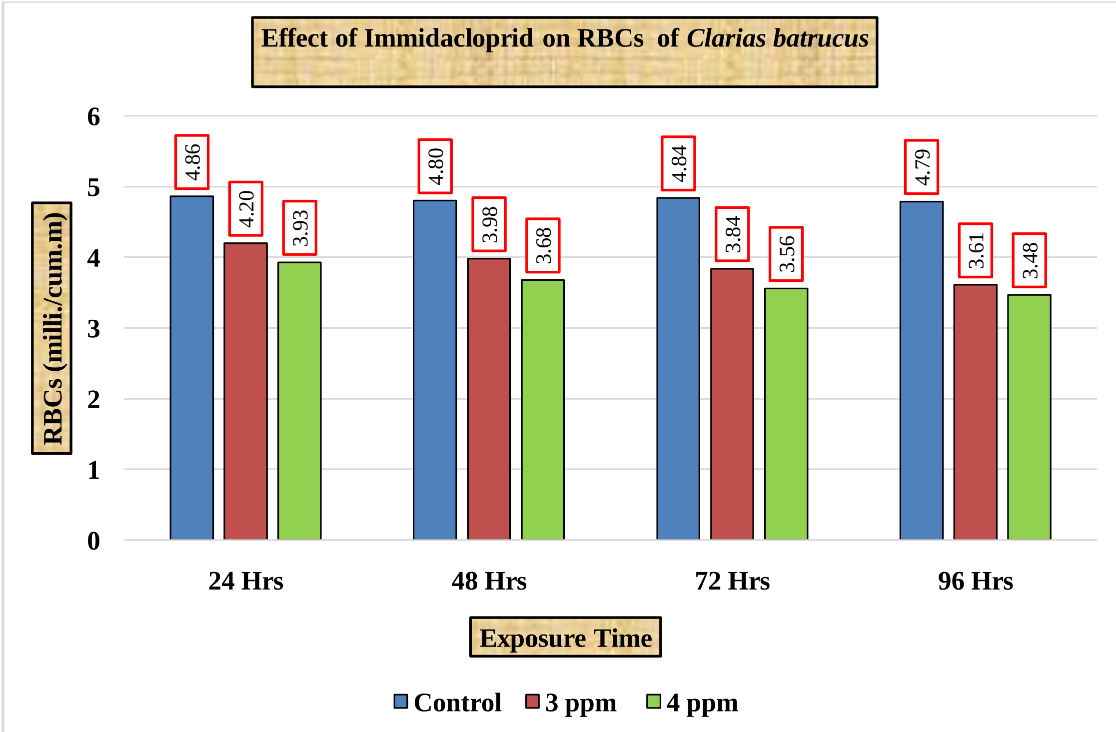

Fig 4:- The variations in RBCs levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Packed Cell Volume (PCV):

The study showed a significant PCV decrease in fish exposed to sub-lethal Imidacloprid concentrations for various durations, as indicated in Table 3 and Figure 5. The results indicate that as the dosage and exposure time increase, the percentage of PCV decreases. The most significant change was observed at 3ppm after 96 hours, resulting in a reduction of 26.50±1.53 (-20.84), while the smallest change was recorded at 3ppm after 24 hours, leading to a decline of 29.88±0.78 (-10.59). Similarly, a substantial change was observed at 4ppm after 96 hours, with a reduction of 26.35±2.16 (-21.29). The smallest change was seen at 2ppm after 24 hours, resulting in a decrease of 28.00±1.92 (-16.21).

Table 3:- Packed Cell Volume (PVC)levels of *C. batrachus* exposed toImidacloprid over different time period.

Exposure Time	Treatment of Imidacloprid: PCV (%)				
	Control	3 ppm	% Decrease	4 ppm	% Decrease
24 hrs.	33.42±1.24	29.88±0.78^{NS}	-10.59	28.00±1.92*	-16.21
48 hrs.	33.33±0.02	27.77±1.80*	-16.68	27.29±1.64*	-18.12
72 hrs.	33.24±0.11	27.35±1.77*	-17.71	25.01±1.83*	-24.75
96 hrs.	33.48±0.92	26.50±1.53*	-20.84	26.35±2.16*	-21.29

Mean + SD (n=3); each value is significant at P<0.05 (Based on t test), - Sign represent decreased value; ^{NS} - represent Non significance.

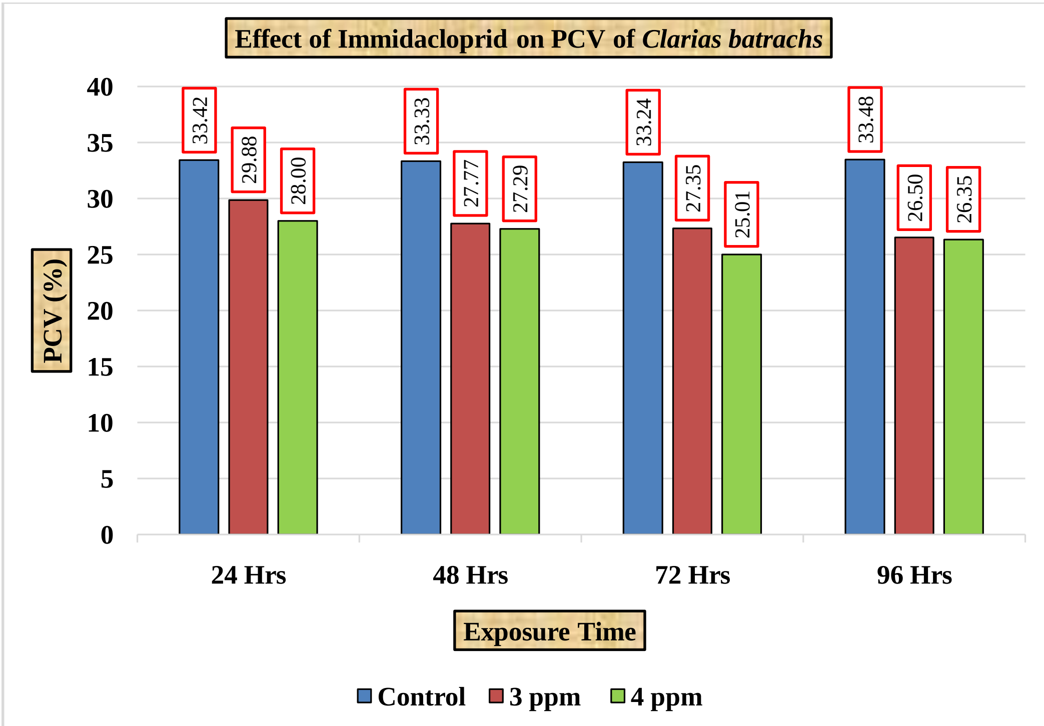

Fig 5:- The variations in PCV levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Mean Corpuscular Volume (MCV):
Table 4 and Figure 6 provide information on MCV values and their percentage changes. The results indicate that MCV decreases as the Imidacloprid dosage and exposure length increase. The most significant MCV change was observed at 3ppm for 96 hours, measuring 57.28±2.71 (-11.3). On the other hand, the smallest change was recorded at 24 hours, with a value of 61.16±1.68 (-4.57). Similarly, at 4ppm, the highest change was observed at 96 hours 56.24±1.12, (-14.46), while the lowest was recorded at 24 hours 58.69±2.16, (-8.42). MCV, which reflects the size of red blood cells, offers valuable insights into their health.

Table 4:- Mean Corpuscular Volume (MCV)levels of *C. batrachus* exposed toImidacloprid over different Time period.

Exposure Time	**Treatment of Imidacloprid : MCV (fl)**				
	Control	**3 ppm**	**% Decrease**	**4 ppm**	**% Decrease**
24 hrs.	64.09±0.84	61.16±1.68^{NS}	-4.57	58.69±2.16*	-8.42
48 hrs.	64.15±0.72	60.11±0.69*	-6.29	58.27±3.52^{NS}	-9.16
72 hrs.	64.29±0.82	58.24±1.03*	-9.41	57.18±2.70*	-11.16
96 hrs.	64.58±0.73	57.28±2.71*	-11.3	56.24±1.12*	-14.46

Mean + SD (n=3);*Value is significant at P<0.05 (Based on t test), - Sign represent decreased value, ^{NS}-represent Non-significance.

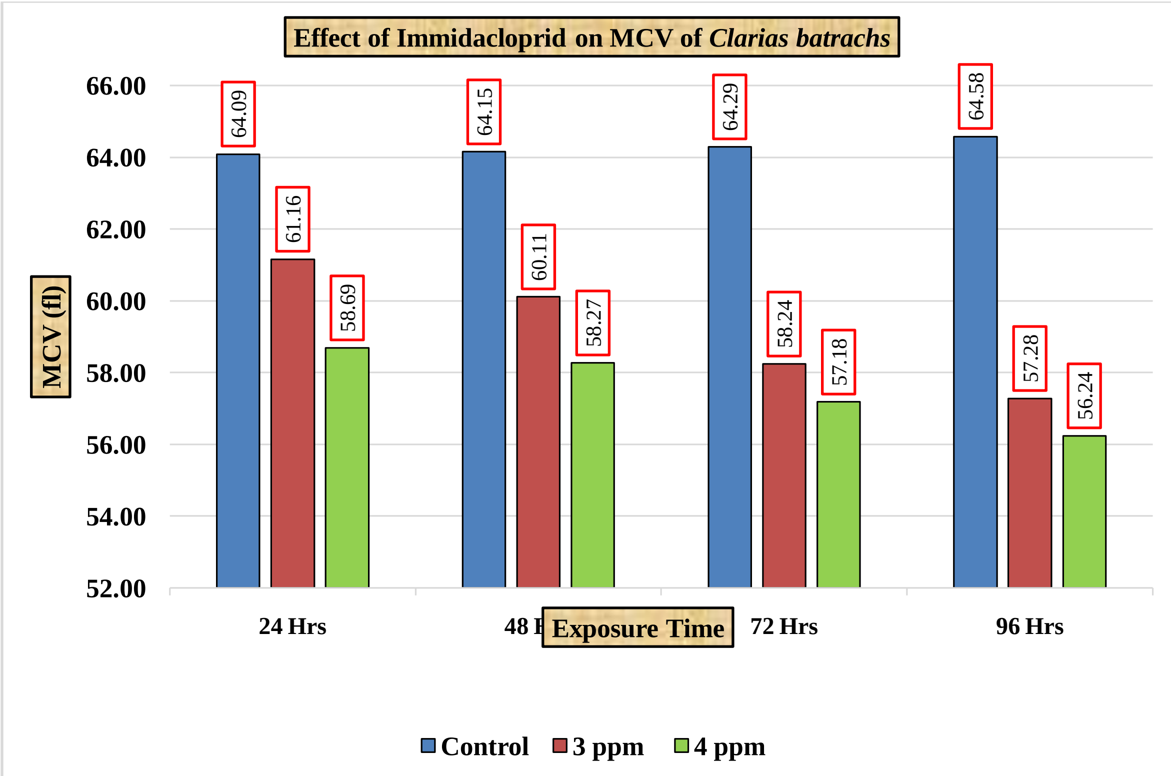

Fig 6:- The variations in MCV levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Mean Corpuscular Haemoglobin (MCH):

In this study, a notable decrease in MCH was observed in Imidacloprid-exposed fish. We find MCH values for both control and exposed fish, along with percentage changes, in Table 5 and Figure 5. The most significant MCH percentage change was at 3ppm Imidacloprid for 96 hours, measuring 18.91±0.21 -10.85), while the smallest change was at 24 hours, with a value of 20.09±0.85 (-9.90) Similarly, at 4ppm, the highest MCH change was at 96 hours, measuring 18.85±0.40 (-11.07) and the lowest change was at 24 hours, 19.34±1.27 (-11.56).

Table5:- Mean Corpuscular Haemoglobin (MCH**)** levels of *C. batrachus* exposed toImidacloprid over different time period.

Exposure Time	**Treatment of Imidacloprid : MCH (pg)**				
	Control	**3 ppm**	**% Decrease**	**4 ppm**	**% Decrease**
24 hrs.	21.87±0.56	20.09±0.85^{NS}	-9.90	19.34±1.27*	-11.56
48 hrs.	21.07±0.60	19.81±0.07*	-5.98	19.31±0.52*	-8.40
72 hrs.	21.82±1.01	19.21±0.42*	-11.96	19.11±0.42*	-12.46
96 hrs.	21.21±0.49	18.91±0.21*	-10.85	18.85±0.40*	-11.07

Mean + SD (n=3), * significant at P<0.05 (Based on t test), - Sign represent decreased value, NS represent Non-significance.

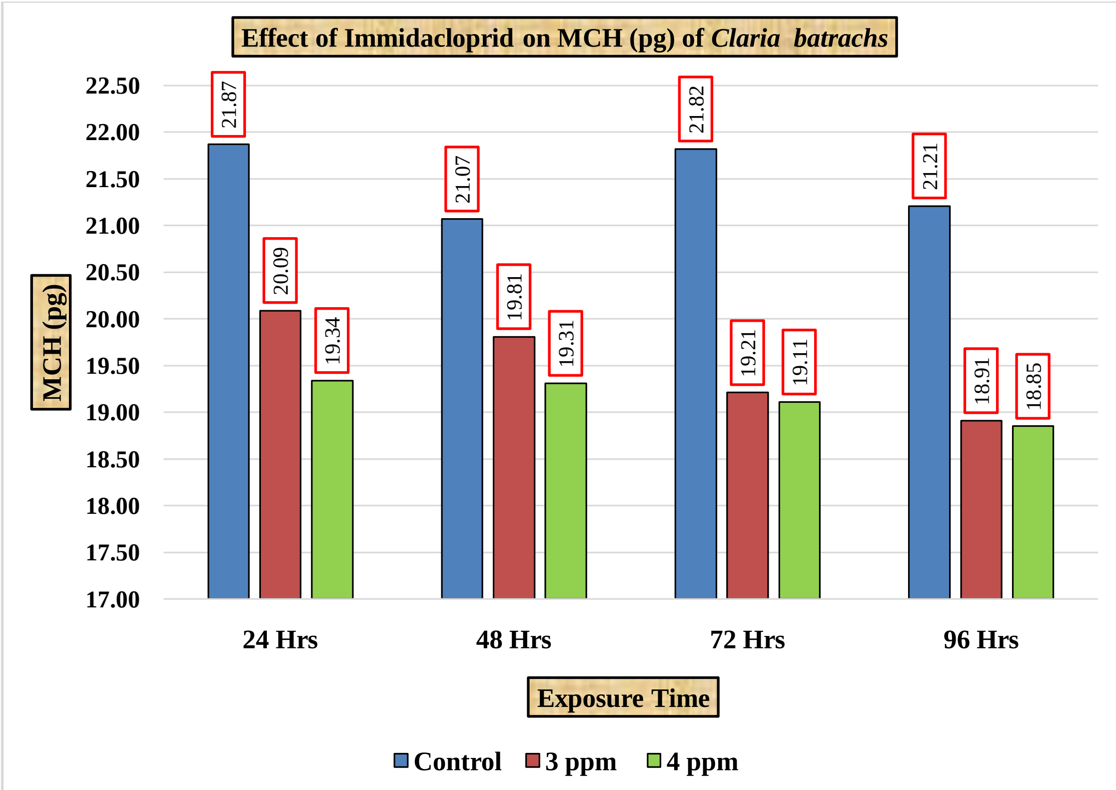

Fig7:- The variations in MCH levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Estimation of White Blood Corpuscles (WBCs) Count:

The study analyzed WBC counts in both control and exposed fish, with percentage changes compared to the control group presented in Table 6 and Figure 6.The most significant increase was observed at 3ppm after 96 hours, with a value of 8.29±0.46 (+44.43%). The smallest change with a value of 7.12±0.09 (+22.79%), occurred at 3ppm after 24 hours. At 4ppm after 96 hours, the highest percentage change, a value of 8.52±0.63 (48.43%), was recorded, while the lowest with a value of 7.30±0.20 (26.07%), was seen at 4ppm after 24 hours. The increase in WBC count is likely due to the immune system becoming more active, which could be attributed to the exposure to Imidacloprid.

Table 6:- White blood cells (WBCs) levels of *C. batrachus* exposed toImidacloprid over different time period.

Exposure Time	Treatment of Imidacloprid WBCs (Cells/cu.mm.)				
	Control	3 ppm	% Decrease	4 ppm	% Decrease
24 hrs.	5.79±0.10	7.12±0.09*	+ 22.79	7.30±0.20*	+26.07
48 hrs.	5.83±0.26	7.73±0.66^{NS}	+ 33.61	8.11±0.65*	+39.10
72 hrs.	5.68±0.11	7.89±0.10*	+ 38.91	8.27±0.60^{NS}	+45.59
96 hrs.	5.74±0.44	8.29±0.46*	+ 44.43	8.52±0.63*	+48.43

Mean + SD (n=3);*Value is significant at P<0.05 (Based on t test), + Sign represent decreased value, NS- represent Non significance.

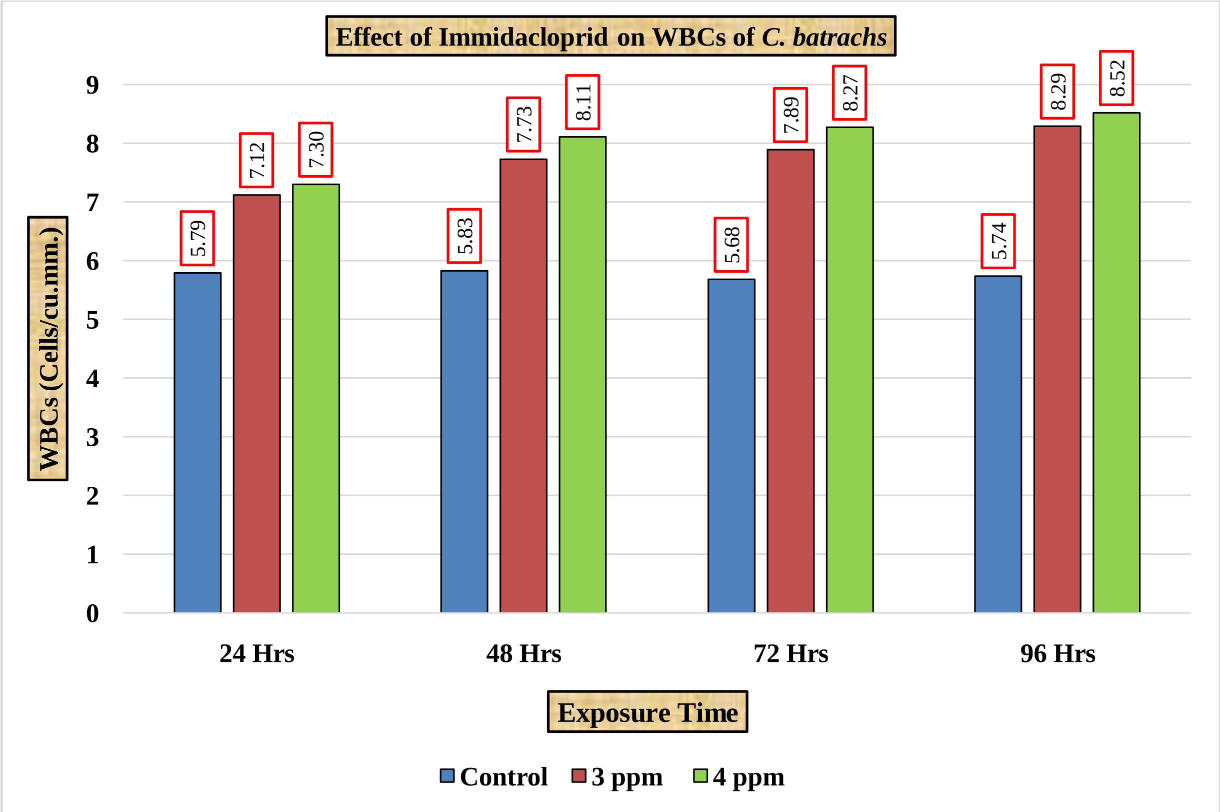

Fig 7:- The variations in WBCs levels in the blood of *C. batracus* after exposure to Imidacloprid for 24, 48, 72, and 96 hours.

Conclusion:-

According to research, pesticides have harmful effects on fish and their blood parameters. Specifically, the Imidacloprid pesticide has been found to cause changes in the hematological parameters of fish, which can significantly impact their overall health. This emphasizes the need for strict monitoring and regulation of pesticide use to safeguard the environment and the well-being of fish. Furthermore, it is crucial for people to use pesticides judiciously and be aware of the need to reduce the utility of Imidacloprid to preserve the aquatic organism and ecosystem.

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