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Assessment of Carbendazim effects on fresh water fish *Cirrhinus mrigala:* Biochemical , behavioural and Haematological Aspects

Pallavi shukla
Indira Gandhi National Tribal University, Amarkantak
M.P. India

Abstract: *Carbendazim is use to protect crops, fruits and vegetable. Carbendazim is a member of the class of benzimidazoles that is 2-aminobenzimidazole in which the primary amino group is substituted by a methoxy carbonyl group. The present study investigated the effect of carbendazim on biochemical, behavioral and hematological parameters on fish species Cirrhinus mrigala. The study was done after 24h, 72 hand 96h during the exposure with 80% of LC50 doses (23.52 mg/l). In hematological study RBC, Hb, platelets decreased in blood, but WBC and leukocyte level increases. Protein, amino acids, glycogen, nucleic acids and enzyme succinic dehydrogenase decreased in liver, but lactic dehydrogenase levels, protease, GOT and GPT increased in the tissues. Behavioral response decreases with time interval from 24h to 96h. A fungicide, carbendazim controls Ascomycetes, Fungi Imperfect, and Basidiomycetes on a wide variety of crops, including bananas, cereals, cotton, fruits, grapes, mushrooms, ornamentals, peanuts, sugar beet, soybeans, tobacco, and vegetables. Present study suggested that the ecotoxicological effect increases when carbendazim is used for pest control.*

Key words: *Cirrhinus mrigala, Carbendazim, LC50, haematological, Behavioral, Biochemical ecotxicological.*

I. Introduction

In the last few years, the excessive increasing of human population and rapid rate of industrialization have created problem of food crop production. Another major problem is crop production is crop damage by various pathogens like insects, weeds, fungus, bacteria, viruses etc. Pesticides are toxic by design-Biocides, designed to kill and reduce pests, unwanted herbs, rodents, fungi or other organisms which impart threat to crop plants. Pesticides are categorized according to their target used and covered a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and others. Among these, three major classes of pesticides are-herbicides used in weed control, insecticides in insect management and fungicides in mycotic or fungal control. So, they are extensively being used by farmers in modern agriculture practices to increase crop production to sustain the human population. The major chemical groups of pesticides that are usually being applied in fields are organophosphate, carbamates, organochlorine, pyrethroids, trizole, and necotenoides [1] But lack of knowledge and injudicious use of the pesticides leads to lethal effects on organisms. After surface runoff these toxicants enter aquatic system and impart hazardous effect on non-target organisms specially fishes. These toxic chemicals change the quality of water that affects the fish and other aquatic organisms health [2,3].

Pesticides are global for all environments and most are synthetic. The mode of exposure to these toxic chemicals in fish and aquatic animals in many primary ways Dermal, direct absorption by the skin in pesticide-contaminated waters, Inhalation, by direct intake of pesticides through the gills Orally by drinking pesticides-contaminated water or feeding on pesticide-contaminated prey. Among different aquatic organisms, fishes are highly sensitive to the environmental contamination of water. Hence, pollutants such fungicide may affect significantly certain physiological processes like biochemical and hematological parameters, alteration in genetic and protein level, changes in histology and particularly oxidative stress in fishes. Thus it serious effect to health status of fishes. As fish is the mostly consumed aquatic food providing high protein in diet hence it also affects human health.

Fungicide Induced Toxicity in Fish Fungicides is substances used to control fungal organisms causing crop damage and prevent fungal plant diseases. Fungicides usage in agricultural fields to control pests is extremely toxic to non target organisms like fish and affects fish health through impairment of metabolism, sometimes leading to mortality [4]. Pesticides toxicity in fish has been studied by previous researchers who have shown that at chronic level, it causes diverse effects including oxidative damage, inhibition of ACHE activity, biochemical changes, histopathological changes as well as hematological and developmental changes, mutagenesis and carcinogenicity. Pesticides present in the environment with other similar compounds, may

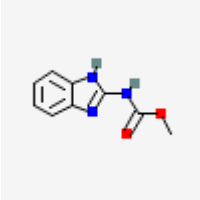
induce lethal or sub lethal effects in fish [5].A fungicides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment.

Exposure of fish and other aquatic animals to pesticides depends on its biological availability (Bioavailability), bioconcentration, biomagnifications, and persistence in the environment. Bioavailability refers to the amount of pesticide in the environment available to fish and wildlife [6]. Persistence (long -standing) pesticides breakdown slowly and may be more available to aquatic animals [7].The information about possible pesticides affect fish and other aquatic life depend upon different factors like, type of Pesticide product, use rates (frequency), weather conditions, aquatic species involved and number of fish killed upon exposure. A lethal dose is the amount of pesticide necessary to cause death because not all animals of a species die at the same dose, a standard toxicity dose measurement, called a lethal concentration 50 (LC50), is used. This concentration of pesticide that kills 50% of a test population of fish within a set period of time is usually determined after 24 to 96 hours.

Carbendazim is a member of the class of benzimidazoles that is 2-aminobenzimidazole in which the primary amino group is substituted by a methoxycarbonyl group. A fungicide, carbendazim controls Ascomycetes, Fungi Imperfecti, and Basidiomycetes on a wide variety of crops, including bananas, cereals, cotton, fruits, grapes, mushrooms, ornamentals, peanuts, sugar beet, soybeans, tobacco, and vegetables. It has a role as an antinematodal drug, a metabolite, a microtubule-destabilizing agent and an antifungal agrochemical.

Chemical Name: Methyl (1H-1,3-benzimidazol-2-yl) carbamate

Chemical formula: C9H9N3O2



Cirrhinus mrigala is a bottom feeding fish. It is an omnivorous type fish. Adults feed on algae and vegetable detritus and debris. Fingerlings feed on vegetable debris, unicellular algae, detritus and mud. Scales are of moderate size; lateral line scales are 40 to 45. Caudal fin deeply forked. Color of the body is silvery, dark gray along the back, sometimes coppery. Pectoral, ventral and anal fins are tinged with black.

II. MATERIALS AND METHODS

Chemical Carbendazim purchased from trading company, and all the other chemicals were of analytical grade and obtained from Indian commercial source. Experimented animal the fresh-water fish *Cirrhinus mrigala* (total size 13-18 cm and weight 36-50 g) for adult brought from local fresh-water pond. They stored in laboratory tank containing 100 liters of de-chlorinated tap water and acclimatized to the laboratory conditions for 2 weeks.

Toxicity test

Toxicity test was applied by the method of Singh and Agarwal [8]. Five fishes kept in glass aquaria containing 1 0 L de-chlorinated tap water. Fishes exposed for 24h to 96h to three different concentrations of pesticides in laboratory. Control fishes kept in similar conditions without any treatment. Each group of fish replicated three times. Mortality recorded after every 24h. Dead animals removed to prevent the decomposition of body in experimental aquarium. The effective doses (LC values, upper and lower confidence limits, slope value, and heterogeneity) calculated by probit log method of Robertson et al. [9].

Experimental designs

Behavioral The behavioral response of C. mrigala on exposure to Carbendazim started after 1-week of treatment. The change in the behavior pattern of *C. mrigala* exposed to these fungicides as well as control group is summarized in (Table 1)

Hematological Five fish were randomly selected from both control and treated groups upon completion of the sub lethal exposure period of 24h, 72h, 96h to fungicide. Blood was collected from the caudal vein by the using heparin coated syringe and shifted to EDTA tubes for estimation of total erythrocyte count, total leucocytes count, hemoglobin percentage, hematocrit, MCV, MCH, MCHC and platelets count through hematology analyzer.(Table 2)

Biochemical Fishes exposed to 80% of 24h LC50 doses (23.52 mg/l).Experiment conducted from 24h to 72h. After completion of treatment, the test fishes removed and washed with water and killed by severe blow on head and operated their liver dissected out in ice tray and used for biochemical and enzymatic analyses. Control

fishes kept in similar condition without any treatment. Each experiment replicated at least 6 times and values expressed as mean ±SE of six replicates. Following parameters tested by different methods. Protein level estimated according to the method of Lowery et al. [10] using bovine serum albumin as standard. Estimation of total free amino acids made according to the method of Spices [11]. Estimation of DNA and RNA performed by method of Schneider [12] using diphenylamine and orcinol reagents respectively. Glycogen estimated by anthrone method of Van Der Vies [13], lactic dehydrogenase by method of Anon [14], succinic dehydrogenase by method of Arrigoni and Singer [15], protease by method of Moore and Stein [16],GOT and GPT by method of Reit. (Table 3).

III. Result

Behavioral Effect-Fish exposed to various concentration of fungicide have shown changes in swimming behaviour, feeding activates, predation, competition, reproduction, and social interactions with species. Behavioural changes are shown in table 1.

Hematological Effect - Hematological assessment was carried in the collected blood of fishes. From the hematological analysis performed on the fishes, some of the important findings emerged which shown in table2 and Fig 2

Biochemical Effect

The toxicity of commonly used fungicides in different organs in fish include gill, heart, kidney, liver, muscle and spleen. Biochemical changes and Enzymatic changes in liver tissue present in (Table 3, Fig. 3)

Table 1: Behavioral effect of Carbendazim on fish species Cirrhinus mrigala at different time interval .

Behavioral Parameters	**Control**	**24hr (80%)**	**72hr (80%)**	**96hr (80%)**
Rate of swimming	+	*	**	***
Surfacing activity	+	*	**	***
Convulsions	+	*	**	***
Rate of opercular activity	+	*	**	***

(+) sign indicate normal while (*) sign indicate abnormal behavioural parameters behaviour of *C. mrigala*.

Table 2: Haematological effect of Carbendazim on fish species *Cirrhinus mrigala* at different time interval

Hematological parameters	**Control**	**24hr (80%)**	**72hr (80%)**	**96hr (80%)**
Hemoglobin (Hb) (g/dl)	11.9 ± .29	10.52 ± .12	9.64 ± .12	7.04 ± .08
Red Blood Cells (RBC) (106 cells/ mm3	5.2 ± .05	4.2 ± .08	3.8 ±.17	2.7 ± .08
White Blood Cells (**WBC**) (/mm3)	17± .43	20.58 ± .48	23.53 ± .17	24.41± .39
Platelets (Lakhs/mm3)	3± .08	2.6 ± .25	1.35 ± .05	1.5± .05
Total Leukocyte Count (TLC/ mm3)	19± .20	20.21± .39	23.43± .10	24.92± .23

Values are expressed as mean ± SE (standard error) of the replicates.

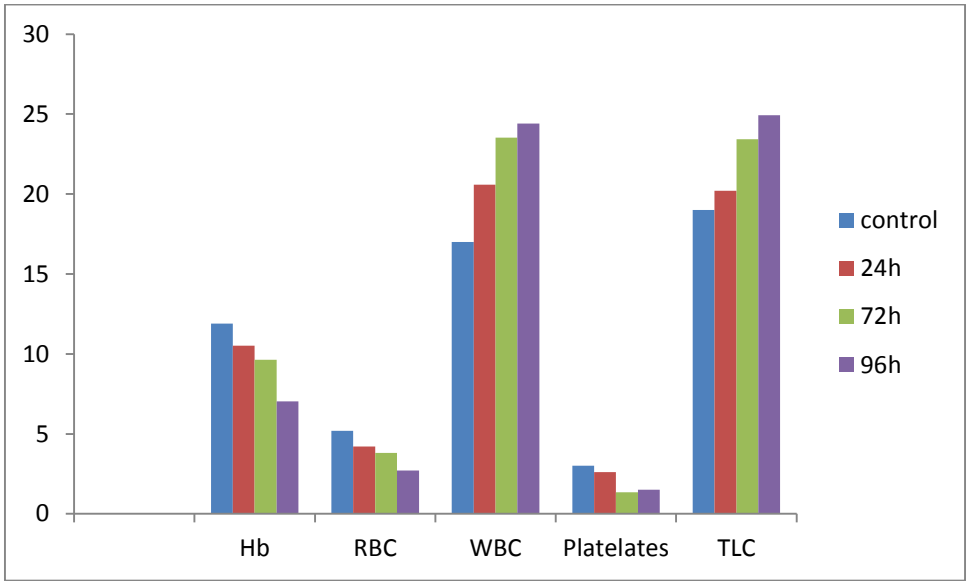


Fig 2: Hematological profile of Carbendazim on fish species *Cirrhinus mrigala* at different time interval .

Table 3 :GOT (Nmoles pyruvate/ mg protein/h) and GPT (Nmoles pyruvate/ mg protein/h), nucleic acids (Ng/mg) level and Total protein (Ng/mg), total free amino acids (Ng/mg), glycogen (mg/g), LDH (pyruvate reduced/min/mg protein), SDH (Nmoles dye/min/mg protein), in different liver tissues of fresh water fish Cirrhinus mrigala exposure to 80% of LC50 of Carbendazim at different time intervals.

Parameter	Tissue	Control	24hr	72hr
Proteases	Liver	13.52± .29	18.23± .10	19.42± .17
GPT	Liver	14.31± .31	18.23± .21	21.41± .12
GOT	Liver	20.13± .12	24.21± .16	26.40± .18
DNA	Liver	13.41± .19	10.15± .13	6.32± .19
RNA	Liver	12.01± .13	9.01± .05	7.31± .10
Protein	Liver	26.21± .14	18.31± .12	15.18± .10
LDH	Liver	20.01± .24	24.04± .17	28.43± .16
SDH	Liver	22.01± .19	13.39± .18	12.00± .22
Glycogen	Liver	35.01± .14	22.01± .16	17.32± .15
Aminoacid	Liver	23.42± .23	28.01± .18	30.41± .04

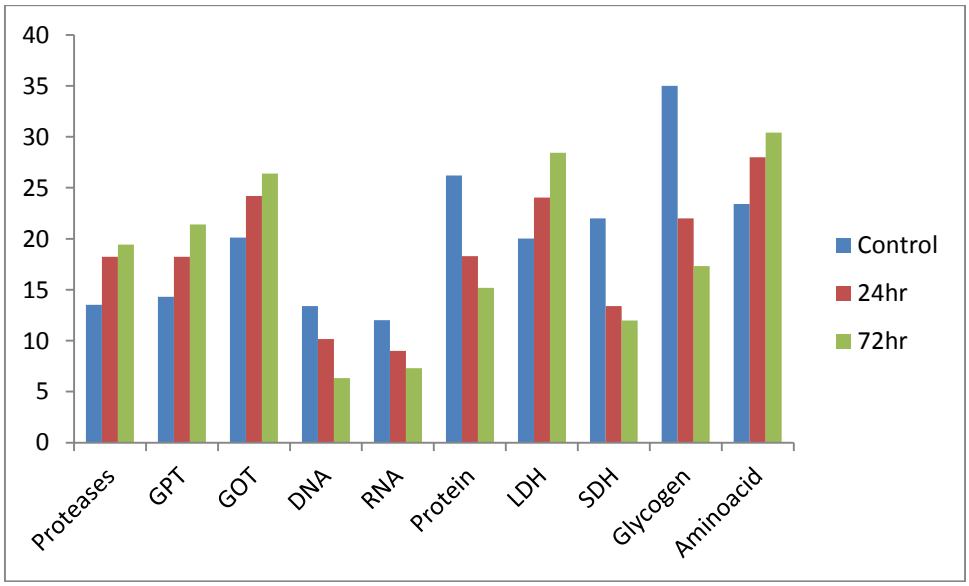


Fig 3: Activity of enzyme protease (tyrosine/mg protein/h), LDH (pyruvate reduced/min/mg protein), SDH (Nmoles dye/min/mg protein), GOT (Nmoles pyruvate/mg protein/h) and GPT (Nmoles pyruvate/mg protein/h) in different tissues of fresh water fish *Clarius batrachus* exposure to 80% of LC50 of Carbendazim at different time intervals.

IV. Discussion

Fungicides causing toxic effects on animals from different types. The main hazard to animals from fungicides arises from their use in agriculture. Some animals poisoning results from careless use of fungicide. Hence fungicides should be used carefully in agriculture. Agricultural plants and animal foods should be monitored for fungicide contamination. The behavioral change is an integrated output of nervous system at the organismal level in response to the underlying biochemical, morphological or physiological disturbances and therefore it is considered as an ideal endpoint reflecting a series of toxic effects and compensatory responses [17].Inaquatic toxicology studies, pesticide induced neurotoxicty and behavioral changes in fish have been the subject of many investigations due to importance of nervous system in maintaining the life of an organism by enabling the monitoring of internal and external environments and response to changes appropriately [18-20].Behavioral action is a sequence of quantifiable actions that operated through the central and peripheral nervous systems [21] and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life such as feeding, reproduction and predator avoidance. Behavior is not a random process, but instead of it, is a highly structured and predictable sequence of activities designed to ensure maximal fitness and survival of the individual. Fish are able to uptake and retain different toxicants dissolved in water via active or passive processes. Sub-lethal concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms and this is more common than mortality [22]. Blood is the indicator of pathological changes induced by the pollutant. 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 [23]. 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 Carbendazim on the hematological parameters of the freshwater fish, *Cirrhinus mrigala* 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 Carbendazim.

Erythrocytes are major and reliable indicators of various sources of stress [24]. Erythrocytes reflect the state of the organism over prolonged intervals of time. 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 Carbendazim. A similar condition was observed in Oncorhynchus mykiss exposed to cypermethrin , Heteropneustes fossilis exposed to deltamethrin [25].The present study showed an increased WBC count in *Cirrhinus mrigala* on exposure to sub-lethal concentrations of Carbendazim. 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 ,Channa punctatus on exposure to deltamethrin, Heteropneustes fossilis exposed to deltamethrin [26]. The low levels of Hb in Cirrhinus mrigala indicate the anemic condition of the fish caused by stress. This induced hemolysis and inhibition of aerobic glycolysis curtailing the synthesis of Hb. Carbendazim interferes with the heme and globin synthetic pathway. Reduction in the Hb concentration can be describe as a good 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 [27].

Reduction in level of protein in experimental fish under pesticide influence is indicates hepatic insufficiency and probably malnutrition. Protein reduction might observe in the present study due to high-energy demand in TCA cycle. The decrease level was also associated with the increase level of protease enzyme in tissues. Decrease in protein content under toxicity stress has already being reported [28]. The decrease in total protein level and increase in free amino acids level in both tissue and liver suggest the high protein hydrolytic activity due to elevation of protease activity [29]. Increase in free amino acids level was the result of breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis and decline in nucleic acids level [30].

In the present study increase, level of amino acids and protease has observed. The metabolites of Carbendazim in ETU, EU and natural products cause depletion of glycogen and fat in fish body. Liver, suggested as an organ for detoxification but under the influence of toxic chemicals, the alternation of their functions may cause. During exposure to sub, lethal concentration of Carbendazim fishes came under stress condition and need more energy to cope the toxicants. Glycogen serves as reserve material. It utilize when body came under stress condition. Depletion of glycogen in liver and tissue may be due to increasement in gycolysis pathway. During stress conditions, the glycogen reserves depleted to meet energy demand [31]. The freshwater fish, Clarius batrachus, has reported to exhibit significant reduction in the level of glycogen [32] studied effects of cypermethrin on various biochemical parameters. Similar results have found in this study. The protein is the

alternative source of energy. Due to the disturbance of arrangement of tissues nucleic acid metabolism also degraded in cells, resulting in the reduction in the DNA content. Furthermore, inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. The regulatory roles of nucleic acid metabolism as observed in the different animals when treated with the different pesticides. Decrease level of RNA also observed by Das and Mukherjee [33]. In this study the level of LDH, GOT and GPT significantly increases under the effect of Carbendazim. Carbendazim has ability to modify the effect of several enzymes. These enzymes are blood soluble enzyme and best indicator of stress conditions [34]. LDH may indicate changes and hypofunction of liver under the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes released from the cells into the blood serum. Increase level of LDH shown by Das et al. [35]. In the present study, the activity of SDH reduces. It is due to the mitochondrial disruption. SDH activity indicated anoxic hypoxic conditions when the fish exposed to toxicant and it was possibly, leading to decrease in the activities of oxidative enzymes and an increase in the glycolic enzymes. Narra et al. [36] reported decreased SDH activity in different tissues of food fish Clarius batrachus exposed to chlorpyrifos. In the present study the level of GOTand GPT inhanced. It might be that GOT and GPT function at the junction between carbohydrateand protein metabolisms. Increase concentration showed probably elevation in gluconeogenesis through transamination of glucogenic amino acids for energy demand in stress condition. Murugesan et al. [37] also found that Sarotherodon mossambicus, when exposed to sublethal and lethal concentrations of carbaryl, showed adaptive elevation in the activity levels of GOT and GPT enzymes, particularly in liver and muscle.

V. Conclusion

In the light of the above findings we may conclude that use of fungicides should be done by the farmers. Fungicide (Carbendazim) which is mostly used by farmer in Indian agriculture is most dangerous for aquatic life. It is dangerous for fishes and its life cycle. It causes severe biochemical, hematological and behavioral effect on fish, so it is necessary to avoid any pesticide in agriculture to save the aquatic life and infect our life also.

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