The effect of the fungicide mancozeb on the haematological parameters of the freshwater fish *clarias batrachus*.

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ABSTRACT: Fungicides bring about variations in the physiological activity of aquatic animals like fishes. Hematological indices constitute a vital tool of research in the field of Ichthyology, fish farming, and environmental monitoring. The present study records the percentage (%) mortality of the fish (*Clarias bratachus)* for 24, 48, 72, 96 hours, LC50 (96 hours) when exposed to Mancozeb. RBC count, WBC count, Hb, PCV, ESR, Platelets count, MCV, MCH, MCHC determinations were done by exposing the fish to two experimental groups (6mg for 5 days and 12 mg for 10 days). The results indicate significant changes in the hematological parameters of the fish. Such fishes when consumed by humans is not good for health. To reduce the pollution from such chemicals, people can make use of biopesticides, or the habitat of aquatic animals should be kept far away from such fungicide exposed environment.

Key word – *Clarias bratachus*, Mancozeb.

1. **Introduction**:

Pesticides are substances meant for attracting, destroying, or mitigating any pest. Pesticides are commonly used in crop protection. Although pesticides have benefits, they do have some drawbacks such as potential toxicity to humans and other animal species. They are the pollutants of the environment with undesirable effects on non-target organisms. The use of these chemicals brings out serious chemical problems, especially in the dry season. During this season, the capacity of the water to dilute these chemicals becomes low. So, there is an increased risk of a high concentration of toxic chemicals. Fishes suffer from natural mortality and high fishing pressure at the end of the dry season. When the water gets polluted by pesticides either directly or indirectly, it leads to a reduction in fish production. This also brings down the number of edible fishes which can affect the health of humans eating those fishes. (Mc Ewen and Stephonson, 1979).

Blood analysis is a vital tool of research in the fields of ichthyology, fish farming, toxicology, and environmental monitoring. This is a good indicator of physiological or pathological changes in fishery management and disease investigation (Adedeji *et al.,* 2009). Hematology indices are important parameters for the evaluation of physiological conditions. These changes depend on the fish species, age, the cycle of sexual maturity (Luskova, 1999). Many workers have assessed the effect of pesticides on the behavioral and hematological responses of various fishes. (Miester ,1992). Pesticides are classified based on the two main aspects namely- chemical properties and on the type of pest they are used upon. Based on their chemical properties, pesticides are of five types. Pesticides that act upon the neurotransmitter of the nervous system are called organophosphate pesticides. These are high in the chrysanthemum and these are named pyrethroid pesticides. Some pesticides were in use a few years back, but due to their toxicity to health and the environment these are banned from the market E.g: - DDT, Alachlor. Such pesticides come under organochlorine pesticides. Sulfonylurea insecticides act upon pests by inhibiting their enzyme acetylcholine neurotransmitter. The effect of this chemical is reversible and this has several subgroups within it. Based on the type of pest they are used upon, pesticides are classified as herbicides, insecticides, biopesticides, rodenticides, and fungicides. Herbicides are used to kill weeds and other plants that grow where they are wanted. Insecticides kill insects and other arthropods. Biopesticides are pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. Rodenticides are used to control mice and other rodents. Fungicides are the pesticides used to kill fungi causing the disease to plants.

In agriculture, fungicides are used to protect tubers, fruits, and vegetables. In industries, numerous fungicides are used to protect products during shipment, suppress the formation of microbes that attack the crops, preserve wood, control, fungal growth in the paper, pulps and protect households, carpet, and fabric (Osweiler *et al.,* 1985). In veterinary medicine, fungicides are used as antibacterial or antiseptic agents in the

treatment of crop diseases and as a molluscicide to repel and kill slugs and snails, (Ortolani *et al.,* 2004). Agricultural fungicide thiabendazole is used against intestinal parasites in both humans and veterinary medicine (Lorgue *et al.,* 1996). Some animals may be more susceptible to poisoning than others due to their physiology or behavior. Some fungicides like copper sulphate have a toxic effect on fishes (Pimentel, 1971 and Tomlin, 2000) and bees (Hardly and Kidd, 1983). In each spring, wild birds (e.g., pigeons, pheasants) are poisoned by mercurial fungicides in the fields sown with treated seeds (Bartok, 1981).

Fungicides cause poisoning in animals such as sheep, poultry (Guitart *et al.* 1996 and Oruc *et al.* 2009), and humans (Israeli *et al.,* 1983 and Kuntz *et al.,* 1997). These pesticides are found to cause injury to the muscular membrane of the skin and mucous membranes, Lorgue *et al.,* (1996) reported that pesticides are the common cause of animal poisoning in France up to 45.5%. In the same way fungicide poisoning up to 8.1% was reported in Italy (Caloni *et al.,* 2004). The application of synthetic pesticides is one of the methods used to increase agricultural production. Pesticides in agricultural runoff affect all aquatic organisms. These pesticides are carried away by rains and floods to the nearby aquatic system, thereby affecting aquatic biota, especially fishes which play a major role as a nutritious food. The main aim for the increased use of agrochemicals such as fertilizers, pesticides, etc is to boost up the productivity of crops and in turn get more yield.

Mancozeb is a type of fungicide belonging to the class of carbamate pesticides called Di-thiocarbamate pesticides. Di-thiocarbamates decompose into a number of compounds such as sulphur,5,6-dihyhydro-3H-imidazole[[2,1-c]-1,2,4-Dithiazole-3-thione], ethylene thiourea (ETU), and ethylenediamine (EDA). Ethylene thiourea has high water solubility and specific toxicity (Srivastava missins & Singh, 2013). These dithiocarbamate fungicides are effective against a broad spectrum of plant diseases caused by fungi. In industries, they are used as slimicides in the water-cooling system, in the manufacture of sugar, pulp, and paper, as vulcanization accelerators, and as anti-oxidants in the rubber industry. They are used as scavengers due to their chelating properties in wastewater treatment. Mancozeb reacts with sulphahydryl groups in the disruption of lipid metabolism, respiration, and production of ATP. Mancozeb affects the nervous system through its main metabolites, disulfide, and ethylene thiourea. It is used to protect many fruits and field crops. Its primary metabolite, ethylene thiourea causes thyroid and carcinogenic effects on organisms. Mancozeb, if applied to the soil will have low mobility based on its high adsorption co-efficient. If it is released into water, it tends to adsorb to sediment and forms suspended solids. It has low soil persistence with a reported half-life of 1-7 days. It spontaneously degrades to ethylene thiourea in the presence of water and oxygen. Ethylene thiourea has a persistence of 5-10 weeks. Mancozeb is insoluble in water, but its metabolite Ethyelenethiourea can get dissolved in soil Mancozeb is not toxic to rats when they are provided with oral doses. They are found to be non-toxic even to honey bees and rabbits. However, it is highly toxic to fishes and other aquatic organisms. It has been reported that mancozeb is carcinogenic and weekly mutagenic to some freshwater species. Mancozeb is used worldwide as a less toxic fungicide in agriculture, but as a chemical, it does have a mutagenic effect on crops if used regularly. In the same way, aquatic animals like fishes that grow in the waters of these agriculture fields do consume the fungicide indirectly which leads to several physiological changes as mentioned above. Thus, mancozeb is unstable in water. Hence, the present study is undertaken to record the hematological features of the freshwater fish Clarias batrachus on exposure to mancozeb.

2.Objective of the study:

The present study deals with the effect of the carbamate pesticide- mancozeb on the hematological parameters of the freshwater fish ***Clarias batrachus.*** The main objectives of the study are

- To study the correlation between the hematological indices and the environmental stress on fishes.
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- To study the acute response of mancozeb on the hematological parameters like red blood cell count, White blood cell count, Haemoglobin, Packed cell volume Erythrocyte sedimentation rate, Platelet count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration of the freshwater fish, *Clarius batrachus*.

3.Review of literature:

The effect of the fungicide mancozeb on the hematological parameters of the freshwater fish *Clarias batrachus* was reviewed in this chapter. Hashim and Zaki, (2005) reported a decrease in the RBC

count and PCV value when *Clarias batrachus* was exposed to 0.5ppm/l of dithane for 30 days. According to Eliska Sudova *et al*., (2009), the common carp *Cyprinus carpio.L* showed a decreased amount of erythrocyte count, hemoglobin, and PCV values after its exposure to praziquantel fungicide.

When rainbow trout (Atamanalp & Yanik, 2002) was exposed to sublethal concentration of mancozeb at 24 hours interval for 3 weeks it showed significant alterations in the hematological parameters with a slight increase in red blood cell count and decrease in the hemoglobin, mean corpuscular volume, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration levels. When the freshwater fish *Oreochromis mossambicus* was exposed to two sublethal concentrations of curzate, there was a significant decrease in the hematological parameters like red blood cells, white blood cells, packed cell volume, and increase in mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin (Bhavika Desai and Pragna Parikh,2012). Burchell, (1822) reported that *Clarius gariepinus* when exposed to 0.03g/1 of diuron for 4 days, showed high values of packed cell volume and hemoglobin. When *Channa punctatus* was exposed to sublethal concentrations of indofil (fungicide) significant reduction in the MCHC occurred (Gunjan Sharma et al., 2007).

Alterations in the red blood cell, white blood cell, differential count, thrombocyte count, erythrocyte sedimentation rate, packed cell volume, mean corpuscular hemoglobin, and cytometric measurements of red blood cell, white blood cell, and thrombocytes were noted in *Cyprinus carpio* exposed to fenthion (Muralidharan,2014).

4.Materials and methods:

4.1 Experimental chemical:

Fungicide – mancozeb

The fungicide used for the present study was mancozeb. Mancozeb was purchased from the "Pest and Green" care agro services, at Thiruvanmiyur.

4.2 Experimental animal

The specimen used for the present study was the freshwater fish *Clarias batrachus*. It was selected to assess the sublethal effect of mancozeb on its hematological features.

4.3 Procurement and management of the fish

About 6 specimens of freshwater fish, *Clarias batrachus* (ranging in size from 20-26 cm) were bought from the local market. They were stored in the laboratory tank containing 20 liters of dechlorinated tap water and were acclimatized for the laboratory conditions for two weeks. The water in the aquarium was aerated continuously & changed once every two days. The fishes were fed daily with commercial feed and boiled eggs.

4.4 Determination of LC50

The determination of the sublethal concentration of any pollutant is the most reliable test for assessing the potential adverse effects of toxicants on aquatic life (Brungs and Mount, 1978). To determine the LC50 concentration, the static renewable bioassay method was adopted (Sprauge, 1971). 10 fishes were used for this and they were starved for 24 hours prior to the experimentation to avoid metabolic differences if any due to different feeding and food reserves. Mortality was taken into account every 24 hours, for a total period of 96 hours. The dead fishes were removed from the experimental group immediately to avoid oxygen depletion. During the period of mortality, test fishes were starved and the medium was not aerated. From this experiment, the median lethal (LC50) and safe or sublethal levels of the respective toxicants were found out for the fish- *Clarias batrachus.* LC50 for 96 hours was determined by probit analysis (SPSS package) of Finney (1975).

4.5 Experimental setup

Three aquarium tanks were used. One aquarium tank was used as control without the addition of fungicide, The other two tanks were used for Experiments I & II. In each tank, two fishes were reared. Two sublethal concentrations of mancozeb were prepared by dissolving 6mg and 12mg of mancozeb in one liter of water Experiment I was conducted for 5 days while experiment II was conducted for 10 days. Fishes were fed with commercial feed.

The experiment, temperature & photoperiod were not taken into account because the experiment was conducted in an open room. After the experimental period, the fishes were sacrificed and blood was drawn from the caudal peduncle of the control and the experimental groups. The blood was collected from the caudal peduncle using 2ml disposable syringes using anti-coagulant, Ethylene diamine tetraacetic acid (EDTA), stored at 4° C and assayed for hematological parameters like red blood cell count, white blood cell count, hemoglobin, packed cell volume, erythrocyte sedimentation rate, platelets, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

5.Procedure and methods:

5.1 Red blood cells (rbc) count

Neubauer hemocytometer was used for RBC counting.(Bhavika Desai and Pragna Parikh, 2014)

5.2 White blood cells (wbc) count:

Total WBC count was done using a Neubauer hemocytometer (Bhavika Desai and Pragna Parikh, 2014).

5.3 Estimation of haemoglobin (Hb):

The hemoglobin content of the fish was estimated using Sahli's method(Bhavika Desai and Pragna Parikh, 2014)..

5.4 Estimation of packed cell volume (PCV)

The PCV was calculated through the microhematocrit method(Bhavika Desai and Pragna Parikh, 2014).

5.5 Estimation of erythrocyte sedimentation rate (ESR)

The ESR was calculated using the Westergren metho(Bhavika Desai and Pragna Parikh, 2014)..

5.6 Platelets – count

For counting platelets or thrombocytes also Neubauer hemocytometer was used.

5.7 Mean corpuscular volume (MCV)

The value of the MCV was calculated from the haematocrit value (PCV%) and the erythrocyte counted using the formula.

MCV = PCV X 10RBC count (fl)

The value was expressed in Fenolitres (fl).

5.8 Mean corpuscular haemoglobin(MCH):

Mean corpuscular hemoglobin concentration expresses the concentration of hemoglobin per unit volume of the erythrocyte. It was calculated from the hemoglobin value and from the erythrocyte count according to the formula.

MCH = Hb X 10RBC count

The value was expressed in pg/cell.

5.9 Mean corpuscular haemoglobin count (MCHC):

This refers to the percentage of hemoglobin in 100 ml of a red blood cell. This was calculated by using the formula.

MCHC = Hb X 100PVC %

The value was expressed in g/dl.

6.Results

LC50 for *Clarias batrachus*

The mortality of *Clarias batrachus* was studied at different concentrations (5mg/L to 20mg/L) of mancozeb and the results were tabulated (Table 1).

TABLE.1. Percentage mortality of *Clarias batrachus* exposed to mancozeb

DOSAGE	NO: OF FISHES	24 HOURS	48 HOURS	72 HOURS	96 HOURS	% OF MORTALITY
CONTROL	10	---	---	---	---	---
5mg	10	0	0	0	0	0
10mg	10	0	0	0	2	20
15mg	10	0	0	2	3	50
20mg	10	0	2	3	4	80

LC50, upper confidence limit, and lower confidence limit for 96 hours of *Clarias batrachus* exposed to mancozeb were determined and tabulated (Table 2).

TABLE 2. Probit analysis for determination of LC50 (96 hrs) of *Clarias batrachus* exposed to mancozeb

Concentration	No: of Subjects	Observed Responses	Expected Responses	Residual	Probit
5.00	10.00	.0	.503	-.503	.05029
10.00	10.0	2.0	1.283	.717	.12827
15.00	10.0	3.0	2.653	.347	.26528
20.00	10.0	4.0	4.524	-.524	.45237

Lower confidence limit: 16.34215

Upper confidence limit: 89.64865

No mortality was recorded at 5mg, 10mg, and 15mg of mancozeb, hence it was considered as sublethal concentration. The experiment was done with 6mg/L and 12mg/L as sublethal concentrations and the LC50 for 96 hours was found to be 15mg/L.

The effect of mancozeb on the red blood cell count, white blood cell count, hemoglobin count, packed cell volume, erythrocyte sedimentation rate, platelets, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration of the freshwater fish *Clarias batrachus* were recorded and tabulated (Table.3).

TABLE.3. Effect of mancozeb on the hematological parameters of the freshwater fish, C*larias batrachus.*

S.No	Hematological Parameters	Control	Experiment-l	Experiment-ll
1.	RBC	1.78 millions/cmm	1.74 millions/cmm	1.56 millions/cmm
2.	WBC	110,220/cmm	65,710/cmm	98,970/cmm
3.	Hb	9.2gms	7.4gms	7.1gms
4.	PCV	14.6%	25.1%	26.2%
5.	ESR	0.3mm for 30mins & 1mm for 60mins.	2.3mm for 30mins & 4mm for 60mins.	3mm for 30mins & 7mm for 60mins.
6.	PLATELETS	2000/cmm	4,600/cmm	25,000/cmm
7.	MCV	187.2 fl	161.0 fl	150.6 fl
8.	M	117.9 pg	47.5 pg	45.5 pg
9.	MCHC	63.0%	29.5%	29.0%

Fig.1 Effect of the fungicide Mancozeb on the RBC count of *Clarias batrachus*

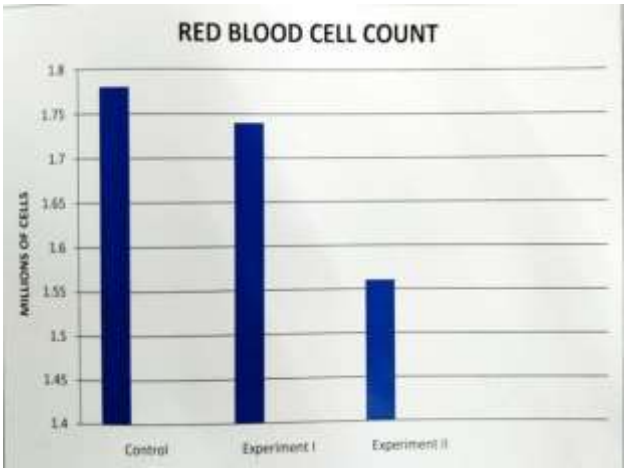


Fig.2 Effect of the fungicide Mancozeb on the WBC count of *Clarias batrachus*

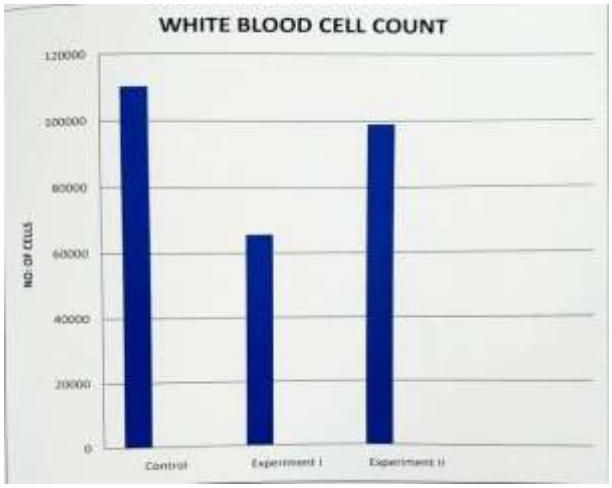


Fig.3 Effect of the fungicide Mancozeb on the Haemoglobin of *Clarias batrachus*

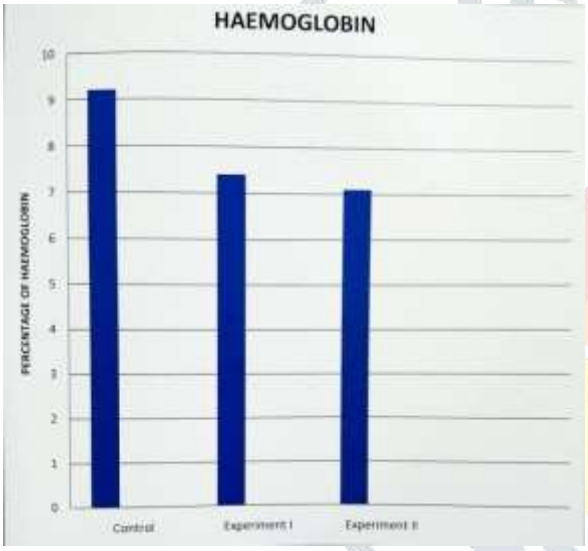


Fig.4 Effect of the fungicide Mancozeb on the Packed Cell Volume of *Clarias batrachus*

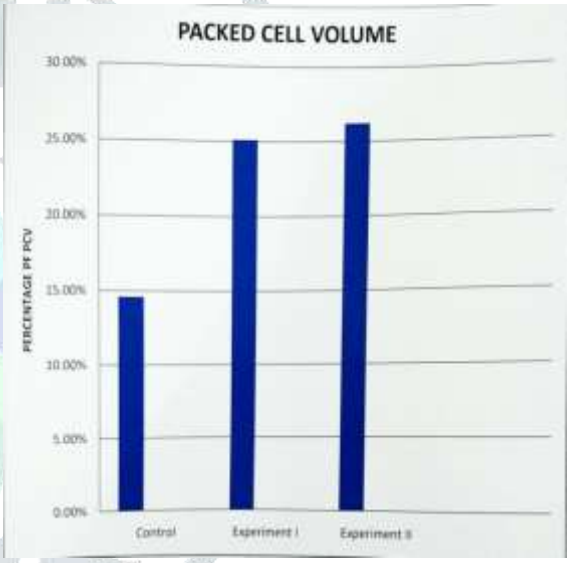


Fig.5 Effect of the fungicide Mancozeb on the Erythrocyte Sedimentation Rate of *Clarias batrachus*

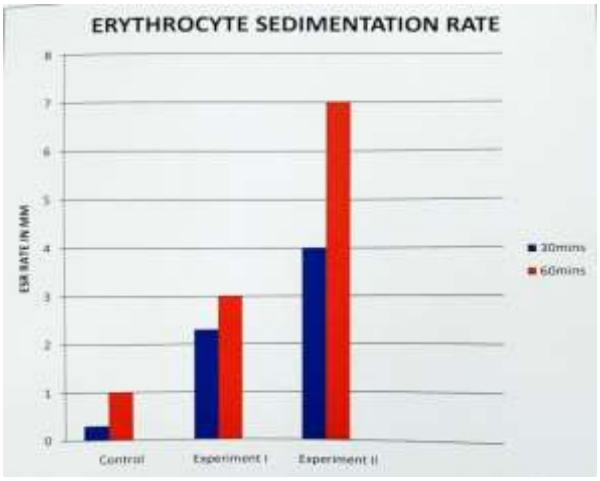


Fig.6 Effect of the fungicide Mancozeb on the Platelets of *Clarias batrachus*

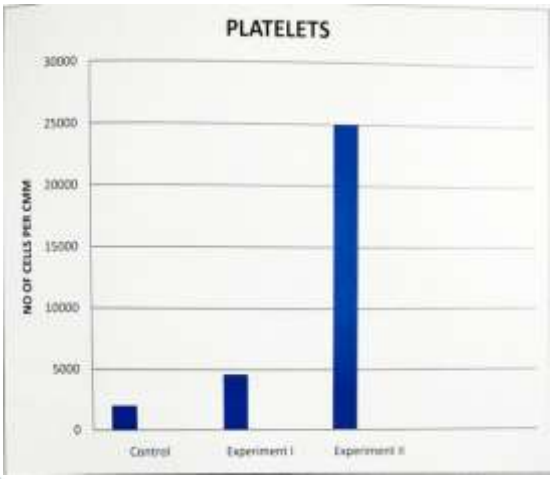


Fig.7 Effect of the fungicide Mancozeb on the Mean Corpuscular Volume of *Clarias batrachus*

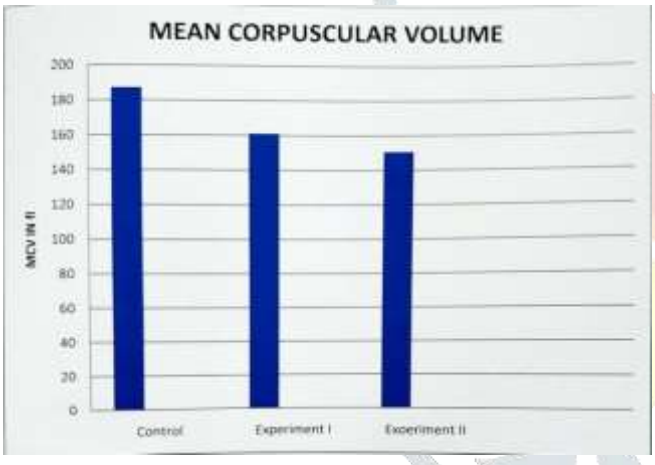


Fig.8 Effect of the fungicide Mancozeb on Mean Corpuscular Haemoglobin (MCH) of *Clarias batrachus*

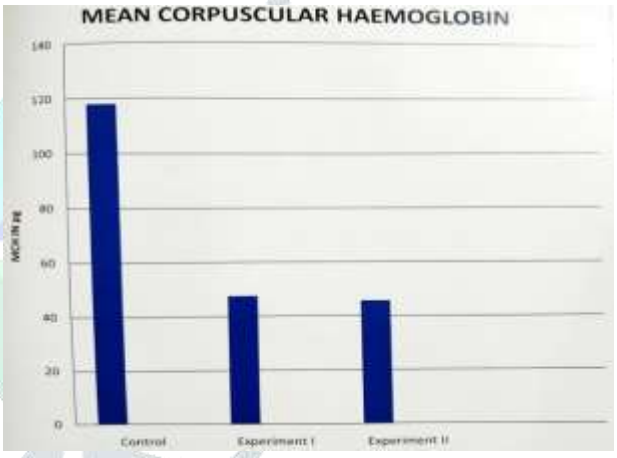
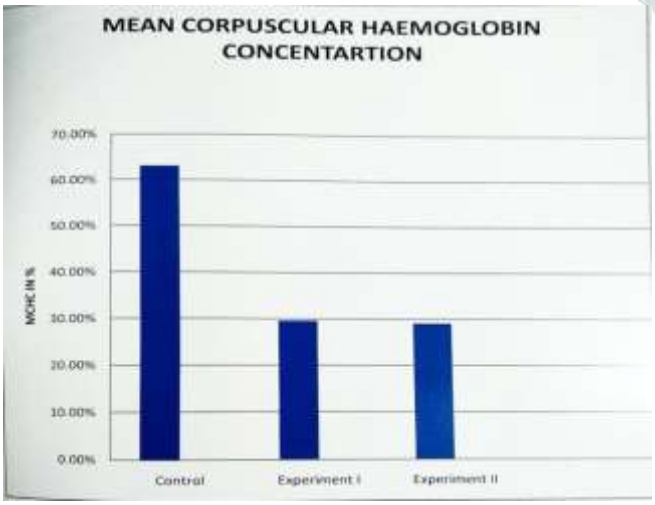


Fig.9 Effect of the fungicide Mancozeb on the MCH Conc. of *Clarias batrachus*



7. **Discussion**:

Fungicides vary in their potential for causing toxic effects on animals. The main hazard to animals from fungicides arises from their use in agriculture. Some livestock poisoning results from overdosing or careless use of fungicide. Hence fungicides should be used carefully in plants, seeds, and trees. Agricultural plants and animal foods should be monitored for fungicide contamination. Blood is the indicator of pathological changes induced by the pollutant. To the onset of any environmental toxicity in the surrounding water, fish blood shows remarkable changes. Hematological parameters are important for toxicological research. They are also used as indicators of environmental stress (Talas and Gurhan, 2009). The hematological studies indicate that acute exposure to fungicide alters the physiological activity of the fish. Hence they are widely used in environmental biomonitoring programs. In view of this fact, the effect of mancozeb on the hematological parameters of the freshwater fish, *Clarias batrachus* was discussed with reference to the works already done on other fishes. The present study indicated significant changes in the hematological parameters of the fish when it was exposed to different sub-lethal concentrations of the fungicide, mancozeb.

Erythrocytes are major and reliable indicators of various sources of stress (Raina Paiva et al ., 2000). Erythrocytes reflect the state of the organism over prolonged intervals of time (Haley and Weister, 1985). High concentration of pesticides or long-term exposure of fish to their sub-lethal concentration, usually decreases the level of RBC count. This is due to the reduced or decreased rate of RBC synthesis. The experiment done in the present study showed a significant change indicating a decrease in the number of RBC on exposure to different sub-lethal concentrations of the mancozeb. A similar condition was observed in *Oncorhynchus mykiss* exposed to cypermethrin (Muhammed Atamanalp and Telat Yanik, 2002), Heteropneustes fossilis exposed to deltamethrin (Kumar *et al* ., 1998), *Tilapia mossambica* when exposed to sumithion and sevin (Koundinya *et al.*, 1980), *Ctenopharyngodon idella* exposed to fenvalerate (Shakoori *et al* ., 1996) *Oreochromis mossambicus* exposed to curzate (Desai and Parikh., 2012).

The present study showed an increased WBC count in *Clarias batrachus* on exposure to sub-lethal concentrations of mancozeb. The increase in WBC indicates the response of the fish to fight against the stress caused by the fungicide. Similar conditions were observed in *Oncorhynchus mykiss* (rainbow trout) exposed to mancozeb (Atamanalp and Yanik,2003), *Channa punctatus* on exposure to deltamethrin (Jayaprakash and Shettu, 2003), *Heteropneustes fossilis* exposed to deltamethrin (Srivastava *et al* ., 2009), *Ctenopharyngodon idella* exposed to fenvalerate (Shakoori *et al* ., 1996).

The low levels of Hb in *Clarias batrachus* indicate the anemic condition of the fish caused by stress. This induced hemolysis and inhibition of aerobic glycolysis curtailing the synthesis of Hb. Mancozeb interferes with the heme and globin synthetic pathway. Reduction in the Hb concentration can be interpreted as a compensatory response that reduces the O2 carrying capacity to maintain gas exchange in the damaged gill lamellae. Similar results were observed in the rainbow trout when it was exposed to mancozeb (Atamanalp and Yanik, 2002), *Channa punctatus* on exposure to deltamethrin (Jayaprakash and Shettu, 2013), *Oreochromis mossambicus* exposed to curzate (Bhavika et al ., 2012).

The present experiment showed a significant increase in the Hematocrit or packed cell volume values of both the sub-lethal concentrations. This result is in accordance with *Oreochromis niloticus* exposed to Fuji-one (Ayyat *et al* ., 2014), *Oncorhynchus mykiss* on exposure to mancozeb (Atamanalp and Yanik, 2002), Tilapia mossambicus on exposure to cadmium chloride (Aziz et al ., 1993). The increased PCV values might have been due to the stress on the fish due to the mancozeb exposure. This confirms the occurrence of hemolytic anemia (Desai and Parikh,2012). The present study showed increased value for Erythrocyte sedimentation rate in the Experiment groups I and II when compared to the control. An increased ESR of blood suggests a possible pathological condition. This result is in accordance with *Channa punctatus* (Bloch.) on exposure to deltamethrin (Jayaprakash and Shettu, 2013), *Clarias batrachus* exposed to sevin (Kumar and Benerje,1990). *Heteropneustes fossilis* exposed to alachlor and rogor (Chaturvedi and Agarwal,1993), *Channa punctatus* on exposure to chlorpyrifos (Sharma et al ., 2009). An increase in the ESR (mm/hr) may be due to an increase in the concentration of fibrinogen which forms into fibrinogenemia (Patnaik and Patra, 2006).

The present study reports increased levels of platelet count. This is in agreement with the increase in the thrombocyte count in (Linn) *Cyprinus carpio* on exposure to fenthion (Leena Muralidharan ., 2014). This may be due to their active role in the coagulation of blood. They are the first group of cells to defend the body against any foreign material as they have the tendency to agglutinate into masses and form deposits upon foreign material. Similarly, an increased thrombocyte count has been found in the African catfish (*Clarius gariepinus*) on exposure to diazinon. Platelets play a major role in blood clotting which prevents blood loss from hemorrhage (Oadedeji et al ., 2008). Increased thrombocytes in sevin treated fishes may be due to internal bleeding. Platelets are phagocytic & their increased number may be to safeguard the fish against toxicity (Sheetu Raina and Anupriya Sacchar, 2014).

The present study showed decreased levels of MCV in Experiment I and Il groups of fishes. A similar condition has been observed in the rainbow trout on exposure to mancozeb(Atamanalp and Yanik,2002). This shows that mancozeb is interfering with the normal physiology of the RBC.

The result of MCH in the present study showed a drastic decrease in the MCH value when compared to the normal. This is in accordance with *Ctenopharyngodon idella* on exposure to fenvalerate (Shakoori et al ., 1996). The decrease in the value of MCH may be a sign of microcytic hypochromic anemia. Similarly, the MCH value decreased in *Oreochromis mykiss* on exposure to mancozeb (Atamanalp and Yanik,2002). The value of MCH depends on the factors of PCV, RBC, and Hb. In the present study, these values are completely altered and so the value of MCH is also affected. This may be due to the stress induced by the fungicide on the fish (Desai and Parikh, 2012).

The MCHC values also decreased in Experiment I and II groups of fishes than the control fishes. Similar results were seen in *Channa punctatus* exposed to deltamethrin (Jayaprakash and Shettu, 2013), *Oreochromis mossambicus* exposed to curzate (Desai and Parikh,2012), *Oncorhynchus mykiss* exposed to mancozeb (Atamanalp and Yanik,2002). Decreased level of MCHC shows the anemic condition of the fish.

The above discussion suggests that the commonly used fungicide mancozeb has a significant impact on the hematological parameters of the freshwater fish - *Clarias batrac*

From this study, it can be known that the commonly used fungicide-mancozeb also has the ability to bring about variations in the physiological activities of aquatic animals like fishes, which may cause a serious impact on their hematological parameters. Such fishes when consumed by humans is not good for health. To reduce the pollution from such chemicals people can make use of biopesticides or the habitat of aquatic animals should be kept far away from such fungicide exposed environment.

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